Bioluminescence

Overview

Notes: For substantial portions of this activity, you need to have access to darkness. I have done this both by presenting the drop-in activity at night-time (which still required ducking under a large tablecloth to view the bioluminescence) and by presenting the full activity across two rooms. The full activity cannot be done at night.

Learning Goals

1. The ocean presents a strong selective environment for bioluminescence. The trait has evolved in the oceans over 40 times.

Set-up

1. Fill up the fish tank with water. (Optional: Add two drops of bleach per quart to the water. This will still be safe to drink, should a kid lick their hands, but will keep the water from being a cesspool of kid germs after the first hour).
2. Put the fish in the tank. A bubbler makes the fish appear to move, but is an optional add-on.

Materials

Items We Provide:

Fish Game
- Fish toys in a variety of colors (red, pink, blue, yellow, etc.)

Circuits
- Construction paper
- Googly eyes
- Glue
- Pipe cleaners
- Circuits
  - Push button switches
  - LED lights (LED light emitting diodes) in a variety of colors
  - Breadboards
  - Wires
  - Battery wires/clips
  - Resistors

Items You Will Need:
- 9v12 batteries (for Circuits)
- AAA batteries (for flashlight)
- Glass fish tank or clear tupperware (for Fish Game)
- Multi-colored flashlight

Procedure

1. **Young Kids:** Most young people will immediately be drawn to the fish tank.
   a. Illuminate it with white light and ask them to identify the colors of fish. You should either have 4 or 5 colors, depending on your variety pack. Next, specify the challenge: You are going to illuminate the fish tank with red light and blue light and they are going to have to find all the pink fish. You are going to then time how long it takes to find the fish under the different lighting conditions.
   b. It will take much longer with the red light than the blue light (unless you have a really large fish tank, this is partially due to the red tones of the fish and less to the length of light, but science outreach isn't perfect). You can ask them which light color was harder (red), and which color would help you illuminate your underwater world (blue). Feel free to explain light wavelengths if the audience is old enough to appreciate it.
   c. (Optional) Ask how a creature might use light to see without giving away their location (flashing). Simulate this with the flashing feature on the flashlight.
   d. Kids like to do this activity over and over. Repeat with all of the colors of fish. They get to grab fish out of the water and they find this delightful.

2. **Older Kids**: (If they aren't drawn to the fish tank).
   a. Explain bioluminescence and how it works in bioluminescent creatures. Ask them to brainstorm animals they know that bioluminesce, and why.

Short Form

1. There are two different modules that accompany the drop-in activity. Viewing bioluminescent organisms works well for all ages and is the backbone of the activity. The aquarium activity works really well for younger kids (though some people never get too old to stick their hand in a fishtank).

Long Form

1. Talk about parallel evolution (or in some cases convergent evolution, but the goal is not necessarily to distinguish). Discuss why bioluminescence has evolved multiple times (at least 40!!!). Ask why it might have been useful.
2. Using the Widder (2010) paper (**attached at the end of the Training Guide** - please don't actually share the paper with the kids), share 2-3 mechanisms for how bioluminescence works. Then share 2-3 reasons for why bioluminescence evolved. Discuss the difference between PROXIMATE vs ULTIMATE.
3. Now hand out the circuit materials (breadboard, wires, switches, battery packs, lights, etc) and some art supplies (googly eyes, paper and pens). Most kids will have never played with circuits before, so make sure you have the manpower to facilitate for each group. Give them 10-15 minutes to design and implement a creature that uses bioluminescence. Tell them that they have to explain the PROXIMATE MECHANISM and the ULTIMATE EVOLUTIONARY GOAL of the bioluminescence. Invite them to share their creations with the group.

Further Resources

- **Proximate and Ultimate**
  https://dash.harvard.edu/bitstream/handle/1/23931958/How%20come%20%20what%20for.pdf?sequence=3&isAllowed=y

NGSS Standards

3-5-ETS1-1 Engineering Design

Define a simple design problem reflecting a need or a want that includes specified criteria for success and constraints on materials, time, or cost.

MS-ETS1-2 Engineering Design

Evaluate competing design solutions using a systematic process to determine how well they meet the criteria and constraints of the problem.

3-LS4-2 Biological Evolution: Unity and Diversity

Use evidence to construct an explanation for how the variations in characteristics among individuals of the same species may provide advantages in surviving, finding mates, and reproducing.

5-LS2-1 Ecosystems: Interactions, Energy, and Dynamics

Develop a model to describe the movement of matter among plants, animals, decomposers, and the environment.

MS-LS2-5 Ecosystems: Interactions, Energy, and Dynamics

Evaluate competing design solutions for maintaining biodiversity and ecosystem services.*

MS-ESS3-3 Earth and Human Activity

Apply scientific principles to design a method for monitoring and minimizing a human impact on the environment.*

MS-LS4-4 Biological Evolution: Unity and Diversity

Construct an explanation based on evidence that describes how genetic variations of traits in a population increase some individuals' probability of surviving and reproducing in a specific environment.

MS. Growth, Development, and Reproduction of Organisms

LS1-4. Use arguments based on empirical evidence and scientific reasoning to support an explanation for how characteristic animal behaviors and specialized plant structures affect the probability of successful reproduction of animals and plants respectively.

MS.Natural Selection and Adaptations

MS.Interdependent Relationships in Ecosystems

Bioluminescence in the Ocean: Origins of Biological, Chemical, and Ecological Diversity

1 author:

Edith A. Widder
Ocean Research & Conservation Association
82 PUBLICATIONS   **2,895** CITATIONS

Some of the authors of this publication are also working on these related projects:

Bioluminescence View project

Bioluminescence in the Ocean: Origins of Biological, Chemical, and Ecological Diversity

E. A. Widder

From bacteria to fish, a remarkable variety of marine life depends on bioluminescence (the chemical generation of light) for finding food, attracting mates, and evading predators. Disparate biochemical systems and diverse phylogenetic distribution patterns of light-emitting organisms highlight the ecological benefits of bioluminescence, with biochemical and genetic analyses providing new insights into the mechanisms of its evolution. The origins and functions of some bioluminescent systems, however, remain obscure. Here, I review recent advances in understanding bioluminescence in the ocean and highlight future research efforts that will unite molecular details with ecological and evolutionary relationships.

The vast majority of bioluminescent organisms reside in the ocean; of the more than 700 genera known to contain luminous species, some 80% are marine (1). These occupy a diverse range of habitats, from polar to tropical and from surface waters to the sea floor (2). The ecological importance of bioluminescence in the ocean is manifest in the dominance of light emitters in open waters; luminescent fish (e.g., mycophids and hatchetfish) and crustaceans (e.g., copepods, krill, and decapods) dominate in terms of biomass, whereas bacteria and dinoflagellates dominate in terms of abundance (3, 4). Its import is also evident in the large number of organisms that retain functional eyes to detect bioluminescence at depths where sunlight never penetrates and in the remarkable degree of diversity and evolutionary convergence among light-emitting organisms (4).

Bioluminescent species are found in most of the major marine phyla from bacteria to fish. As a phylum, comb jellies have the highest proportion of bioluminescent species, whereas other phyla such as diatoms and arrow worms have none or few luminescent representatives (2, 4).

Rivaling its diverse distribution is its impressive array of colors, intensities, and kinetics. Measurements of bioluminescent emission spectra have revealed a rainbow palette of hues that extend over the full visible range (Fig. 1) (5–8). Because most bioluminescence has evolved in the open ocean, most emission spectra are blue, centered on the wavelength that travels farthest through seawater ($\lambda_{max} \sim 475$ nm) (4). Green is the next most common color and is more often found in benthic and shallow coastal species, possibly because increased turbidity from particles in the water scatters blue light and favors the transmission of longer wavelengths (6, 9). Violet, yellow, orange, and red occur only rarely, and in most of

these cases their functions and chemistries remain obscure (1, 5–8).

Photon fluxes span at least nine orders of magnitude, from about 10^3 photons per second for a single bioluminescent bacterium to more than 10^{12} photons per second for some krill and fish (10–12). Emission kinetics range from the persistent glow of bioluminescent bacteria to flashes as brief as 43 ms from lanternfish light organs (10). Luminescent chemicals may be released directly into the water or retained within cells called photocytes. The angular distribution and waveband of light emitted by photocytes may be adjusted by means of muscles and complex optical components that reflect, refract, or filter the light, in which case the photocytes and accessory structures are called photophores or light organs. Emitters may also produce spatial patterns of light displayed over the surface of their bodies or by swimming patterns during light emission (4). All of these parameters carry information to the eyes of potential predators, prey, or members of the same species.

Understanding what function bioluminescence serves in a particular organism provides insight into what selection pressures imposed by the environment and by intergroup competition may have favored the evolution of bioluminescence in one group over another. Wide diversity among light-emitting chemistries has long confounded efforts to trace evolutionary origins. Here, I review new evidence centered on alternative cellular functions for light-emitting molecules and genomic analyses of light emitters that further illuminates the evolutionary origins of bioluminescence.

What Are the Functions of Bioluminescence?

The many functions of bioluminescence reflect the unique nature of the visual environment in which they have evolved. The open ocean is a world without hiding places, where sunlight filtering down through clear water diminishes approximately 10-fold for every 75 m of decent, until all

visible light disappears below 1000 m (12). Under sunlight or moonlight, the light field is dim, blue, and highly directional. In order to hide, many animals vertically migrate downward into the dark depths during the day and only venture into food-rich surface waters under cover of darkness (4). This results in what some consider the most massive animal migration pattern on the planet (12). As a consequence of this migration, most open ocean inhabitants live their lives in dim light or darkness, where bioluminescence can aid animal survival in at least three critical ways: (i) It can serve as an aid in locating food, either by means of built-in headlights or by the use of glowing lures. (ii) It can be used to attract a mate by means of species-specific spatial or temporal patterns of light emission. (iii) It can function as a defense against predators (4). The last is probably the most common use and takes many forms. Some animals, including crustaceans, squid, jellyfish, and fish, release their light-emitting chemicals into the water, producing clouds or particles of light that serve to distract or blind a predator (2, 4, 12). Other animals mark their predators with luminescent slime, making them easy targets for secondary predators (2). Alternatively, when caught in the clutches of a predator, some luminescent prey produce bright and often elaborate displays, which attract secondary predators that will attack the first attacker, thereby affording them an opportunity for escape (2, 4). Luminescence may also be used as a warning to predators, signaling the unpalatability of the prey (2). It is also used extensively as camouflage, in a process called counterillumination, whereby the silhouette of an opaque animal is replaced by bioluminescence of comparable color, intensity, and angular distribution to downwelling ambient light. This latter use of bioluminescence is common among fishes, crustaceans, and squid that inhabit the twilight depths of the ocean where many predators have upward-looking eyes adapted for locating the silhouettes of prey (2, 4, 9).

In most cases, the presumed function of the light emission has its basis in inference from morphological and physiological characteristics rather than experimental studies or in situ observations (13). For example, in the case of fish with red-emitting light organs (Fig. 1), the location of the light organ just below the eye and the unusual long-wavelength sensitivity of the eye suggest that their red luminescence may be used to illuminate prey that are blind to red light (14). In the case of luminous bacteria that form specific symbioses with certain marine fishes and squid, the adaptive value of the light emission is generally evident: The bacteria provide the host with light that can be used to attract prey, evade predators, or attract a mate, while the host provides the bacteria with an ideal growth environment (15). For free-living bacteria where the adaptive value is less evident, the most generally accepted hypothesis is that luminous bacteria growing on fecal pellets may serve as

Ocean Research and Conservation Association, Fort Pierce, FL 34949, USA. E-mail: ewidder@teamorca.org

an attractant, causing the pellets to be consumed and thereby introducing the bacteria to an animal's nutrient-rich gut (2).

Experimental evidence for function has been largely confined to studies of physiological control, such as experiments demonstrating the ability of counterilluminators to adjust the physical characteristics of their ventral light emissions to match those of experimentally manipulated downwelling light fields (4, 9). Behavioral experiments are far less common, with the most extensive studies being those with dinoflagellates demonstrating that their light emission reduces grazing by nocturnal predators (4).

Opportunities for direct in situ observation are rare. Explorations with submersibles and remote-operated vehicles are regularly revealing new luminescent organisms, such as the newly discovered bombardier worms: swimming deep-sea annelids that release green light bombs when disturbed (16). But many behaviors can only be observed unobtrusively by using methodologies that have recently become possible with far-red

illumination and intensified imaging technologies (17).

What Is the Biochemical Variability of Bioluminescence?

The chemical reaction involved in bioluminescence must be sufficiently energetic to produce an excited singlet state molecule that will generate a visible photon as it relaxes back down into its ground state (in contrast to fluorescence and phosphorescence, which depend on absorption of sufficiently energetic photons). Chemical oxidation reactions involving molecular oxygen fit this criterion (1), which may explain why the primary mechanism operating in bioluminescent reactions involves the breakdown of a peroxide bond (18). In fact, the generic terms for the enzyme (luciferase) and substrate (luciferin) involved in light-producing reactions require taxon prefixes to distinguish the different bioluminescent systems (Fig. 2).

In bacteria, two simple substrates [a reduced flavin mononucleotide ($FMNH_2$) and a long-

chain aliphatic aldehyde (RCHO)] are oxidized by molecular oxygen and luciferase. The aldehyde is consumed during the reaction but is continuously synthesized by the bacteria, resulting in a persistent glow (15). Alternatively, the chemical structure of dinoflagellate luciferin bears a striking similarity to chlorophyll (Fig. 2), which suggests that it originated in photosynthetic species. Although the biosynthetic pathway of luciferin is unknown in dinoflagellates (19), a dietary dependence on dinoflagellate luciferin has been suggested in krill (2). Ostracod luciferin is an imidazopyrazinone synthesized from three amino acids (Trp-Ile-Arg) as is coelenterazine (Phe-Tyr-Tyr) (Fig. 2), but in both cases the details of biosynthesis are unknown (2). In the case of coelenterazine, its manner of biosynthesis has recently become of particular interest with the discovery that coelenterates require it as a dietary source (20). Although there is some circumstantial evidence for its synthesis in crustaceans (21), such a linkage remains to be confirmed. In some bioluminescent systems, accessory proteins serve as secondary emitters, which shift
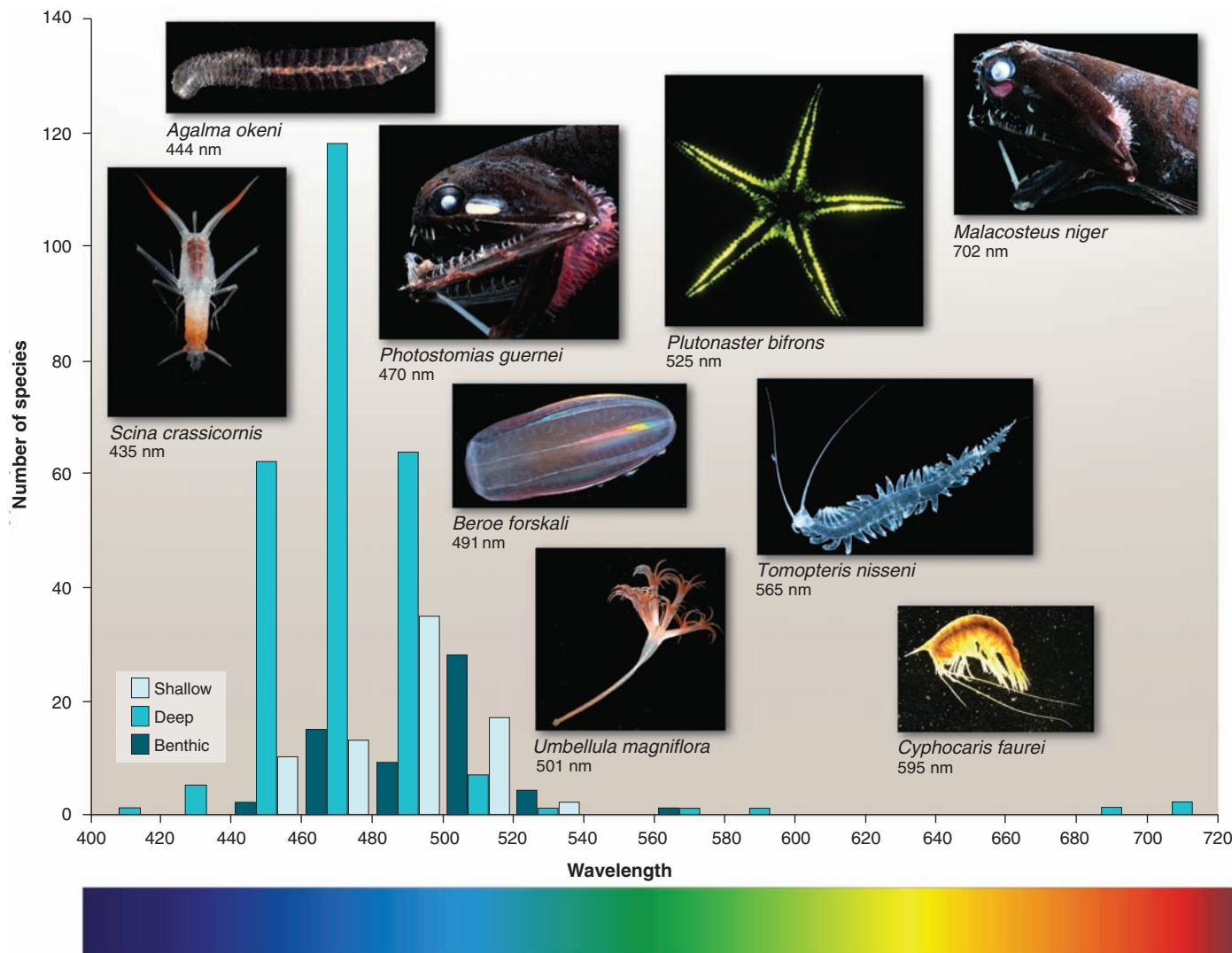

Fig. 1. The distribution of bioluminescence emission maxima varies by marine environment and organism type. Bioluminescent emissions extend over the full visible range and beyond. [Photo credits: J. Cohen for the photograph of *S. crassicornus*; P. Herring, *P. bifrons*; and P. Batson (DeepSeaPhotography.com), *C. faurei*]
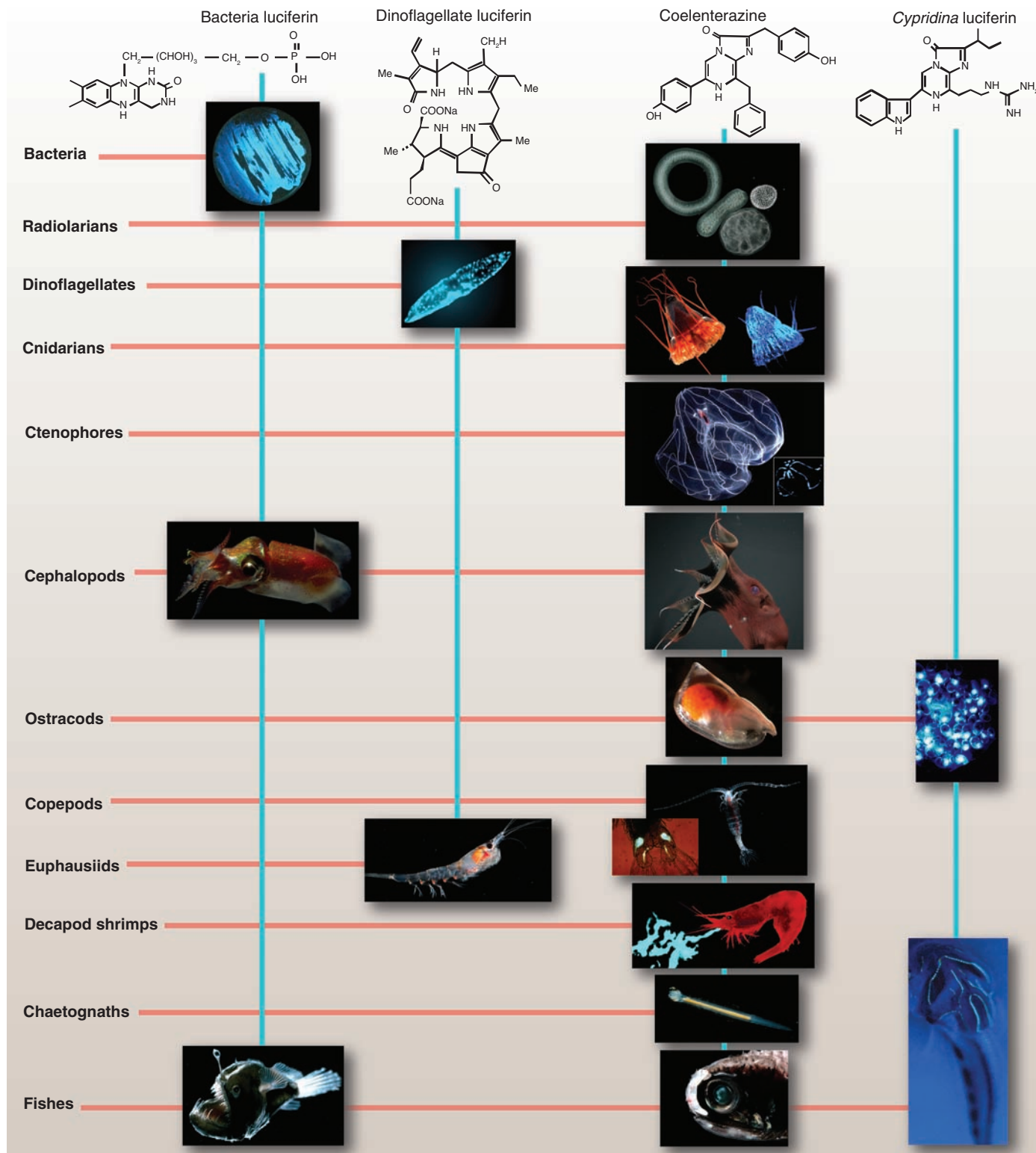
Fig. 2. The chemical structures of the four best-known luciferins are as diverse as their phylogenetic distribution. Bacterial luciferin may occur in free-living or symbiont bacteria (e.g., in squid such as *Heteroteuthis dispar*) or in fish such as *Melanocetous johnsoni*. Dinoflagellate luciferin occurs not only in dinoflagellates (e.g., *Pyrocystis fusiformis*) but also in euphausiids (e.g., *Meganyctiphanes norvegica*). Some of those using coelenterazine as luciferin include radiolarians (e.g., unidentified polycystine radiolarians), cnidarians (e.g., scyphozoan *Periphylla periphylla*, as seen in the light and photographed by its own light), ctenophores (e.g., *Bathocyroe fosteri*, with bioluminescence display shown in inset), vampire squid (e.g., *Vampyroteuthis* *infernalis*), ostracods (e.g., *Orthoconchoecia agassizi*), copepods (e.g., *Gaussia princeps* releasing its bioluminescent chemicals from glands on its tail, shown in inset), decapods (e.g., *Acanthephyra purpurea* spewing luciferin and luciferase out of its mouth), chaetognaths (e.g., *Caecosagitta macrocephala*), and fish (e.g., the myctophid *Diaphus* sp. has a large preorbital light organ). *Cypridina* luciferin, which is an imidazopyrazinone like coelenterazine, is found in ostracods such as *Vargula hilgendorfii* and is the dietary source of luciferin for the midshipman fish *Porichthys notatus*. [Photo credits: S. Haddock, radiolarians and chaetognath; K. Reisenbichler, *V. infernalis*; J. Case, copepod luminescent glands and midshipman fish photophores]

the color of the bioluminescent emission to longer wavelengths. The best known of these is green fluorescent protein (GFP), which was isolated and cloned from a bioluminescent jellyfish and has been used extensively as an in vivo fluorescent marker of gene expression, protein synthesis, and cell lineage (*1*).

Besides the four best-known luciferins used by marine organisms (Fig. 2), there are some that are partially defined, such as that of the rock-boring clam *Pholas dactylus*, the parchment tube worm *Chaetopterus variopedatus*, and the syllid fireworm *Odontosyllis enopla*, as well as many more yet to be elucidated, most notably among mollusks, echinoderms, and hemicordates (acorn worms) (*1*).

What Are Evolutionary Processes That Lead to Bioluminescence?

Based on the number of light-producing chemistries across the monophyletic lineages, bioluminescence is estimated to have evolved independently at least 40 times (*2*). Remarkably, not only is there evidence of independent origins within taxa (e.g., ostracods have two known chemistries: coelenterazine and vargulin) but even within individual species (e.g., the deep-sea anglerfish, *Linophryne coronata*, has two different light-emitting systems in adult females: bacterial luminescence in the dorsal lure and an intrinsic, unidentified chemistry in the chin barbel) (Fig. 3A).

Most hypotheses put forth to explain the evolution of luminescent systems fall into two basic categories related to selection acting on either substrates or enzymes. In the first case, selection driving the evolution of luciferin substrates may have resulted from pressures to protect organisms from photochemically generated reactive oxygen species such as H_2O_2 and O_2^- (*18*). For example, the luciferin coelenterazine, which is found in at least nine phyla, is a strong antioxidant (*2, 18*). As vision-dependent animals migrated to greater depths to escape detection by visual predators, the reduced oxidative stress in deeper waters shifted the selection pressure from the antioxidative to the chemi-luminescent properties of this molecule (*18*).

The alternative enzyme-centric explanation suggests luciferases originally acted as mixed-function oxygenases (*22*). In this case, as visual animals were driven into darker waters, natural selection may have favored the development of more-sensitive eyes and enhanced visibility of visual signals (*4*). As a consequence, a mutation in an oxygenase enzyme involved in the break-down of pigment molecules associated with spots displayed to attract a mate or repel a predator could result in external luminescence that caused imme-diate selective pressures for the light emitter (*22*). Although the oxygenase hypothesis has been questioned by genetic and biochemical evidence (*18*), there remains support for enhanced visual signaling selection pressures. For example, there is evidence that the deep-sea finned octopod, *Stauroteuthis syrtensis*, developed light organs from suckers because the selective advantage of

the visual display of suckers (i.e., to attract a mate) superceded the advantage provided by their adhesive properties (Fig. 3B) (*23*).

Although the chemical basis for light produc-tion in octopods has not been determined, light emission at suckers or any other localized photo-phore would require an enzyme mutation coupled with substrate availability. Therefore, by combining the substrate-centric and enzyme-centric hypothe-ses, one might envision a scenario where reduced oxidative stress in deeper waters freed up anti-oxidants, such as coelenterazine, as substrates for chemiluminescent reactions that resulted from specific enzyme mutations. This view gains support from the fact that, although light-emitting substrates are relatively few in number and con-served across phyla, the bioluminescent enzymes are unique and independently derived (*2*).

There may also be a connection to protection from oxidative stress with GFP because similar proteins exist in bioluminescent jellyfish and sea pens, as well as in non-bioluminescent corals, copepods, and lancelets (*24*). In corals and lance-lets, it has been suggested that GFP-like proteins could function as antioxidants to detoxify reactive oxygen species (*24, 25*).

In bioluminescent bacteria, the question of evolutionary origins has recently gained new focus with the reclassification of members of the *Vibrio fischeri* species group as a new genus, *Aliivibrio* (*26*). The taxonomy of luminescent bacteria has been revised often in efforts to better define evolutionary relationships and origins. The distribution of bioluminescent species among bacteria is not even; all species in the terrestrial genus *Photorhabdus* are luminescent, but marine genera with bioluminescent species (*Aliivibrio*, *Photobacterium*, *Shewanella*, and *Vibrio*) include many closely related nonluminous species (*15*). Nonetheless, comparative genomics have revealed that all luminous bacteria share a common gene sequence: the lux operon that encodes for the bio-synthesis of luciferase and its substrates (*15*). This highly conserved sequence appears in bacteria from very different ecological niches, suggesting a strong selective advantage despite the energetic costs of producing light. In mixed cultures of lumi-nescent and dark mutants of *Vibrio harveyi*, the dark mutants rapidly overrun the culture unless the mixture is irradiated with ultraviolet (UV) light, in which case the balance tips the other way, apparently because bioluminescence stimulates DNA repair (*27*). If DNA repair was the initial selective advantage for light production in bacteria, then the lux operon may have been lost in bacteria that evolved more efficient DNA repair systems but retained in those where visible light became a selective advantage. Further selective advantage would have been afforded with the evolution of quorum sensing, which conserves energy by as-suring that luminescent bacteria do not synthesize their light-producing chemicals unless a sufficient concentration are present to be visible. Although once considered confined to bioluminescent bacte-ria, quorum sensing is widespread in nonluminous
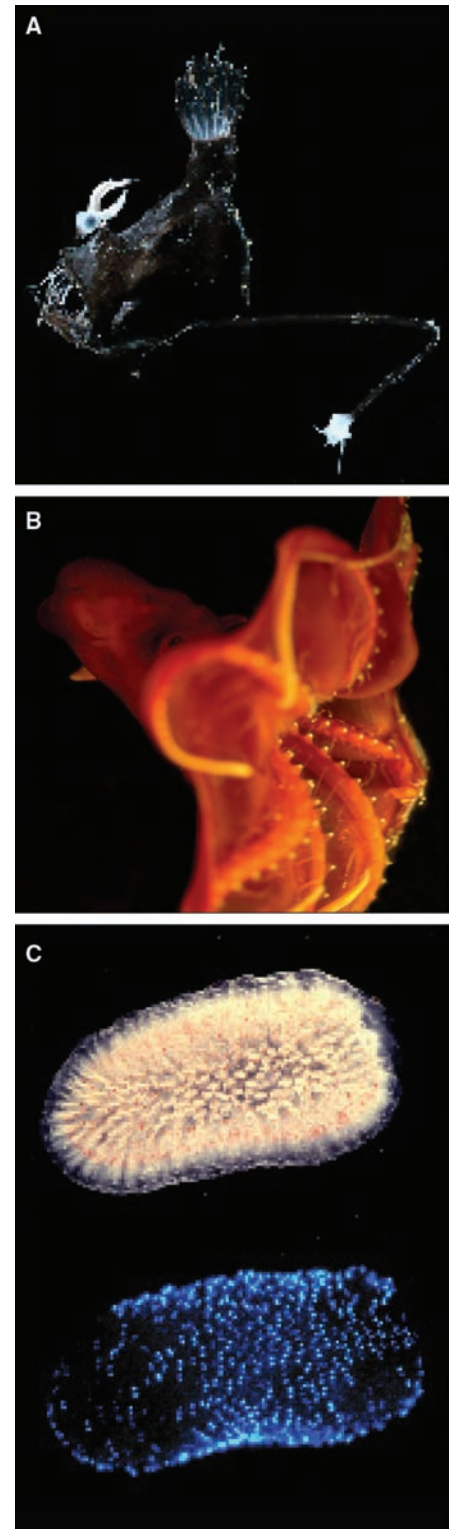
Fig. 3. Bioluminescence has resulted from some intriguing evolutionary adaptations. (**A**) In the deep-sea anglerfish *Linophryne coronata*, bioluminescence from the esca is bacterial in origin, whereas that from the chin barbel is an unidentified intrinsic chemistry. (**B**) In the octopus *Stauroteuthis syrtensis*, its suckers are photophores. (**C**) In the tunicate *Pyro-soma atlanticum*, luminescence originates from pu-tative bacterial endosymbionts.

Gram-negative bacteria, where it serves multiple functions, such as enhancing pathogenicity of bacteria by delaying toxin production until population densities are high enough to overwhelm the host's defenses (*28*). The question, therefore, arises: Which came first, quorum sensing or bioluminescence?

The details of bacterium-host interactions in bioluminescent symbionts [e.g., *A. fischeri* and the Hawaiian bobtail squid *Euprymna scolopes* (*29–31*)] reveal unique insights into the co-evolution of bioluminescence among distinct classes of organisms. For example, the recent discovery of light perception capabilities within the light organ of *E. scolopes* explains how the host has the means to reject nonluminous strains of *A. fischeri* (*32*). Although it was once thought that such complex and tightly coupled associations must have coevolved, recent phylogenetic analyses of bacteria isolated from two squid families (*33*) and seven teleost families (*34*) revealed deep divergences among the hosts that are not reflected in the symbionts, pointing to evolutionarily independent origins of these symbioses. It will be interesting to see whether the same differences exist between hosts and symbionts in flashlight fishes and deep-sea anglerfishes, where the bacterial symbionts, which are re-acquired from the environment with each generation, are as yet unidentified and unculturable (*15*).

Further along the continuum toward greater integration of symbiont and host are the the colonial tunicates known as pyrosomes (Fig. 3C). On the basis of demonstrated bacterial luciferase activity, their luminescent organs may be bacterial endosymbionts (*1*). However, they do not produce the persistent glow characteristic of luminous bacteria but emit light in response to either mechanical or photic stimuli (*12*). Pyrosomes are exciting candidates for genomic-level analysis and possibly gaining new insight into the mechanisms that underlie endosymbiosis.

Some of the most detailed information on the evolution of bioluminescent enzymes in the ocean comes from work on marine dinoflagellates. Although there are no sequenced dinoflagellate genomes, eight dinoflagellate luciferase genes have been fully identified (*19*). Phylogenetic analysis indicates that the most primitive luciferase of these eight is from the large heterotroph *Noctiluca scintillans*. This luciferase gene codes for a single catalytic domain (*19*), whereas in the seven other sequenced luciferases—all from photosynthetic dinoflagellates—there are three homologous catalytic sites (*35*). Although these sites are highly conserved across this group, there are some differences in their genetic structure; for example, the luciferase gene in *Pyrocystis* has a large unique noncoding region (*36*). *P. lunula* also differs substantially from the other dino-flagellates in this group in that it lacks luciferin

binding protein and exhibits no circadian rhythm in the breakdown of luciferase, luciferin binding protein, and its light-producing organelles and it produces approximately two orders of magnitude more light in response to mechanical stimuli (*12*). Given these differences, it seems likely that the selective pressures shaping the luminescent capacities of these dissimilar dinoflagellates may have diverged at some point in evolutionary history. Relating comparative genomic analysis to variable luminescent capacities and control mechanisms in different dinoflagellates is an intriguing new approach to comprehending the adaptive importance of bioluminescence. Dinoflagellates may also provide valuable insight into how gene duplication and gene loss function in generating biodiversity. For example, luciferase gene loss can now be examined by using oligonucleotide primers recently developed for specific luciferase genes. Use of these primers revealed a strain of *Gonyaulax spinifera* that produces bioluminescence at such low levels it is undetectable to the human eye, suggesting that either the luciferase is not expressed or that only remnants of the genes remain (*37*).

Seeing the Light

The many examples of evolutionary convergence related to bioluminescence are a testament to the survival value of the trait, whereas its abundance and ubiquity in the ocean attests to its importance in marine ecosystems. In situ imaging systems used to document the vertical distribution of planktonic emitters from surface to sea floor (*38*), as well as calculate their nearest-neighbor distances (*12*), will become increasingly important to providing a more detailed understanding of animal distributions and population dynamics in ocean ecosystems. Satellite sensor systems may also contribute, as with the recent detection of a $15,400$-km^2 bioluminescent "milky sea" in the Indian Ocean (*39*). This eerie phenomenon may be due to a luminous bacterium (e.g., *V. harveyi*) growing on the remains of a monsoon-induced algal bloom (*12*). However, this hypothesis awaits confirmation until in situ collections are made in an active milky sea. Improved low-light sensors deployed from aerial platforms may facilitate more targeted sampling efforts. It is hoped that with improved in situ sensor technology and additional observation platforms, such as autonomous underwater vehicles and undersea observatories, new insights from the field will be combined with more detailed genomic and physiologic studies in the laboratory to better understand the ecological importance and adaptive value of bioluminescence in the ocean.

References and Notes
1. O. Shimomura, *Bioluminescence: Chemical Principles and Methods* (World Scientific, Toh Tuck Link, Singapore, 2006).
2. S. H. D. Haddock, M. A. Moline, J. F. Case, *Annu. Rev. Mar. Sci.* **2**, 443 (2010).
3. P. J. Herring, E. A. Widder, in *Encyclopedia of Ocean Science Vol. 1*, J. H. Steele, S. A. Thorpe, K. K. Turekian, Eds. (Academic Press, San Diego, CA, 2001), pp. 308–317.
4. E. A. Widder, in *Adaptive Mechanisms in the Ecology of Vision*, S. N. Archer, M. B. A. Djamgoz, E. Loew, J. C. Partridge, S. Vallerga, Eds. (Kluwer Academic, Dordrecht, Netherlands, 1999), pp. 555–581.
5. S. H. D. Haddock, J. F. Case, *Mar. Biol.* **133**, 571 (1999).
6. P. J. Herring, *Proc. R. Soc. Lond.* **220**, 183 (1983).
7. M. I. Latz, T. M. Frank, J. F. Case, *Mar. Biol.* **98**, 441 (1988).
8. E. A. Widder, M. I. Latz, J. F. Case, *Biol. Bull.* **165**, 791 (1983).
9. S. Johnsen, E. A. Widder, C. D. Mobley, *Biol. Bull.* **207**, 1 (2004).
10. A. F. Mensinger, J. F. Case, *J. Exp. Mar. Biol. Ecol.* **210**, 75 (1997).
11. S. D. Miller, S. H. D. Haddock, C. D. Elvidge, T. F. Lee, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 14181 (2005).
12. E. A. Widder, *Mar. Freshwat. Behav. Physiol.* **35**, 1 (2002).
13. P. J. Herring, *J. Mar. Biol. Assoc. U.K.* **87**, 829 (2007).
14. E. A. Widder, M. I. Latz, P. J. Herring, J. F. Case, *Science* **225**, 512 (1984).
15. P. V. Dunlap, in *Encyclopedia of Microbiology*, M. Schaechter, Ed. (Elsevier, Oxford, 2009), pp. 45–61.
16. K. J. Osborn, S. H. D. Haddock, F. Pleijel, L. P. Madin, G. W. Rouse, *Science* **325**, 964 (2009).
17. E. A. Widder, *Oceanography* **20**, 46 (2007).
18. J. F. Rees *et al.*, *J. Exp. Biol.* **201**, 1211 (1998).
19. L. Liu, J. W. Hastings, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 696 (2007).
20. S. H. D. Haddock, T. J. Rivers, B. H. Robison, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 11148 (2001).
21. C. M. Thomson, P. J. Herring, A. K. Campbell, *J. Mar. Biol. Assoc. U.K.* **75**, 165 (1995).
22. H. H. Seliger, *Nav. Res. Rev.* **XLV**, 5 (1993).
23. S. Johnsen, E. J. Balser, E. A. Widder, *Nature* **398**, 113 (1999).
24. D. D. Deheyn *et al.*, *Biol. Bull.* **213**, 95 (2007).
25. C. H. Mazel *et al.*, *Limnol. Oceanogr.* **48**, 402 (2003).
26. H. Urbanczyk, J. C. Ast, M. J. Higgins, J. Carson, P. V. Dunlap, *Int. J. Syst. Evol. Microbiol.* **57**, 2823 (2007).
27. A. Czyz, K. Plata, G. Wegrzyn, *Luminescence* **18**, 140 (2003).
28. J. W. Hastings, E. P. Greenberg, *J. Bacteriol.* **181**, 2667 (1999).
29. M. J. McFall-Ngai, in *Gut Microbiota and Regulation of the Immune System*, G. Huffnagle, M. Noverr, Eds. (Landes Bioscience, Austin, TX, 2008), pp. 102–112.
30. S. V. Nyholm, M. J. McFall-Ngai, *Nat. Rev. Microbiol.* **2**, 632 (2004).
31. E. G. Ruby, *Nat. Rev. Microbiol.* **6**, 752 (2008).
32. D. Tong *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 9836 (2009).
33. R. C. Guerrero-Ferreira, M. K. Nishiguchi, *Cladistics* **23**, 497 (2007).
34. P. V. Dunlap *et al.*, *Cladistics* **23**, 507 (2007).
35. L. Liu, T. Wilson, J. W. Hastings, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 16555 (2004).
36. O. K. Okamoto, L. Liu, D. L. Robertson, J. W. Hastings, *Biochemistry* **40**, 15862 (2001).
37. A. Baker, I. Robbins, M. A. Moline, M. D. Iglesias-Rodriguez, *J. Phycol.* **44**, 419 (2008).
38. I. G. Priede, P. M. Bagley, S. Way, P. J. Herring, J. C. Partridge, *Deep-Sea Res.* **53**, 1272 (2006).
39. S. D. Miller, S. H. D. Haddock, C. D. Elvidge, T. F. Lee, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 14181 (2005).
40. I am grateful to P. Herring for helpful comments and the Office of Naval Research for its long-standing support of bioluminescence research.