# Bioluminescent Microbes

***Mum: I saw a wonderful show of blue waves on the shoreline last night, do these have anything to do with fireflies?***

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## Bioluminescent Microbes

### Storyline

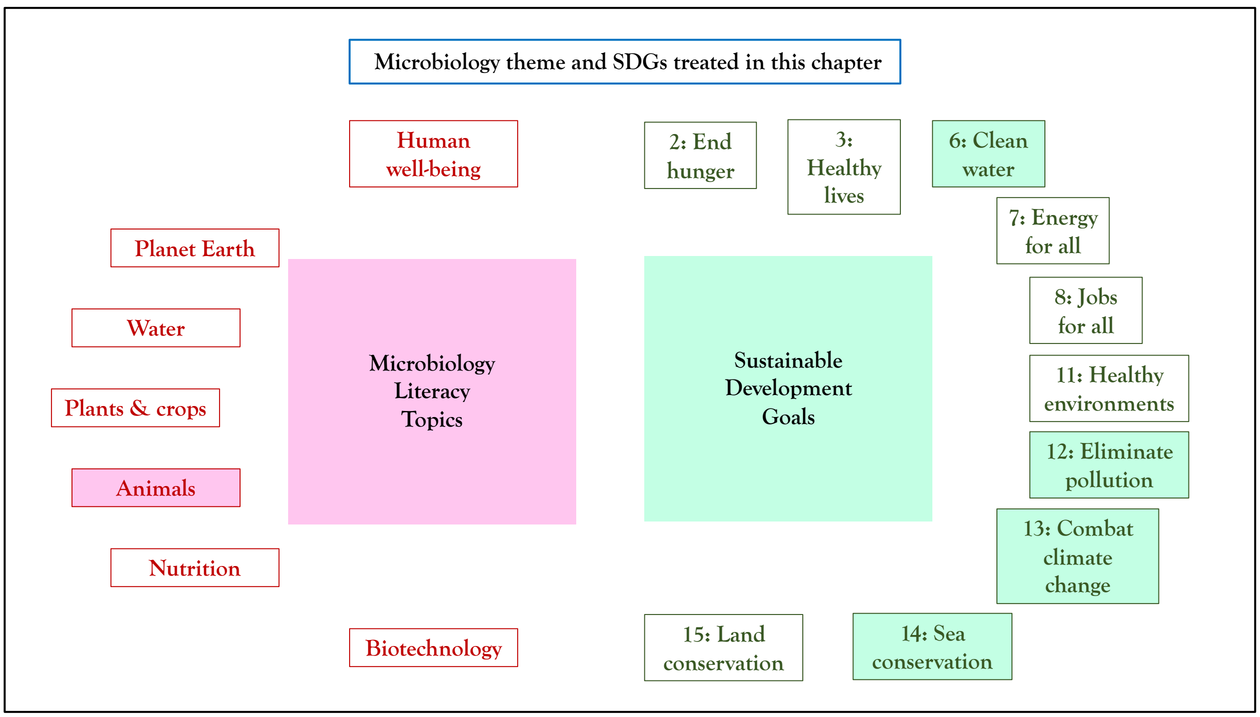
Headlines about scary "flesh-eating bacteria" and "deadly superbugs" invariably attract more attention than a headline about a glowing bacterium that lives peacefully within an obscure species of squid. Despite the disproportionate attention harmful microbes receive, the vast majority of animal-microbe interactions are actually mutually beneficial. The bioluminescent bacteria are an example of a charismatic group of beneficial microbes. Members of this group can grow inside fish and squid, trading light production for food and a safe place to live. At first glance, it seems paradoxical that a microbe would benefit from expending loads of energy to make light. How could glowing possibly benefit a tiny little microbe that's swimming alone in the ocean? Bioluminescent single-celled dinoflagellates can reach exceptionally high densities in warm, nutrient-rich water. This is called a dinoflagellate "bloom," in which cell numbers increase so much that we no longer need a microscope to see them. Not surprisingly, these microbes thrive on anthropogenic pollution and climate change. Dinoflagellates use their collective bioluminescence to confuse predators.

Figure 1. Bloom of bioluminescent dinoflagellates at Morro Rock (Morro Bay, CA)

However, compared to dinoflagellates, bioluminescent bacteria are much smaller, never reach such high cell densities in the ocean, and their predators do not have eyes that can be confused by collective flashes of light. So, how does bioluminescence benefit them? The bioluminescent bacterium *Vibrio fischeri*, which is closely related to many kinds of harmful bacteria, has a clever answer to this question. First, *V. fischeri* cells make *pheromone signals* – chemicals that enable bacteria to communicate with one another and that allow them to detect their siblings. If few siblings are detected, then the cells will not make light. This form of cooperation is called quorum sensing and it assures that only when enough tiny flashlights are put together, will they make sufficient light to be visible. "How do they prevent the pheromone signals from floating away?" and "visible to whom?" are the right questions to ask. Of all the bacteria in the ocean, only *V. fischeri* can navigate the gauntlet of defenses and swim into the specialized light organ of the Hawaiian bobtail squid, *Euprymna scolopes* (Box 3). Once inside, the bacteria re-model their new home and are encouraged to adopt a "stay at home" lifestyle as squid-derived food is delivered right to them. In exchange, the happily fed bacteria combine oxygen and other chemicals to make light, thanks to the build-up of their sibling-sensing pheromone signals within the confined space. The squid then uses its specialized tissues to project the bacterial light into the environment to illuminate its nocturnal activities. When the sun rises, the squid will bury themselves in soft sand until nightfall, when they re-emerge.

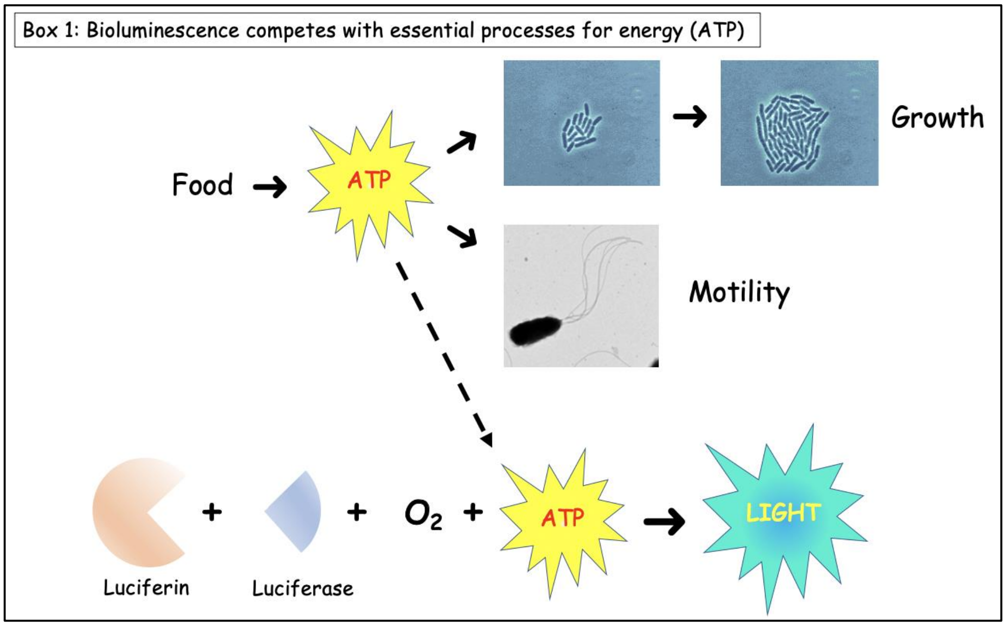
## The Microbiology and Societal Context

*The microbiology*: Evolution of harmful and beneficial bacteria; influence of beneficial bacteria on immune system, nutrition, and tissue development in animals; influence of bacterial genetics and physiology on animal behavior and ecology; cooperation among beneficial bacteria; cooperation between beneficial bacteria and their animal host. *Sustainability issues:* animal health; quorum quenching as alternatives to antibiotics and a way to inhibit biofouling; ability of animals to adapt to climate change; water quality and toxic bioluminescent dinoflagellates; Microtox bioassay for detecting environmental toxins; studying toxic bioluminescent dinoflagellates as a way to understand effects of climate change

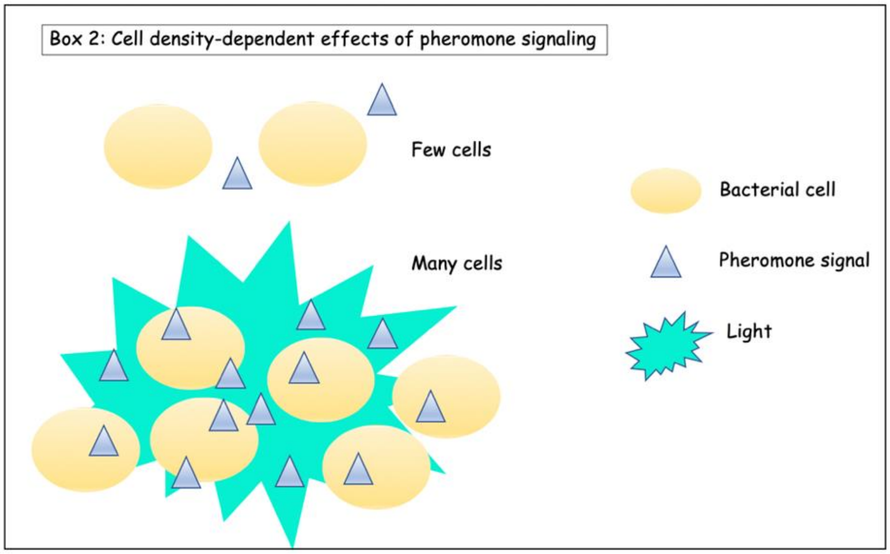



## Bioluminescent Microbes: The Microbiology

**1. *Bioluminescence is energetically costly.*** Bioluminescence in bacteria, fire flies, and dinoflagellates is mediated by oxygen, a substrate generically referred to as "luciferin," and an enzyme generically referred to as "luciferase." However, their respective luciferins and luciferases are very different chemicals, and so are the light generating reactions they perform. One consistent theme in these very different reactions is the expense of energy, and thus, the need for careful control. Bacteria are efficient little machines that prefer not to waste energy. Each bit of energy (ATP) they get from their food must be spent wisely on essential processes, such as motility and growth. However, there is an extra cost associated with being a bioluminescent bacterium. These bacteria must divert some of their energy away from essential processes in order to make light (Box 1). How do bioluminescent bacteria make sure they are not wasting energy on light production?




2. ***Bacteria use pheromone signals to regulate important processes, such as bioluminescence.*** Cells of bioluminescent bacteria are not only good at cooperating with animals, they are also quite good at cooperating with one another through a process called "quorum sensing." Each tiny cell produces "pheromones" that get released into the surrounding environment. The pheromones freely move into neighboring cells and stimulate them to make sparks of light. The pheromone signal amplifies, especially in a closed space, as the number of neighboring cells increases. Under the right conditions, the fleeting sparks in a few scattered cells eventually amplify in the growing bacterial population and become *flames*. In this way, bioluminescent bacteria can carefully orchestrate light production, and maintain synergy among a cohort of genetically related cells (Box 2).



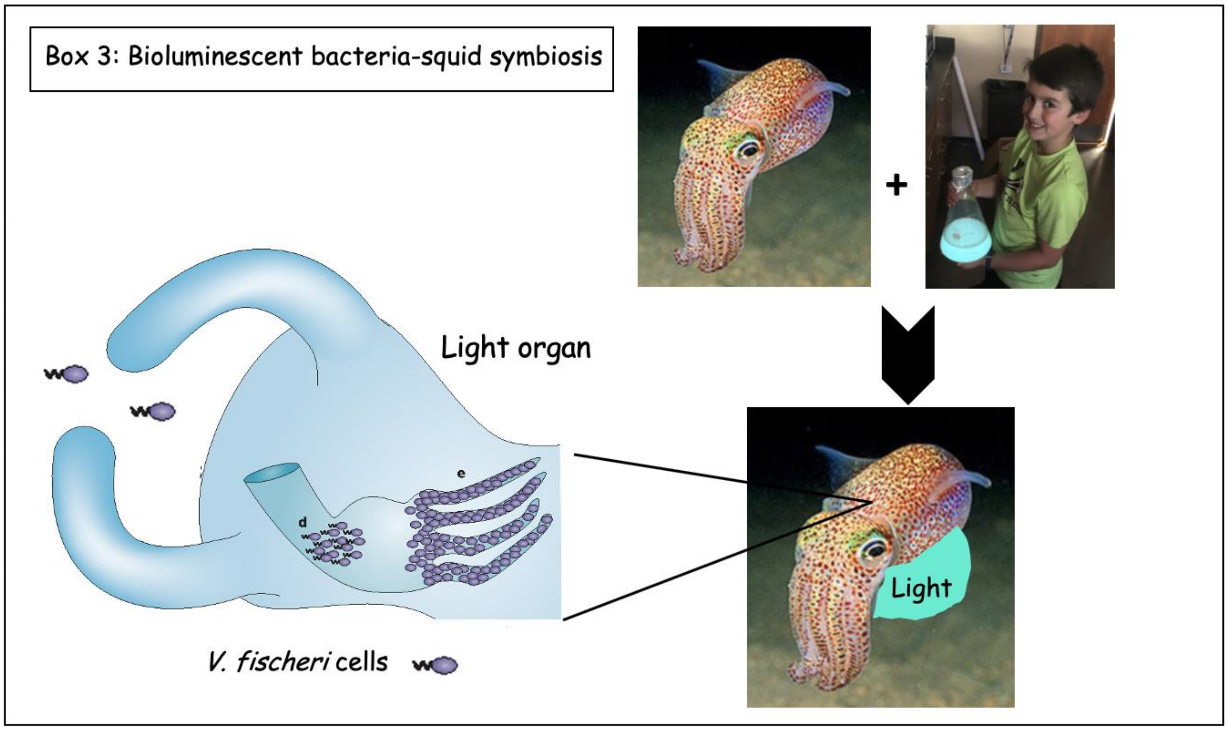

Box 2: Cell density-dependent effects of pheromone signaling

Quorum sensing was first recognized in bioluminescent bacteria, but we now know that this form of chemical communication is used by many kinds of bacteria to orchestrate diverse activities requiring or benefitting from a coordinated population effort, such as an attack on body defenses by pathogenic bacteria. Similar quorum sensing circuits can be found in both beneficial bioluminescent bacteria and their pathogenic relatives. For example, while *Vibrio fischeri* uses a particular quorum sensing circuit to regulate bioluminescence, its relative *V. cholerae* uses a similar circuit to regulate deadly diarrhea-causing toxins and sticky biofilm slime production.

3. ***Bioluminescence is also controlled by the environment.*** Bioluminescent bacteria swimming in the ocean never reach the population density of the much larger single-celled plankton, called dinoflagellates, whose oxygen-dependent bioluminescence can light up the surf at night. Bioluminescent bacteria are only visible when they are held captive in laboratory culture, or they congregate in animal tissues, such as when cells of *Vibrio fischeri* colonize the specialized squid light organ. Being in a closed space, whether a laboratory flask or a light organ, allows bacterial pheromones to accumulate and stimulate collective light production among growing cells. Bioluminescent bacteria require the right environmental conditions (i.e. oxygen, nutrients, temperature, etc.) or bioluminescence will be turned off. Delivery of oxygen and nutrients to the light organ is carefully controlled by the animal. However, the squid has limited control over temperature or oxygen content of the environment. At night, when the squid are active, they can use their bacterial flashlight to help them find food and ideal environmental conditions. In these ways, the animal partner in a bioluminescent symbiosis can control the amount and intensity of bacteria-derived light to suit its needs.

4. ***Bioluminescent bacteria-animal relationships have been perfected over millions of years.*** There are thousands of different kinds of microbes in seawater, but only cells of *V. fischeri* are able to navigate the fraught pathway to the specialized light organ of the squid. Their journey takes them through ducts filled with toxic, viscous mucus, and a vigorous immune response, before they reach the interior chambers called "crypts" (Box 3). Within these crypts, *V. fischeri* cells grow on squid-derived nutrients, they secrete pheromones, and produce the desired amount of light, otherwise the squid will expel them. The squid light organ clearly evolved over millennia for the sole purpose of

housing this particular population of bioluminescent bacteria. The light organ colonization process parallels bacterial colonization of gut tissue in many types of animals, particularly those with an enlarged hindgut, which like the light organ, probably evolved to house beneficial microbes. However, in the case of the hindgut, the microbes aren't encouraged to make light, they instead help the animal digest recalcitrant bits of food (see also Topic Framework *The Rumen*). To colonize the gut, microbes must side-step a vigorous immune response as they pass through mucus-lined tissues of the esophagus, low pH stomach, and intestines.



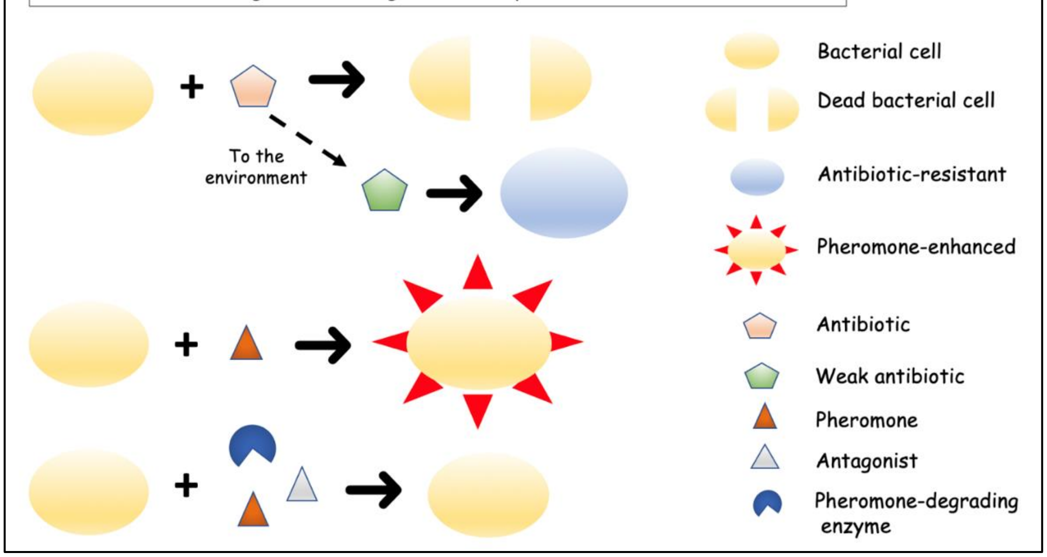

## Relevance to Sustainable Development Goals and Grand Challenges

**Goal 6. Ensure availability and sustainable management of water and sanitation for all** (*assure safe drinking water, improve water quality, reduce pollution, protect water-related ecosystems, improve water and sanitation management)*. Membrane bioreactors (MBRs) are a highly effective technology in wastewater treatment. The ability of MBRs to remove particles, including microbes, from wastewater results in a high-quality effluent that can be re-used for many purposes, including irrigation. Over time, microbes in wastewater will activate biofilm slime production using similar quorum sensing circuits as *V. fischeri*. Slime production encourages a process called "biofouling," in which microbes stick to membranes in an MBR and diminish filtration. In order to combat "biofouling," pheromone-digesting enzymes are being used to disrupt quorum sensing circuits. This cleaning process improves the function and longevity of membrane filters.

**Goal 12. Ensure sustainable consumption and production patterns** (*achieve sustainable production and use/consumption practices, reduce waste production/pollutant release into the environment, attain zero* *waste lifecycles, inform people about sustainable development practices)*. No environment on earth is too remote to be spared from anthropogenic pollution. Pesticides, metals, and other contaminants can be found in all of our rivers, lakes, oceans, sediments, and soils. Even the deepest parts of the ocean are not removed from human impact. As terrestrial mineral resources are depleted, there is a growing interest in deep sea mining. How will the ecological impacts of these deep-sea mining operations be quantified? An important strategy in combating pollution in any environment is detection. Notably, basic research on bioluminescence led to a cost-effective, fast, and reliable method to detect and quantify environmental pollution: the Microtox assay. Energy for bioluminescence comes from the metabolism of food. Toxic substances in environmental samples will disrupt metabolism. In the Microtox assay, cells of *V. fischeri* are exposed to environmental samples, and disruptions in bioluminescence are correlated to the degree of toxicity of an environmental sample.

Antibiotics pollution, mainly from wastewater treatment plants, farms, and aquaculture, is an enormous public health crisis that receives little attention. Widespread antibiotic contamination at sub-lethal levels in the environment encourages antibiotic resistance and threatens global public health. What if we could use therapeutic pheromones instead of antibiotics to fight bacterial infections? Bacteria produce pheromones that can trigger enhanced capabilities among their population, like biofilm slime or toxin production. Targeted changes to the chemical structure of pheromones can result in antagonistic molecules that confuse quorum sensing circuits of deadly microbes while having minimal effect on non-target circuits. Plants are known to produce a wide variety of quorum sensing antagonists, presumably to prevent colonization by pathogens. These natural pheromone antagonists could become a new class of antimicrobials. Alternatively, or in addition, pheromone degrading enzymes could be used to disrupt quorum sensing circuits of pathogens. Thus, pheromones could mitigate antibiotic usage, thereby reducing these environmental pollutants. Presumably, because therapeutic pheromones primarily modify bacterial behavior without killing them, they might be less likely to encourage the development of resistance (Box 4).

Box 4: Pheromone antagonists and degraders as replacements for antibiotics



**Goal 13. Take urgent action to combat climate change and its impacts** *(reduce greenhouse gas emissions, mitigate consequences of global warming, develop early warning systems for global warming consequences, improve education about greenhouse gas production and global warming)*. Climate change is already having measurable effects on everything from the frequency and intensity of hurricanes, droughts, and wild fires, to the fundamental chemical properties of our oceans. As ocean temperatures rise, bioluminescent bacteria and their animal hosts must adapt synchronously, or these relationships will not be sustainable, resulting in the loss of unique biodiversity. Rising ocean temperatures also means declining levels of dissolved oxygen. What will be the long-term effects of oxygen depletion on the ability of bioluminescent bacteria to carry out oxygen-dependent light production within their animal host?

Warmer waters have increased the home range of bioluminescent dinoflagellates. Not only do these microbes produce toxins, they are voracious predators of diatoms. Diatoms are photosynthetic plankton that fix enormous quantities of $CO_2$ into organic carbon (e.g. sugar). Furthermore, as diatoms die, they drag tons of carbon into the deep ocean. Thus, diatoms are an important firewall against rising $CO_2$ levels and serve an essential role in carbon cycling. While these bioluminescent dinoflagellates create beautiful displays of light, they symbolize an ocean in distress. The role of bioluminescent dinoflagellates in exacerbating the effects of climate change cannot be underestimated. Studying dinoflagellate blooms will be an essential component of climate-related fisheries and aquaculture management, as well as a warning system for climate-related public health issues.

**Goal 14. Conserve and sustainably use the oceans, seas and marine resources for sustainable development** (*prevent and significantly reduce marine pollution of all kinds, in particular from land-based activities, including marine debris and nutrient pollution*). Blooms of toxic bioluminescent dinoflagellates are typically preceded by warming waters and an infusion of nutrient pollution from anthropogenic sources such as farm run-off and sewage spills. During the day, these blooms can appear as "red tide" events, in which photosynthetic pigments color the water. By tracking these blooms, we should be able to identify and mitigate sources of pollution.

## Potential Implications for Decisions

1. ***Individual***

a. Bioluminescent waves are beautiful, but they are formed by toxin-forming dinoflagellates. Dinoflagellate toxins can cause neurological problems, such as disorientation, nausea, vomiting, and paralysis. The urge to immerse yourself in bioluminescent waves to capture the perfect "selfie" must be weighed against the potential for toxic side-effects.

b. Bioluminescent bacteria form associations with squid that live in shallow waters with soft sandy bottoms in places like Hawaii, Japan, France, and Australia. By trampling on these habitats, what impact can bathers have on these symbiotic associations?

2. ***Community and National policies***

a. Local governments should use bioluminescent waves as an indicator of environmental degradation.

b. Regular testing and observation (e.g. Microtox technology, tracking bioluminescent blooms) should inform policies that mitigate pollution and limit public exposure to contaminated environments.

c. We should continue to leverage research on the regulation of bacterial bioluminescence for the sake of a cleaner environment (e.g. "quorum quenching" microbes in water purification filters to prevent biofouling)

d. Government funding for applications in bioluminescence research and environmental health (e.g. Microtox technologies, pheromone alternatives to fungicides and pesticides; controlling dinoflagellate blooms)

e. Government funding for applications in bioluminescence-related research on animal health (e.g. pheromone-degrading enzymes and pheromone analogs as alternatives to antibiotics; ways to control dinoflagellate blooms)

**f.** Bacteria-squid associations are typically found along the shore in areas with soft, sandy bottoms. What effect might bathers have on these environments? Should these environments be protected?

## Pupil Participation

1. ***Class discussions on the science of bioluminescence and bioluminescent symbioses***

2. ***Pupil stakeholder awareness***

*a.* How do beneficial microbes help animals? (e.g. role of bioluminescence in animal-bacteria associations)

*b.* How might bioluminescent microbes threaten public health?

*c.* In what ways can bioluminescence research improve public health? (e.g. model systems for studying beneficial colonization of tissue; bioluminescence regulators, their homologs, and degraders as a new classes of antibiotics and pesticides; bioluminescence as a way to detect toxins and sources of pollution, etc.

*d.* How might climate change affect bioluminescent microbes?

3. ***Exercises***

a. How might we use technology to observe the ways animals use bioluminescence in their daily lives? Connect with an aquarium that maintains animals with bioluminescent bacterial symbionts (e.g. Tennessee Aquarium, Waikiki Aquarium flashlight fish exhibits)

b. Design a plan for monitoring red tides by day and bioluminescent surf by night. What actions can be taken to notify the public of the potential dangers and identify the source(s) of pollution encouraging the bloom of dinoflagellates?

c. Set up a meeting (virtual or in-person) with experts in the field of bioluminescence to discuss model systems and contributions of quorum sensing and bioluminescence in public health

## The Evidence Base, Further Reading and Teaching Aids

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8. How flashlight fish swim together in the dark. https://www.youtube.com/watch?v=h4legu-KB_E
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## Glossary

Anthropogenic- caused by humans
Antibiotic- natural or synthetic chemical used to kill bacteria or inhibit their growth
ATP- Chemical source of energy in living cells (Adenosine Tri-Phosphate)
Biofilm- A group of microbes bound together by sticky slime
Biofouling- Biofilm formation on a surface
Bioluminescent- Production and emission of light by a living organism
Diatom- Microscopic algae that use sunlight to make energy and food
Dinoflagellate- Microscopic plankton, some can produce bioluminescence and toxins
Fungicide- A chemical that kills yeast, mold, or other fungi
Flesh-eating bacteria- Bacteria able to damage skin and underlying tissue
Light organ- A specialized structure in certain animals that is colonized by bioluminescent bacteria
Luciferase- A chemical needed for bioluminescence
Luciferin- A chemical needed for bioluminescence
Membrane bioreactor- A combination of methods, including filters, to clean wastewater
Mucus- Slime produced by animals to protect certain body surfaces
Nocturnal- occurring at night
Pesticide- A chemical used to kill harmful insects or other "pests"
Pheromone- A chemical produced by an organism that changes the behavior of another organism
Pheromone analog- A chemical that closely resembles a pheromone but differs in activity
Quorum sensing- Cell-density-dependent regulation of behaviors using pheromones
Quorum quenching- Inhibiting cell-density dependent behaviors using pheromone analogs
Red tide- Blooms of toxic dinoflagellates
Super-bug- Microbes that are resistant to many kinds of antibiotics