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GUEST COMMENTARY

Quorum Sensing on a Global Scale: Massive Numbers of Bioluminescent Bacteria Make Milky Seas

Kenneth H. Nealson[1] and J. Woodland Hastings[2]*

Department of Earth Science, 223 Zumberge Hall, University of Southern California, Los Angeles, California 90089-0740,[1] and Department of Molecular and Cellular Biology, 16 Divinity Avenue, Harvard University, Cambridge, Massachusetts 02138-2020[2]

Many in the field would not have believed that luminous bacteria could be responsible for continuous and substantial light emission from the surface of the ocean extending over an area as large as the state of Connecticut and detectable from space. But in a recent paper in the *Proceedings of the National Academy of Sciences*, Steve Miller and colleagues report such a luminescence event detected by a satellite sensor system (25); this event persisted for three consecutive nights in the northwestern Indian Ocean near Somalia in 1995 (see cover), and they judged it to be due to light-emitting bacteria.

How did the authors uncover this remarkable display? As Miller tells it, during a lunchtime chat with his colleagues Tom Lee and Carl Schueler at an American Meteorology Society Conference held in Seattle in 2003 the question was raised as to whether bioluminescence might be detected by satellite sensors. No one thought that the well-known and readily observed dinoflagellate emission would be detectable from space, but Miller kept wondering if there might be some other kind of bioluminescence that could be.

On returning to his home base at the Naval Research Laboratory in Monterey, Calif., he searched the World Wide Web and found descriptions of so-called milky seas. Although this phenomenon is not often mentioned in the scientific literature (15, 27), there have been hundreds of strikingly similar reports by mariners over the last several hundred years of eerie displays dubbed milky seas consisting of continuous and widespread luminescence from the surface of the ocean, many of which are recorded in logs of merchant ships (19, 33). In searching these reports, Miller found a reference to a likely and ultimately fruitful milky sea sighting logged by the *S.S. Lima*, transiting an area on the night of 25 January 1995, which reported that, "At . . . (2200 local time) on a clear moonless night a whitish glow was observed on the horizon and, after 15 min of steaming, the ship was completely surrounded by a sea of milky-white color with a fairly uniform luminescence. The bioluminescence appeared to cover the entire sea area, from horizon to horizon . . . and it appeared as though the ship was sailing over a field of snow or gliding over the clouds.. The bow waves and the wake appeared blackish in color . . ."

The Internet also led him to the bioluminescence web page of the University of California, Santa Barbara, and ultimately to Steve Haddock, a specialist in bioluminescence at the nearby Monterey Bay Aquarium Research Institute in Moss Landing. They enlisted the help of Chris Elvidge of the National Geophysical Data Center in Boulder, CO, to retrieve archival data acquired at that time from the U.S. Defense Meteorological Satellite Program constellation of satellites. Miller and Haddock independently saw a faint feature in the raw data in the vicinity of the area described in the ship report. When they enhanced the imagery in that area and overlaid the ship coordinates, there was a eureka moment. The agreement with the coordinates of the *S.S. Lima* was exact!

Icing on the cake came when Miller discovered that milky seas were described in a discussion between two crew members in Jules Verne's classic *Twenty Thousand Leagues under the Sea* at a level which indicated that Verne used actual ship logs for some of his raw material. Curiously, the "milk sea" described by Verne (32) occurred on the same day of January as the third night of the satellite imaging.

> The 27th of January, at the entrance of the vast Bay of Bengal . . ., about seven o'clock in the evening, the Nautilus . . . was sailing in a sea of milk . . . Was it the effect of the lunar rays? No: for the moon . . . was still lying hidden under the horizon . . . The whole sky, though lit by the sidereal rays, seemed black by contrast with the whiteness of the waters.
>
> "It is called a milk sea," I explained . . .
>
> "But sir, . . . can you tell me what causes such an effect? for I suppose the water is not really turned into milk."
>
> "No, my boy: and the whiteness which surprises you is caused only by the presence of myriads of infusoria, a sort of luminous little worm, gelatinous and without color, of the thickness of a hair whose length is not more than seven-thousandths of an inch. These insects adhere to one another sometimes for several leagues."
>
> " . . . and you need not try to compute the number of these infusoria. You will not be able, for . . . ships have floated on these milk seas for more than forty miles."

Miller et al. failed to heed Verne's admonition; by knowing the sensitivity of their detector, the spatial extent of the milky sea, and the amount of light emitted by a single luminous

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* Corresponding author. Mailing address: Department of Molecular and Cellular Biology, 16 Divinity Avenue, Harvard University, Cambridge, MA 02138-2020. Phone: (617) 495-3714. Fax: (617) 496-8726. E-mail: Hastings@FAS.Harvard.edu.

bacterium, they were able to estimate the total bacterial population of this milky sea to be on the order of 4×10^{22} cells.

In describing milky seas Herring and Watson (19) referred to the phenomenon as a "bioluminescent puzzle." But what exactly was the nature of this puzzle? It was the source of the light. Although the existence of the milky seas could not really be doubted, the origin of the light emission remained elusive. By all accounts it could be inferred that the origin was biological, but none of the observers, including scientists on the one research vessel that encountered and investigated a milky sea in 1986 in a region in the northwest Indian Ocean (20), could specify with certainty an organism in the water that was clearly responsible for the light emission.

Assuming that the light is due to bioluminescence, and there seems no reasonable alternative, it must be recalled that almost all marine bioluminescent organisms emit light as brief flashes (milliseconds or seconds) or discontinuous bursts lasting for minutes at most. This includes, most importantly, the ubiquitous dinoflagellates, which were considered a possible cause for milky seas because of their brilliant flashing luminescence that is emitted upon mechanical stimulation (17, 34). The possibility that strong surface winds might somehow stimulate their emission uniformly over a wide area for extended periods was suggested (19), but such an event has never been observed in areas where dinoflagellates were abundant. Moreover, milky seas have been reported in very calm seas, and scientists on the research vessel sampled water everywhere in the milky seas that they encountered, but they were completely unable to implicate dinoflagellates in the phenomenon (20).

So why could bacteria, which are the only marine organisms known to emit light continuously for hours or days at a time and without mechanical stimulation, not be credited as being responsible for the light? Because of the phenomenon of autoinduction, now referred to as quorum sensing (10), which was, in fact, first discovered in luminous bacteria (28) and since then has been shown to occur in all species studied (26, 29). In this phenomenon, the transcription of specific genes, in this case the bacterial luciferase gene (*lux*), is triggered at concentrations above a threshold concentration of a low-molecular-weight molecule referred to as autoinducer, first discovered in *Photobacterium fischeri* to be an acyl-homoserine lactone (6) produced by the cells themselves.

With pure autoinducer at hand (7) and a model of autoinduction to test (15, 26, 27), a chemostat was used to maintain cells at low densities. Luminescence disappeared, but it was promptly reestablished after the addition of acyl-homoserine lactone (29). Quorum sensing (by another name) was born!

The discovery of the genes responsible for this phenomenon (*luxI* and *luxR*) and the study of mutants defective in these genes solidified the notion of the mechanism (8), although it did not begin to anticipate the complexities discovered later by Bassler and colleagues, which include multiple inducers and multiple receptors (5, 22, 24). Nor did the initial work anticipate the widespread occurrence of autoinduction, which controls genes in symbionts and pathogens of many kinds (11), as well as the roles that the mechanism plays in controlling organismal interactions in biofilms and other communities (9, 23).

Thus, the possible involvement of bacteria in milky seas was discounted previously because it seemed that the autoinducer could scarcely accumulate at the concentration required in the open ocean. In this scenario bacteria were always considered to be "free floating" or planktonic, and it was indeed shown that individual bacteria under such conditions do not emit light, whereas emission from a single induced bacterium could be detected (4, 12).

However, luminous bacteria do emit bright light on surfaces as colonies (2) or when they are cultured as symbionts in light organs of some higher organisms, including fishes or squid (14, 18, 21, 30, 31), where the bacteria grow in a confined space and the autoinducer can accumulate. Might there be some analogous niche or an association with a host organism in milky seas where bacteria are confined and autoinducer accumulates?

Logs of merchant ships quoted by Herring and Watson (19) indicate that this is so. In several cases water samples were collected in buckets and inspected closely, which revealed fine luminous thread-like structures. Among these reports is one from the *Trojan Star* in 1930 ("Sample seemed to contain thousands of very thin lines of light, some approximately 13 mm long, others were shorter"), as well as others from the *Sofla* in 1949 (". . . in the bucket thin white threads, one inch long and smaller . . . Ordinary phosphorescence was also observed which was altogether different from the white threads, which gave off a continuous white light or glow") and from the *Sarpedon* in 1961 ("Hair-like objects, khaki in color, about 1.25 cm long").

Such continuous light emission from surface samples of the water from a milky sea was also observed by Lapota et al. (20) (and recorded photometrically); these workers reported that the emission continued over 10 h of observation. They attributed the luminescence to the luminous bacterium *Vibrio harveyi* in association with colonies of the microalga *Phaeocystis*, but when the algae were incubated on marine nutrient agar, only two luminous bacterial colonies (identified as *V. harveyi*) were observed, along with many nonluminous colonies.

A massive bloom of *Phaeocystis* or some similar alga that supports the growth of associated luminous bacteria to high local densities capable of inducing the luminous system might thus explain the milky sea phenomenon. Blooms of many phytoplankton types commonly extend for hundreds of miles, consistent with all reports of milky seas. But whether *Phaeocystis* is the alga responsible is not at all certain, although *Phaeocystis* is indeed a genus with a worldwide distribution and is known for dense spring blooms in regions with high nutrient contents (1). *V. harveyi* is similarly not well established as the bacterium responsible, although it is evidently well suited for such a role. It is a cosmopolitan species that is able to grow on a broad range of substrates and was the species in which autoinduction was discovered. A single favored niche for *V. harveyi* has never been identified, and the milky sea community might constitute one (21, 27), although luminous bacteria are not generally associated saprophytically with surfaces of plants, as they are with animals (15).

Another feature of milky seas confirmed by Lapota et al. (20) was that the luminescence was not distributed vertically through the water column. It appeared to be present only at the surface, thus explaining why a wave or other displacement of the surface water creates a dark area, as reported by earlier observers. However, healthy *Phaeocystis* and other phytoplankton types are usually not restricted to the surface water (3, 13).

Thus, assuming that the bacteria are not solitary in the seawater, as seems quite certain, what could the nature of the biological community be? Two possibilities can be considered. (i) A species of luminous bacteria specifically associates with an algal species, gaining nutrients from material produced by the alga and growing on the alga in colonies so that the autoinducer accumulates and the bacteria emit light. When algal blooms occur, these bacteria bloom. (ii) Milky seas occur as large algal blooms begin to break down and as massive amounts of the released lipids and hydrocarbon-rich microbial material accumulate as a surface film, where the growth of a luminous bacterial species is favored. Confined to the film, which might contain particulates derived from lysed algae, the autoinducer accumulates and luminescence is induced. In either case one of the clear predictions is that in contrast to the rhythmic (circadian) luminescence of dinoflagellates (16), light emission from bacteria should occur both during the daylight and nighttime hours, which is true for all luminous bacteria. This is a strong and readily testable prediction.

In the end, the report by Miller et al. puts a phenomenon discovered in test tubes, thought to operate on scales of micrometers to millimeters, on a truly global scale. It also provides hope that, with vigilant monitoring and an ability to respond rapidly, as Miller et al. hope can be done with satellite monitoring, it may be possible to get to a site and obtain samples for examination and culturing, leading to an understanding of this intriguing and still quite puzzling phenomenon.

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