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Dynamics of *V. fischeri* and *E. scolopes* Under Environmental Stress

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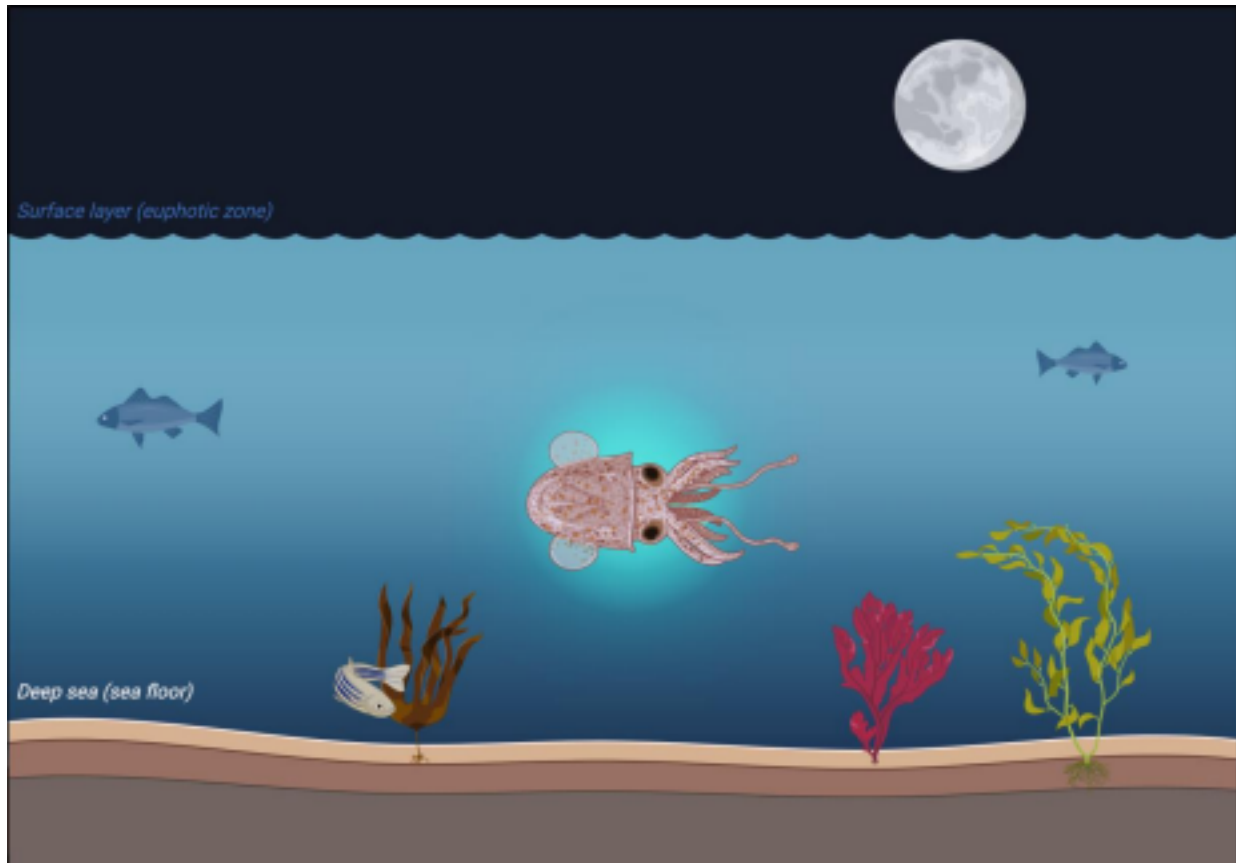
Abstract

Symbiotic relationships are fundamental to ecological systems, shaping species interactions and influencing survival, reproduction, and evolutionary pathways. One well-studied example of a mutualistic symbiotic relationship occurs between the Hawaiian bobtail squid (*Euprymna scolopes*) and the gram-negative bacterium *Vibrio fischeri*. This partnership enhances the survivability of both organisms through a specialized form of camouflage known as counterillumination. In this process, *V. fischeri* utilizes quorum sensing to regulate bioluminescence, producing light that matches the surrounding environment and conceals the squid's silhouette from predators below. In return, the squid maintains a specialized light organ that provides rich nutrients, creating a stable environment for the bacteria to proliferate. While notably efficient and stable, the effectiveness of this relationship can be influenced by external environmental factors. Conditions such as temperature, pH, and salinity play crucial roles in determining the success of bacterial colonization, bioluminescence efficiency, and host-symbiont interactions. Since *E. scolopes* and *V. fischeri* are highly sensitive to environmental changes, they serve as valuable bioindicators for assessing the broader impacts of climate change on oceanic symbioses. Climate change impacts, such as ocean acidification and increasing temperature, have been demonstrated to be detrimental to this intricate mutualism. This review paper will examine the effects of climate change factors on the symbiotic relationship and the surrounding environment, and analyze how each factor can promote or prevent the growth of the microbe *V. fischeri*. The possible implications and adaptations made by these organisms in the face of progressing environmental stressors will also be discussed and analyzed throughout the review.

Keywords: quorum sensing, symbiosis, counterillumination, bioluminescence

Dynamics of *V. fischeri* and *E. scolopes* Under Environmental Stress

A symbiotic relationship can be defined as a close, prolonged interaction between two or more different species. The dynamic between *E. scolopes* and *V. fischeri* is often referred to as a mutualistic symbiosis and has been studied as a model system in which the microbe infects the light organ of the squid, providing it with luminescence and camouflage at night; in return, the squid provides the microbe with a habitat (Figure 1). This infection occurs through pores in the light organ, which communicate with the ambient environment and allow accumulated, or aggregated, *V. fischeri* bacterial cells to enter the organism as a cluster of microbes (Lee & Ruby, 1994). The host squid utilizes luminescence to perform counterillumination, a process by which marine species employ bioluminescence to camouflage. This relationship is ecologically obligate, meaning that aposymbiotic hosts are not observed in the wild. Despite this, it is observed that the host squid can develop independently of infection and relies on *V. fischeri* primarily for light production (Claes & Dunlap, 2000). Additionally, the ability of the microbe to emit bioluminescence relies on the cell density exceeding a certain threshold within the light organ, where individual cells communicate via quorum sensing (Scheerer et al., 2006). Quorum sensing refers to the bacterial cell signaling phenomenon in which the cells produce extracellular signaling molecules known as autoinducers. These autoinducers allow the mass of bacterial cells to interact with their environment as a collective whole, enabling *V. fischeri* bacteria to act uniformly. Researchers refer to this relationship as a model system due to its replicability within the laboratory and the significant number of juvenile hosts available for infection.

Figure 1*Nocturnal Counterillumination in the Hawaiian Bobtail Squid*

Note. The Hawaiian bobtail squid (*Euprymna scolopes*) uses bioluminescent bacteria housed in a specialized light organ to match the brightness of moonlight and starlight from above, creating counterillumination that camouflages it from predators below, helping it avoid detection. Created in BioRender.com.

Because of rising temperatures in the face of climate change, organisms like these are prone to heat shock due to temperatures exceeding their thermal safety margin—the allowance of an organism to survive in a maximum environmental temperature (Hector et al., 2022). While immensely adaptable, different environmental factors can change how the relationship is formed

between juvenile squid and *V. fischeri*, and it is important to explore how these factors are changing over time. Further research into how these species and their symbiosis may be used as a flagship system for their environment will allow researchers to make decisions about environmental safety and sustainability, as well as enable people to make conscious efforts toward mitigating the effects of climate change in the underwater community.

Flagship species are species that are identified by researchers to be symbols of a given habitat, raising support for the conservation of that environment. By having this system act as a flagship, more public awareness may be brought to environmental stressors as cephalopods like the bobtail squid maintain important roles in trophic food webs and may be physiologically affected by rising temperatures resulting from climate change (Otjacques et al., 2024). The status of these species depends largely on environmental factors that have been developing into detrimental inhibitors, such as the increased acidification of the ocean and rising temperatures, which can heavily influence how these organisms interact. Through this exploration, we are investigating the question: How can pH, salinity, and temperature affect the evolution of the symbiotic relationship between *V. fischeri* and *E. scolopes* in different environments?

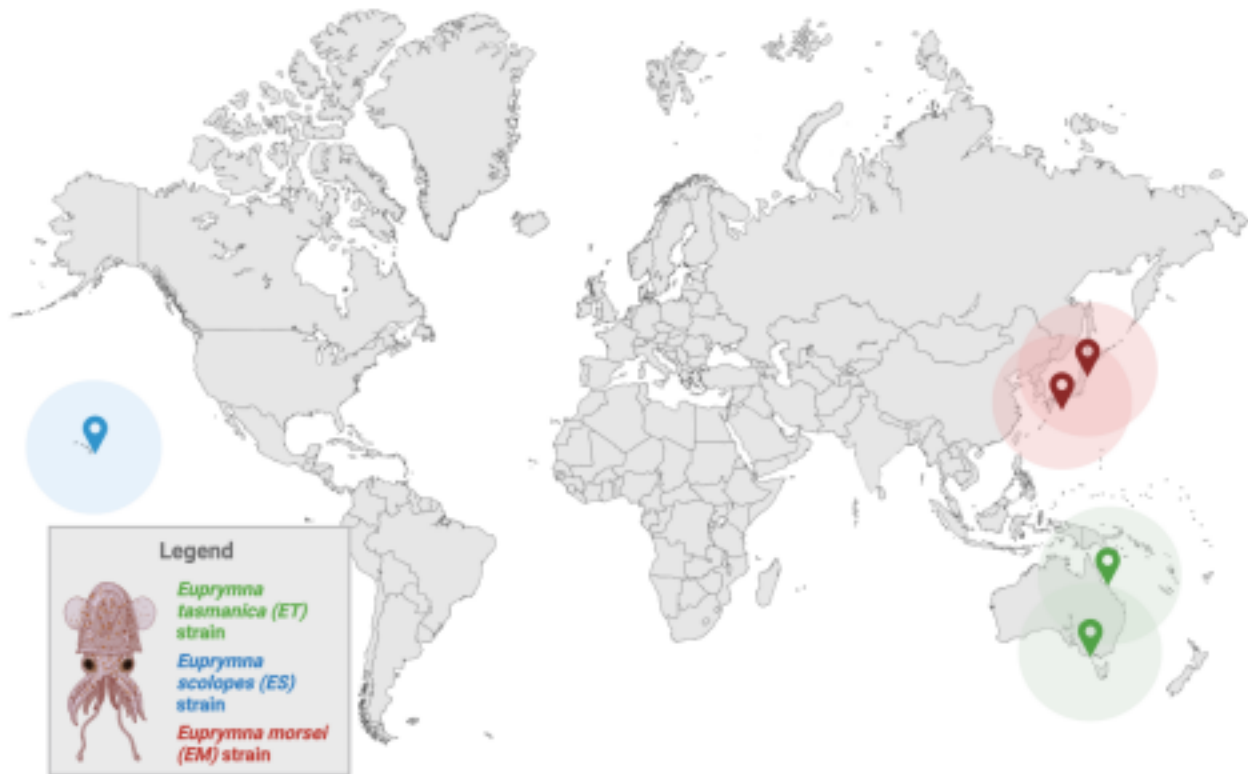
Effect of pH on Symbiosis

The pH, or the measure of acidity in a substance, can play a significant role in the interaction of this symbiotic system. In systems involving microbes such as *V. fischeri*, even the smallest shift in pH can lead to a drastic effect on the microbial community (Cohen et al., 2020). Despite this, *V. fischeri* has evolved to adapt to the fluctuating environment of the light organ (Pipes and Nishiguchi, 2022). When researching pH and its influence on this symbiotic system, Cohen et al. determined the upper and lower pH limits on the *V. fischeri* strains EM17 and

EM17Tn7 to be in the range between pH 6.0 and 10.0 (Cohen et al., 2020). When analyzing the pH adaptations of the microbe, it is important to note that the pH level in the light organ of the host squid experiences several fluctuations throughout the day. The adaptability of *Vibrio fischeri* may be attributed to its ability to survive in the optimal environment provided by the light organ. There are several implications of these results; an increase in acidity could be detrimental to the survival of both species, as a pH under 6.0 could prevent *V. fischeri* from infecting the host squid, leading to the inability to camouflage by counterillumination. Regardless, pH levels in the extremes may allow for microbial strain adaptation, as seen in acid specialists derived from ancestral *V. fischeri* that may thrive in pH levels at about 5.6, significantly more acidic than the average oceanic pH of 8.1 (Cohen et al., 2020). This may be due to the geographic location of the strain, as it may impact the strain's ability to withstand more extreme conditions brought upon the environment by climate change factors (Figure 2). As atmospheric carbon dioxide (CO₂) increases due to the burning of fossil fuels among other human activities, ocean acidification increases correspondingly. This increased amount of CO₂ is a contribution to the impact of climate change, and not only affects marine life, but also the vitality of terrestrial organisms. While *V. fischeri* remains resistant to the lowering pH, drastic changes in ocean acidity may influence its ability to act as a symbiote with *E. scolopes* (Ocean Acidification, n.d.). This is due to the internal pH environment of the host squid light organ, which maintains a pH range of 7.0 to 8.0 upon hatching (Cohen et al., 2020).

Figure 2

Geographical distribution of varying V. fischeri strains.



Note. *E. scolopes* (ES) strains are found in Hawaiian oceans, while *E. tasmanica* (ET) strains are found off the coast of Australia, and *E. morsei* (EM) strains can be found off the Japanese coast. Incorporating environmental differences based on geography may be important, and analyzing threshold tolerance levels in *V. fischeri* strains. Created in BioRender.com.

Effect of Temperature on Symbiosis

Temperature can dramatically impact the dynamic relationship between *E. scolopes* and *V. fischeri* in shaping the evolution of this host-microbe symbiosis; it is often the deciding factor of the relationship rather than squid-host specificity due to its influence on the survivability of

either organism (Soto et al., 2008). When analyzing the mechanisms of evolution involved in environmental changes, it is crucial to examine the natural habitats and niches of each species. It is known that all extant Euprymna species are allopatric, which indicates that they naturally inhabit separate locations that do not overlap geographically. These species are mainly found in the Indo-Pacific, and native *V. fischeri* strains are likely to outcompete non-native symbionts (Soto et al., 2008). A study concluded that *V. fischeri* adapts well to low, high, and fluctuating temperature stress, but lacks significant adaptation to benign temperature stress, where the temperature oscillates only slightly when compared to the aforementioned more drastic changes (Cohen et al., 2019). This conclusion indicates that microbial adaptations to temperature stress could promote coevolution, as demonstrated by how monophyletic groups of *V. fischeri* in Hawaii are more efficient at infecting the Hawaiian variant of the bobtail squid (*E. scolopes*) compared to other groups of *V. fischeri* that are encountered in differing geographic locations (Wollenberg and Ruby 2011).

Effect of Salinity on Symbiosis

Altering the salinity levels in an aqueous environment can also play a role in impacting the dynamic between *E. scolopes* and *V. fischeri*. Soto et al. (2008) discovered that varying strains of *V. fischeri* reacted differently under different salinity conditions (Soto et al., 2008). *V. fischeri* strain ES114 grew optimally under low salinity conditions, where the percent of NaCl concentration was under 3.0% (Soto et al., 2008). Contrastingly, the ET401 strain grew optimally under high salinity conditions, where the percent NaCl was higher than 4.0% (Soto et al., 2008). These results indicate that an extreme salinity condition may prevent some strains of *V. fischeri* from continuous growth, which would inhibit the model system from working toward

counterillumination. This inhibition would be detrimental to the production of counterillumination, as it relies heavily on the ability of the microbes to communicate via quorum sensing and amplify the signal to bioluminesce. A decreased number of *V. fischeri* would not produce as intense of a glow as a larger amount of the microbes would due to the positive feedback loop generated by quorum sensing. One may attribute these differences to geographic location as well, as the salinity may differ in the oceans of Hawaii and Australia (Figure 2). In their growth experiments of *V. fischeri* under differing salinity conditions, Soto et al. indicated that even minute salinity changes in the environment could significantly influence the host-microbe relationship; this has dramatic implications in the face of climate change, which could alter marine salinity with changes to the global water cycle (Soto et al., 2008).

Outstanding Questions & Limitations

Despite the uniformity of this model system, it may still be a challenge to utilize these organisms as flagship species in the event that they adapt to regulate their symbiotic relationship in the face of changing environmental conditions. In many symbiotic relationships, the microbe employs the ability to protect the host from factors like salinity, pH, or heat by increasing host tolerance, which may be the case with *V. fischeri* and its host (Hector et al., 2022). As concluded by Cohen et al., some strains of *V. fischeri* have adapted to growth under more extreme temperature conditions, making it more difficult to observe behavioral changes due to factors like global warming (Cohen et al., 2019). Additionally, eliminating outside factors that influence the symbiotic relationship and its reaction to pH, temperature, and salinity is crucial in understanding whether adaptations are actually made in response to these environmental

conditions. This may initially prove to be an obstacle, but it may be mitigated by ensuring a sterile laboratory environment with only the factors of interest incorporated into the experiment.

Concluding Remarks

The interaction between the bioluminescent bacterium *Vibrio fischeri* and the bobtail squid *Euprymna scolopes* is a widely studied ecological system due to its adaptability, specificity, and consistency. Despite limitations in research generated by its adaptability, the system could serve as a threshold for the impacts of climate change, and when it may be more drastic, which may provide policymakers with more reason to take action against these changes. This relationship has served as a model for understanding microbial colonization, host-microbe interactions, and the regulatory mechanisms of quorum sensing. However, it is crucial to address the impact of environmental changes on the functionality of this relationship. Factors such as pH, salinity, and temperature fluctuations can disrupt microbial communities, alter host physiology, and challenge the delicate balance required for successful symbiosis.

Investigating the *E. scolopes* and *V. fischeri* symbiosis provides researchers with critical insights into how climate-related changes impact both individual species and broader ecological systems. By analyzing how environmental fluctuations influence microbial colonization, bioluminescence efficiency, and host survival, scientists can accurately predict potential cascading effects on marine food webs and biodiversity. Additionally, studying this system may help identify early warning signs of ecosystem destabilization, reinforcing the need for conservation strategies to the public. Raising awareness of these effects among policymakers and the scientific community can promote initiatives aimed at mitigating climate change, implementing marine conservation measures, and preserving biodiversity in ocean habitats.

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