

Is the Vibrio fischeri–Euprymna scolopes Symbiosis a Defensive Mutualism?

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6.1 Introduction

The light-organ symbiosis between the bioluminescent bacterium *Vibrio fischeri* and the Hawaiian bobtail squid *Euprymna scolopes* is a fascinating association rife with intriguing biology. This sepiolid squid is a small nocturnal inhabitant of shallow sandy reefs in the Hawaiian archipelago that allows *V. fischeri*, and only this bacterium, to colonize epithelium-lined crypts of a specialized light-emitting organ. This “light organ” is located just ventral to the squid’s ink sac in the mantle cavity. Once they have colonized the light organ, the bacterial symbionts emit a bluish light, and adaptations by the host allow it to direct and modulate the emitted light. Below, we will discuss how the light organ’s architecture suggests that it functions in the camouflaging behavior referred to as counterillumination (Figure 6.1), wherein marine animals emit light downward, roughly matching the downwelling light from above to obscure their silhouette from animals beneath them in the water column (Clarke, 1963; Dahlgren, 1916; Harper and Case, 1999; Latz, 1996; McFall-Ngai and Morin, 1991; Warner et al., 1979).

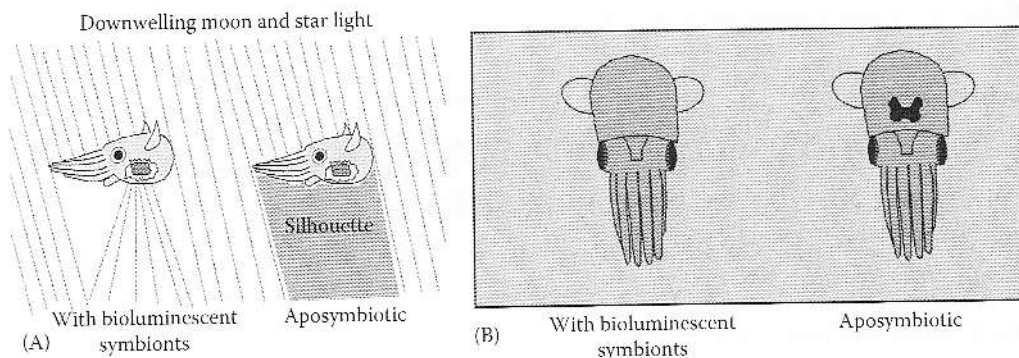


FIGURE 6.1 Proposed counterillumination mechanism of *E. scolopes*. Panel A: It has been proposed that *E. scolopes* with bioluminescent symbiotic *V. fischeri* can emit a controllable, ventrally directed luminescence thereby obscuring their silhouette from a predator beneath them in the water column. Panel B: A cartoon of *E. scolopes* viewed ventrally and backlit illustrates in an idealized way how the light organ and integral ink sac might be obscured by bioluminescence and how the rest of the animal may have different degrees of translucence.

Research on the *V. fischeri*–*E. scolopes* symbiosis has gathered momentum, but many big-picture questions remain unanswered. *E. scolopes* was first described by Berry nearly a century ago (Berry, 1912), and detailed studies of bacterial infections in *Euprymna morsei* light organs were provided by Kishitani in the 1920s. Although the original reports are now difficult to find, they are nicely reviewed in E. Newton Harvey's book *Bioluminescence* (Harvey, 1952). While early studies focused on the behavior, ecology, and embryology of *E. scolopes*, in the last 20 years research into the *V. fischeri*–*E. scolopes* association has increasingly focused on using this symbiosis as a model for mechanistic studies of mutualistic host–bacteria interactions (Stabb, 2006). This focus stems in large measure from experimental tractability. Most notably, this symbiosis can be reconstituted in the laboratory, allowing detailed analyses of how a very specific host–bacterium relationship is initiated. Many exciting discoveries have been made, yet fundamental questions about the ecology of these symbiotic partners and the significance of the bioluminescence resulting from their association remain relatively obscure.

The purpose of this chapter is first to orient the reader to key background information in the field, encompassing some mechanistic studies as well as what is known about the ecology of the partners and the evidence for mutualism in the symbiosis. Then we will describe the premise and basic model of counterillumination. We will next present the evidence for and against counterillumination being the selective advantage of this symbiosis for the host squid, and weigh this evidence along with other hypotheses that seek to explain the ecological significance of light-organ bioluminescence. Finally, we will close by highlighting gaps in our current knowledge and future experimental strategies that promise to yield important new insights.

6.2 Background

6.2.1 Reconstitution of the Symbiosis in the Laboratory Enables Experimental Studies of Early Infection

V. fischeri symbionts are acquired by *E. scolopes* through horizontal transmission, with each new generation of hatchling squid obtaining *V. fischeri* from the environment. Thus, *V. fischeri* and *E. scolopes* exist for some period of time outside the symbiosis, although at least for the host this aposymbiotic stage is brief. The ability to reconstitute this symbiosis experimentally stems from the fact that both partners can be maintained without great difficulty in the laboratory. *V. fischeri* can be cultured readily, and we have observed doubling times of less than 30 min in standard rich media.

When samples from *E. scolopes* light organs are plated onto solid media, *V. fischeri* colonies are recovered with high efficiency, and the light-organ infections are monospecific, meaning that they contain only *V. fischeri* and no other bacteria (Boettcher and Ruby, 1990, 1994; Ruby and McFall-Ngai, 1991). *E. scolopes* can also be kept in the laboratory, where it readily produces eggs and hatchlings. *E. scolopes* was initially bred in aquaria for embryological studies (Arnold et al., 1972), but later the posthatching initiation and development of their light-organ symbiosis was also studied (Montgomery and McFall-Ngai, 1995; Wei and Young, 1989). Eventually, *E. scolopes* was raised through a full life cycle (Claes and Dunlap, 2000; Hanlon et al., 1997).

E. scolopes eggs are symbiont free, and exposing the hatchlings to *V. fischeri* inocula in seawater results in symbiotic infection (Ruby and McFall-Ngai, 1991; Wei and Young, 1989). Arguably, this infection in the laboratory approximates natural horizontal transmission of symbiont. Populations of *V. fischeri* have been examined in *E. scolopes* habitats, so inoculum levels that approximate environmental populations of *V. fischeri* can be used. When fresh hatchlings are placed in nearshore Hawaiian seawater, the onset of luminescence is detectable within 8 h (Ruby and McFall-Ngai, 1991), a time frame that parallels infections in the laboratory. Colonization by small numbers of *V. fischeri* that have not yet begun to bioluminesce, occurs even earlier (Asato, 1993; Ruby and Asato, 1993).

Numerous articles have reviewed the advances in our mechanistic understanding of this symbiotic infection (Geszvain and Visick, 2006; McFall-Ngai, 2000; Nyholm and McFall-Ngai, 2004; Ruby, 1999; Ruby and McFall-Ngai, 1999; Stabb, 2006; Visick, 2005; Visick and McFall-Ngai, 2000; Visick and Ruby, 2006), and we will provide only a brief overview of the infection process. The bilobed *E. scolopes* light organ resides in the mantle cavity, where it is exposed to environmental seawater. Upon hatching, two fields of cilia on the light-organ surface begin beating and shedding mucus (Nyholm et al., 2002). Bacteria in the seawater get stuck in this mucus, thereby concentrating them from the dilute environment (Nyholm et al., 2000). Motility by the bacteria is then required as they swim from aggregates in the mucus to pores on the surface of the light organ (Graf et al., 1994; Millikan and Ruby, 2002, 2004; Nyholm et al., 2000). Symbionts enter the pores and travel through ducts and eventually into epithelium-lined pockets referred to as antechambers and crypts (Sycuro et al., 2006). Although many *V. fischeri* cells can be concentrated in aggregates outside the light organ, relatively few cells appear to survive this journey and initiate colonization (Dunn et al., 2006; McCann et al., 2003; Ruby and Asato, 1993).

The initial *V. fischeri* colonists proliferate, and both the increase in their cell density and some environmental cue in the host trigger them to begin producing bioluminescence (Boettcher and Ruby, 1990; Bose et al., 2007). *V. fischeri* mutants that do not produce bioluminescence are attenuated in colonization (Bose et al., 2008; Visick et al., 2000), suggesting that the host either detects dark infections and sanctions them or generates an environment in the light organ in which bioluminescence is physiologically advantageous for the symbionts. This underscores the importance of bioluminescence in the symbiosis and can be interpreted as an indication that the host limits the success of dark “cheaters” among the symbionts.

6.2.2 Ecology and Behavior of *V. fischeri*

V. fischeri has been isolated from a variety of marine environments, and is perhaps best known for colonizing light-emitting organs of specific fish and squid. The “bobtail” squid that serve as hosts for *V. fischeri* are in the genera *Sepiolo* or *Euprymna*, which are found in the Mediterranean Sea and Pacific Ocean, respectively (Fidopiastis et al., 1998; Nishiguchi, 2002; Nishiguchi et al., 1998). *V. fischeri* also colonizes the light organs of monocentrid “pinecone” fish (Fitzgerald, 1977; Ruby and Nealson, 1976). However, *V. fischeri* is not restricted to monospecific associations and has been isolated from the gut consortia of fish in nonspecific symbioses (Makemson and Hermosa, 1999; Ruby and Morin, 1979; Sugita and Ito, 2006). Moreover, *V. fischeri* is found free living in the water column and in sediments (Lee and Ruby, 1994b; Ruby et al., 1980). Numerically, it is not particularly abundant in these environs, but *V. fischeri* is widespread and occurs in habitats where it has no known light-organ hosts. The metabolic and genomic flexibility of *V. fischeri* suggest that in contrast to obligate symbionts this bacterium probably has an important environmental lifestyle (Ochman and Moran, 2001; Ruby et al., 2005).

To adjust to the host and other environments, *V. fischeri* possesses an impressive array of regulatory systems (Geszvain and Visick, 2006; Hastings and Greenberg, 1999). *V. fischeri* is well known for a group behavior known as “quorum sensing,” wherein a diffusible pheromone mediates changes in gene expression when populations reach high cell density (Hastings and Greenberg, 1999). Quorum sensing and the redox-responsive ArcA/ArcB regulatory system tune the luminescence of *V. fischeri* to express maximally when cells are crowded, yet in relatively oxidative conditions (Bose et al., 2007). Because regulation of luminescence is governed in part by a diffusible pheromone, the potential exists for a sub-population of *V. fischeri* to incite a group decision to luminescence in the light organ. Understanding the basis for the behavior of *V. fischeri* in the light organ should help elucidate the role of luminescence for the bacterium (Stabb, 2005) and may reveal mechanisms by which the host manipulates the symbionts’ luminescence.

6.2.3 Ecology and Behavior of *E. scolopes*

E. scolopes is a solitary nocturnal predator that feeds mainly on shrimps and polychaetes, and grows from hatchlings just a few millimeter in length to thumb-sized adults (Moynihan, 1983; Shears, 1988). The adults probably live less than a year (Hanlon et al., 1997; Singley, 1983). Females lay clutches of tens or hundreds of eggs, and as mentioned above, each new generation must acquire *V. fischeri* from the surrounding marine environment (Wei and Young, 1989), although infection is so rapid that no uninfected *E. scolopes* individual has ever been found.

Most observations of *E. scolopes* are made in shallow sandy reef areas, but it is unclear how confined it is to such habitats. We, and others, typically use flashlights and nets to collect animals at night by wading in knee-deep water. It is not uncommon to find animals less than a meter from shore, sometimes in water no more than 10 cm deep. However, there are reports of *E. scolopes* being collected offshore near the surface (R. Young, personal communication) and even at depths around 200 m (Berry, 1912). So while most observations of *E. scolopes* are in nearshore shallow water, it is not clear the extent to which this reflects the fact that this is where researchers tend to look for them. Similarly, we tend to find *E. scolopes* adults on the sandy bottom, not up in the water column; however, almost certainly the animals are aware of our presence before we spot them, so their behavior may be perturbed. In aquaria, *E. scolopes* spend most of their time on the bottom (Moynihan, 1983), and we tend to assume that this is their habit; however, it would be most accurate to say that little is known about their natural behavior.

Camouflaging seems to be a general strategy for *E. scolopes* (Anderson and Mather, 1996; Shears, 1988), which routinely buries or coats itself with sand and uses chromatophores to change colors from a palette that is similar to the background (Figure 6.2). The squid can even be observed swimming with a sand coat, which they can apparently discard with remarkable speed and control (Shears, 1988). Even without the sand coat, their coloration blends in well with the sandy reef bottoms; as anyone who has collected these animals can attest. The animals also sometimes emit ink blobs and then jet away, when threatened. However, in our observations and those of Anderson and Mather (1996), the animals rarely jet very far from the site of an encounter.

Little is known about which predators *E. scolopes* may be hiding from. Hawaiian monk seals occasionally feed on *E. scolopes* (Goodman-Lowe, 1998), and we have observed lizard fish struggling with a catch of *E. scolopes*; however, we have a poor understanding of the threats it faces. Also lacking are observations of luminescence by these squid in their natural habitat. Every wild-caught *E. scolopes* tested has been found to emit bioluminescence; however, this was determined upon placing the animals in a sensitive luminometer and not by observations in the wild. There are reports that the animals “flash” their luminescence when disturbed (Moynihan, 1983; A. Wier and M.J. McFall-Ngai personal communication; R. Young, personal communication), but this has not been documented in a natural setting.

6.2.4 Evidence of Mutualism in this Symbiosis

The selective advantage of the symbiosis for *V. fischeri* appears clear. *V. fischeri* is provided a privileged niche in the *E. scolopes* light organ, where it grows rapidly (Ruby and Asato, 1993). Not only are they provided nutrients to support this growth, but because the squid can prevent infection by other

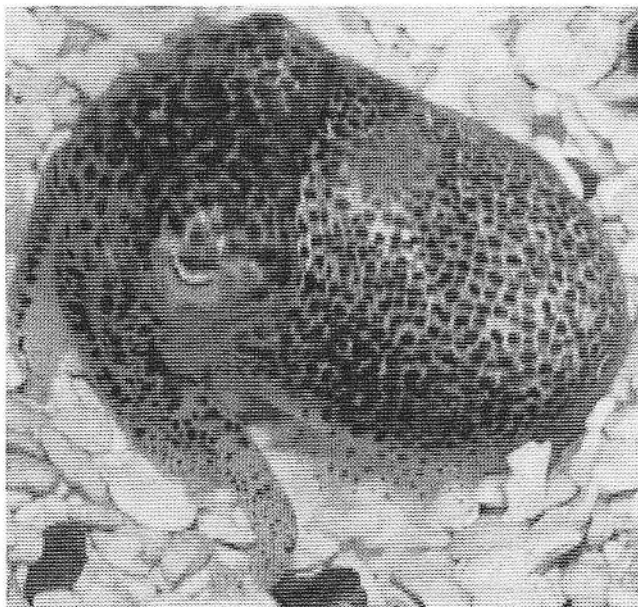


FIGURE 6.2 An adult *E. scolopes*. An adult Hawaiian bobtail squid sits on the coral sand bottom of an aquarium. The mottled appearance is due to controllable chromatophores that are expanded to produce a relatively dark pattern of reddish and yellowish brown pigments in this picture. Chromatophores can also be retracted to small points, rendering the mantle largely translucent. The mantle length of this typical adult specimen is ~2.5–3 cm. (Photo credit of Jeffrey L. Bose).

microorganisms, *V. fischeri* cells would appear to have the host immune system effectively protecting them from predatory grazing or competitive antibiosis by other microbes. Moreover, colonization of the *E. scolopes* light organ is not a dead end. Each morning these squid settle to the bottom, cover themselves with sand, and the light of dawn triggers the expulsion of most *V. fischeri* cells into the environment (Boettcher et al., 1996a). The *V. fischeri* left behind grow to repopulate the light organ by dusk. Presumably, because of this diurnal “venting” process, *V. fischeri* populations are relatively high in habitats occupied by *E. scolopes* (Lee and Ruby, 1994b). Further ecological studies (Lee and Ruby, 1994a) support the theory that in the Hawaiian reefs occupied by *E. scolopes*, the ability to colonize this host is a strong selective force on *V. fischeri*. So, while *V. fischeri* can be found in many marine environments, its populations appear to be enhanced by *E. scolopes*.

Presumably, the host also benefits from the association, and the *V. fischeri*–*E. scolopes* symbiosis is a mutualism. The *E. scolopes* light organ grows and maintains a monospecific culture of *V. fischeri* and has the means to control the release of its bioluminescence. Moreover, we (and others) have observed that stressed animals appear to clear their light organs of *V. fischeri* symbionts altogether, suggesting that the host has ultimate control and is not suffering an unavoidable infection. The symbiosis probably provides a benefit to *E. scolopes* by giving it a controllable source of bioluminescence for some behavior, and this is usually attributed to a camouflaging “counterillumination” behavior mentioned above and discussed at greater length below. Although an additional nutritional or other benefit of the symbionts cannot be ruled out, *E. scolopes* raised through a complete life cycle in the absence of *V. fischeri* did not appear to suffer due to lack of symbionts (Claes and Dunlap, 2000).

6.3 Counterillumination Model

Strategies to avoid predation are numerous and varied in the marine environment and can involve highly specialized adaptations. Familiar examples include animals with the ability to camouflage themselves through pigmentation or shape that mimics the surrounding substrate, such as rock or kelp beds, or the

ability to bury into sand or hide among rocks. However, in the open ocean, the ability to “hide” from predators is difficult, given the lack of substrate. In spite of this difficulty, many mesopelagic organisms including luminous fishes, crustaceans, and cephalopods have found a way to camouflage themselves by producing light in discrete regions of their body (Clarke, 1963). This use of light is called counterillumination, which is most often used to describe the ability to match downwelling light using ventrally directed luminescence (Dahlgren, 1916; Harper and Case, 1999; Latz, 1996; McFall-Ngai and Morin, 1991; Warner et al., 1979; Young, 1977). In this way, light production along the ventral surface of an organism can be used to hide the animal’s silhouette if viewed from below in the water column (e.g., Figure 6.1).

Downwelling light in the mesopelagic zone is unidirectional and predictable in its wavelength and low intensity, but in shallow water light is more variable both in quantity and quality. Moreover, light in shallow water is multidirectional owing to reflection, shadows, and surface effects. This potentially renders counterillumination a more difficult strategy for shallow-water animals to use effectively. However, McFall-Ngai and Morin provided convincing evidence that counterillumination behavior does occur in shallow-water leigognathid fishes, although they suggest that “disruptive illumination” might be a better description of the mottled appearance that the ventral bioluminescence imparts on the animals (McFall-Ngai and Morin, 1991).

The concept of “disruptive illumination” is an important distinction from most discussions of counterillumination, and it may be especially relevant to the role of the *E. scolopes* light organ. However, for most part we will use “counterillumination” to encompass partial or complete illumination, disruptive illumination, or intermittent countershading, with the commonality to all of these being the ability of the animal to produce light that mimics their surroundings to whatever extent, in order to camouflage themselves. One exception is that of the cookie cutter shark discussed next, where camouflage is not the apparent role of counterillumination. In general though we will use “counterillumination” in reference to hiding behaviors, and we will sometimes use “camouflaging” in conjunction with “counterillumination” to reinforce this distinction.

Several factors may contribute to the effectiveness of counterillumination. It has been argued that animals should have many evenly distributed light sources, because those with fewer or less-distributed sources of bioluminescence would be less able to produce an even field of light, minimizing or negating any camouflaging effect of counterillumination (Johnsen et al., 2004). Consistent with this argument, counterillumination is usually attributed to an array of ventrally directed photophores. In *Histioteuthis* squid, for example, many small light organs are scattered over the ventral surface of the body, head, and arms. However, the majority of shallow-water luminous animals, including *E. scolopes*, have a single light-emitting organ. It has also been pointed out that effective counterillumination should mimic both the quantity and the quality (e.g., wavelength) of downwelling light (Johnsen et al., 2004; McFall-Ngai and Morin, 1991). Otherwise, luminescence could have the opposite effect and become an attractant for predators. Finally, the habits and visual acuity of the predator being hidden from are important.

6.4 Weighing the Evidence for Counterillumination and Other Models

6.4.1 Does Light-Organ Anatomy Suggest Counterillumination?

The idea that the *E. scolopes* light-organ functions in counterillumination gathered momentum from close examination of its architecture (McFall-Ngai and Montgomery, 1990). The light organ is situated in the mantle cavity just ventral to the bulk of the ink sac, and it is oriented such that light is emitted ventrally. The light-organ crypts occupied by bioluminescent symbionts are situated between a reflective layer of reflectin protein (Crookes et al., 2004) and a muscle-derived lens (Montgomery and McFall-Ngai, 1992), which together appear to direct and control ventral light emission. In the adult animals, the ink sac apparently can be drawn around the light organ, perhaps in the manner of a shutter, and anatomical observations of several fixed specimens suggest that controlled movement of reflective tissue and ink sac diverticula could modulate the emission of light (McFall-Ngai and Montgomery, 1990). The animals may also be able to control luminescence by modulating the oxygenation of the light organ (Boettcher et al., 1996b). This could effectively control luminescence because oxygen is one of the reactants

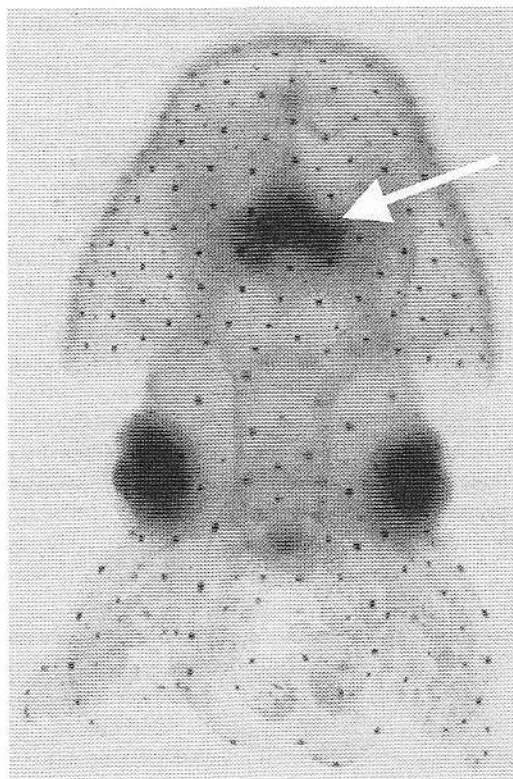


FIGURE 6.3 Backlit juvenile *E. scolopes*. This animal has retracted its chromatophores to small points rendering its mantle largely translucent. A white arrow points to the light organ and ink sac. The other prominent dark (opaque) shaded structures are the eyes. The mantle length of this typical hatchling juvenile is ~1–1.5 mm. (Photo credit of Dawn M. Adin).

of the *V. fischeri* luciferase reaction. Thus, the *E. scolopes* light organ has elements consistent with counterillumination, specifically a controllable ventrally emitted light.

In at least one respect, the architecture of the light organ seems imperfectly adapted for camouflaging by counterillumination. The *E. scolopes* light organ does not cover the entire underside of the animal the way multiple photophores of some mesopelagic squid do. This would arguably represent a poor or incomplete counterillumination apparatus. On the other hand, symbiotic luminescence would provide counterilluminating cover for a large opaque structure in the squid, the ink sac integral to the light organ (this is illustrated with a juvenile in Figure 6.3), which might provide a selective advantage.

6.4.2 Does Host Behavior Fit the Counterillumination Hypothesis?

It is difficult to say whether *E. scolopes* behavior supports the counterillumination hypothesis simply because very little is known about this animal's behavior. As discussed in the final section, answering many questions about the behavior and ecology of *E. scolopes* would help put light-organ function in a natural context and inform our discussion of its potential role(s). At least it seems clear that *E. scolopes* is nocturnal and spends at least some time in the water column, which is consistent with counterillumination.

One mysterious behavior of *E. scolopes* deserves special mention. Multiple investigators have seen *E. scolopes* “flash” bioluminescence (Moynihan, 1983; A. Wier and M.J. McFall-Ngai personal communication; R. Young, personal communication). Typically, in situations when these animals were

severely disturbed or roughly handled, they often released ink. This suggests that flashes might be an attempt to distract or confuse predators that get too close, perhaps as part of an escape response. Obviously, visible flashes are inconsistent with camouflaging, and it may be that flashes reflect the real role of the light organ or that the light organ has two very distinct roles.

6.4.3 How Conclusive Are Attempts to Measure Counterillumination in *E. scolopes*?

Jones and Nishiguchi (2004) have provided the most important and convincing data for evaluating counterillumination in the *V. fischeri*–*E. scolopes* symbiosis. They placed adult *E. scolopes* in small chambers and plotted the amount of light emitted from the animals, as a function of the intensity of the downwelling light. To differentiate the room lights from animal bioluminescence, the overhead lights were turned off and animal luminescence was measured immediately thereafter. Controls that included chambers without animals ensured that phosphorescence of the seawater, which might be expected to correlate with overhead light intensity, was not mistaken for bioluminescence. As predicted by the counterillumination hypothesis, bioluminescence released from animals was more intense as the intensity of the overhead illumination was increased (Jones and Nishiguchi, 2004). An exception to this was observed at the highest light intensities, when animals began to dim the luminescence they emitted. Although not predicted by the counterillumination hypothesis, it seems plausible that at uncommonly perhaps artificially high light intensities the animals would not be able to match the downwelling light and would abandon counterillumination behavior. More importantly, this observation helps to validate the experimental setup, because it is unclear how any nonbiological explanation for the data could yield this result at high light intensity.

Two results of the Jones and Nishiguchi study hint at either the imperfection of counterillumination or the technical difficulty of such experiments. First, although luminescence emitted by the animals correlated with the intensity of downwelling light, the animal luminescence appeared dimmer than that of the downwelling light (Jones and Nishiguchi, 2004). However, even imperfect matching of the downwelling light (imperfect counterillumination) might have a selective advantage over no counterillumination at all. Also, it is important to remember that animal luminescence was measured after the lights were turned off, but presumably before the squid could react and alter the luminescence. Therefore, an unexpectedly quick reaction by the squid could explain this aspect of the data. This brings up a second result not predicted by the counterillumination hypothesis; sometimes animals were remarkably slow in dimming their luminescence after the lights were turned out. In such animals, luminescence continued for a minute or more after the lights were out. This seems to be a counterproductive behavior for an animal trying to be stealthy, but then again the animals may never (or rarely) encounter such a rapid and absolute change in light intensity, in nature.

Overall, the work of Jones and Nishiguchi strongly supports the counterillumination hypothesis; however, skeptics can still point to an obvious gap in our observations. Specifically, to our knowledge nobody has photodocumented counterillumination by *E. scolopes*, either in the laboratory or in nature. At least in the laboratory this might seem easy, by simply putting *E. scolopes* in a clear-bottom container and viewing it from underneath with dim diffuse light overhead. In our experience, however, animals put in this situation are prone to simply sitting on the bottom, with their arms curled under them and their chromatophores darkened. Presumably, counterillumination would be most useful when the animals are up in the water column, and as discussed below future work aimed at viewing animals behaving in this way could provide important information.

It is also worth noting that to our knowledge the wavelength of light emitted from *E. scolopes* has not been compared to the background light in its habitat(s). This is important, because for counterillumination to be an effective camouflage it must presumably match the background both in intensity (as Jones and Nishiguchi measured) and in quality. Moynihan (1983) reported that flashes seen from disturbed squid appeared “green,” although we have observed the expected bluish light emitted by bacteria in *E. scolopes* juveniles (Stabb, 2005). It seems unlikely that a green light would effectively match the backgrounds we are familiar with in the shallow sandy reefs of Hawaii; however, measurements of the emitted light and environmental light are needed to resolve the issue.

6.4.4 Counterillumination Is Not Necessarily a "Defensive" Camouflage

If counterillumination is used as camouflage by *E. scolopes*, this might reflect an offensive, rather than defensive, strategy. *E. scolopes* might use counterillumination to hide from potential prey. However, at least in aquaria we have not observed *E. scolopes* approach the prey from above. Rather they seem to attack horizontally with their tentacles to capture shrimps. It is also uncertain whether the prey that *E. scolopes* feeds on would be easier to catch if the squid possesses a ventral counterillumination mechanism; however, with so much unknown about *E. scolopes* behavior (e.g., its feeding habits in deeper water), it is impossible to rule out camouflaging counterillumination as an offensive strategy.

6.4.5 Alternatives (or Amendments) to the Counterillumination Camouflage Hypothesis

If *E. scolopes* does not use bioluminescence in a camouflaging counterillumination behavior, or if this is not the primary selective advantage of symbiotic bioluminescence, then what might be the main role of the light organ? Proposing alternate answers to this question may prove to be a useful exercise, both by preventing tunnel-visioned interpretation of existing data and by pointing future research in new directions. Below, we discuss a few possible uses for the light organ other than as a silhouette-obscuring camouflage.

E. scolopes might use counterillumination as a prey attractant. Widder presented evidence that the cookie cutter shark, *Isistius brasiliensis*, uses counterillumination not to obscure its entire ventral surface, but rather to obscure all but an image shaped like a smaller fish (Widder, 1998). She convincingly argues that this might be used as a lure to attract predators of small fish, including the swordfish, tunas, and porpoises that *I. brasiliensis* attacks. It is not clear to us whether a distinct luring image would be produced by the shape of the *E. scolopes* light organ, but we cannot rule this out. Notably, this model predicts the same sort of correlation between ambient light and emitted luminescence that was observed by Jones and Nishiguchi (2004).

Alternatively, luminescence *per se*, and not the counterillumination of a particular alluring shape, might be used to attract prey. This would seem to be a risky proposition for *E. scolopes* hunting in the water column. *E. scolopes* may have defensive strategies other than avoiding detection, but these are not immediately apparent (e.g., it lacks spines or known predator-detering toxins), making stealth an important asset for *E. scolopes*. On the other hand, it seems plausible that the shrimp or polychaetes that *E. scolopes* feeds on might display phototaxis, and with so little knowledge about the behavior and ecology of *E. scolopes* we cannot rule out the possibility that *E. scolopes* exploits such a behavior of its prey.

Perhaps, the most plausible alternative model for the role of the light organ center is its potential to flash brightly. As mentioned above, such flashes have been observed when animals were collected or held in captivity (Moynihan, 1983; A. Wier and M.J. McFall-Ngai personal communication; R. Young personal communication). The ability to flash visible luminescence could suggest a role in intraspecific communication; however, the fact that it has been observed when the animals are disturbed (e.g., physically grabbed) suggests a role as a startling tactic (Herring, 1977). Another sepiolid squid in Hawaii, *Heteroteuthis hawaiiensis*, found in open ocean waters at depths of up to 600 m, can use its light organ to create a glowing cloud of bacteria mixed with mucus (Dilly and Herring, 1978; Young, 1995; Young and Roper, 1976). If *E. scolopes* can emit a brief flash of light with or without expelling bacterial symbionts, this might be used to startle or confuse the predators. Like the expulsion of ink blobs by *E. scolopes* (Anderson and Mather, 1996), this behavior could be used once camouflage has failed.

The bioluminescent defense response of *H. hawaiiensis* may be used in addition to the likely use of its light organ (and multiple light organs on its arms and body) to camouflage its silhouette during the low-level light of dusk and dawn when it migrates to shallower waters (150–200 m) to feed (Dilly and Herring, 1978; Young, 1995; Young and Roper, 1976). This underscores the point that *E. scolopes* may use its light organ for dual roles, and both counterillumination and another behavior may have selective advantages. Or from another perspective, a flashing behavior might be the selective force driving *E. scolopes* light-organ evolution, but this might come at the cost of a relatively large opaque structure that would make the squid more visible to predators, but by emitting a dim counterilluminating light

when the flashing behavior is not in use *E. scolopes* might ameliorate the disadvantage of having a light organ. Again, such a scenario would be consistent with the data. Jones and Nishiguchi (2004) reported data supporting counterillumination, and yet counterillumination may not be the primary purpose for the *E. scolopes* light organ.

6.5 Future Directions

Many big-picture questions regarding the *V. fischeri*–*E. scolopes* symbiosis remain unanswered. Notably, as discussed in this chapter, the functional significance of the light organ for the host is uncertain, although evidence points to a role in camouflaging by counterillumination. Future behavioral and ecological research on *E. scolopes* would be welcome and could shed light on one of the most central issues of the symbiosis.

6.5.1 The Life and Times of *E. scolopes*

Many fundamental questions about the ecology and behavior of *E. scolopes* remain opaque. How much of their life is spent in shallow water? Do they often venture deeper? Can reports of *E. scolopes* in the mesopelagic zone be substantiated? What are they feeding on in such habitats? How much time do they spend in the water column or on the bottom? What is the quality of the light in their environs? Does this match the light they emit? Do they hunt exclusively in the water column? What are their main predators? What are the hunting techniques and visual acuity of these predators? Answering these questions may not be easy. Observing the natural habits of these nocturnal well-camouflaged animals has obvious barriers. For example, it will be difficult for divers to follow the nighttime behavior of these animals without disrupting their routine. Remote tagging and tracking devices are probably not feasible, although the advent of nanotechnologies and miniaturization could facilitate such approaches.

One technology that could provide a powerful tool for studying *E. scolopes* in the environment is the use of autonomous underwater vehicles (AUVs). Fidopiastis and Clark (personal communication) have proposed that a robot with the capacity to swim and crawl might be trained to follow *E. scolopes* individuals, monitoring and recording their behavior. This promises to be much less disturbing to the animals than observations by humans, and could yield unparalleled insights into the behavior of *E. scolopes* over a range of environments. Remotely operated vehicles have been used to monitor marine animals such as jellyfish, and AUV technology has been applied to tracking bass (Zhou et al., 2007). Moreover, an AUV was used to measure patterns of marine bioluminescence (Blackwell et al., 2002). If an AUV can be trained to track *E. scolopes* individuals and potentially to monitor their luminescence, this has great potential for answering many unresolved questions about their behavior.

6.5.2 What about the Flashes?

The fact that *E. scolopes* occasionally emits bright flashes may belie the true function of the light organ; however, observations of this behavior are rare, and to our knowledge it has only been observed in disturbed captive animals. A squid-tracking AUV might be able to document flashes in the wild, and to determine the behavior of the animal before and after the flash. Is it used when the animals are threatened by a predator? Is it used in conjunction with the release of ink? Do the animals change their swimming direction in an evasive manner following a flash? Do the flashes have a temporary blinding or distracting effect on a would-be predator? Or are flashes used as intraspecies signals perhaps eliciting some altogether unknown behavior? Any observation of light-organ flashing in the wild would be tremendously important.

Observations of flashing in the laboratory might also provide insight into the function of the light organ for *E. scolopes*. Because the flashes seem to be rare, it may be useful to set up a digital image recording system that monitors the animals constantly, detects flashes, and then saves the data before and after a flash event. A clear-bottom tank could be used with a camera (or array of cameras) so that observations could be made of the animal's ventral surface, although flashes should be detectable regardless of

how the animals direct them. To test whether flashes are used in defensive responses or communication, it will probably be necessary to place multiple squid or predators in the same tank, which could require a relatively large setup. Although such an experiment is not without obstacles, it could yield important data and might be easier than observations, in nature.

6.5.3 More Tests of Counterillumination

One conspicuously missing piece of evidence for counterillumination by *E. scolopes* is the lack of an image documenting this effect. A squid-tracking AUV might capture such an image in the environment, which would provide strong support for this theory. However, carefully crafted laboratory studies might provide similar and compelling evidence. As noted above, animals observed in clear-bottomed containers tend to simply sit on the bottom. A clear-bottomed raceway with water flowing through it (e.g., a clear pipe) might force the animals off the bottom, allowing visualization from underneath the swimming *E. scolopes*. Alternatively, a useful setup might include a camera underneath a clear-bottomed container set to capture several hours worth of images, or to be triggered by the motion of the animals. In the absence of a human investigator, the animals may eventually leave the bottom (e.g., to capture prey or mate) and could then be visualized. Sensitive digital cameras capable of capturing low-light images could document the squid's silhouette and whether or not it is obscured by counterillumination.

A more advanced apparatus for measuring counterillumination would also be useful, if counterillumination can be elicited by animals going about relatively normal behaviors in the laboratory. One such methodology that has been employed productively is to use an overhead light that provides illumination that is seen as constant the animals, by that is actually "chopped" into short bursts of light, such that a photomultiplier detector set "out of phase" with the incident light will measure only the counterillumination (Latz and Case, 1992). In this way, quantitative measurements of counterillumination can be made on animals that are not forced into cramped confines as they were in the apparatus used by Jones and Nishiguchi (2004), and it may provide a telling picture of natural counterillumination behavior. For example, the apparent under-illumination by animals observed by Jones and Nishiguchi may simply reflect a perturbation of the animals associated with putting them in the experimental apparatus.

The ultimate proof that camouflaging provides an antipredatory selective advantage would be an ecological prey study in which *E. scolopes* with luminescent symbiotic bacteria and animals raised with nonluminescent symbionts or no symbionts are presented to an appropriate predator and in an environment that mimics their natural one. Such experiments are obviously difficult to set up; however, such an approach was used to demonstrate an antipredatory effect of counterillumination in the fish *Porichthys notatus* (Harper and Case, 1999). A potential predator in these experiments could be the lizardfish of the Synodontidae family, which we suspect may be an important natural predator of *E. scolopes*, although as noted above more research needs to be done in this regard. Such experiments would need to be done with pools of animals isolated from one another, so that cross contamination of luminescent bacteria to nonluminescent animals cannot occur. The animals would also have to be monitored closely for potential flashing behavior, to distinguish their possible antipredatory value from that of counterillumination. If done convincingly, such experiments could settle the issue of the selective advantage of symbiotic bioluminescence in the *V. fischeri*–*E. scolopes* mutualism.

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REFERENCES

- Anderson, R. C. and J. A. Mather. 1996. Escape responses of *Euprymna scolopes* Berry, 1911 (Cephalopoda: Sepiolidae). *Journal of Molluscan Studies* 62:543–545.
- Arnold, J., Singly, C., and L. Williams-Arnold. 1972. Embryonic development and post-hatching survival of the sepiolid squid *Euprymna scolopes* under laboratory conditions. *Veliger* 14:361–364.
- Asato, L. M. 1993. Morphological and physiological changes in *Vibrio fischeri* during the initiation and release from the mutualistic association with its sepiolid host, *Euprymna scolopes*. Masters thesis, University of Southern California.
- Berry, S. S. 1912. The Cephalopoda of the Hawaiian islands. In *Bulletin of the United States Bureau of Fisheries*. Government Printing Office, Washington, pp. 255–362.
- Blackwell, S., Case, J., Glenn, S., Kohut, J., Moline, M. A., Purcell, M., Schofield, O., and C. VonAlt. 2002. A new AUV platform for studying near shore bioluminescence structure. In *Proceedings of the 12th International Symposium on Bioluminescence and Chemiluminescence*, eds. P. Herring, L. J. Kricka, and Stanley, P. E., pp. 197–200. London: World Scientific.
- Boettcher, K. J. and E. G. Ruby. 1990. Depressed light emission by symbiotic *Vibrio fischeri* of the sepiolid squid *Euprymna scolopes*. *Journal of Bacteriology* 172:3701–3706.
- Boettcher, K. J. and E. G. Ruby. 1994. Occurrence of plasmid DNA in the sepiolid squid symbiont *Vibrio fischeri*. *Current Microbiology* 29:279–286.
- Boettcher, K. J., Ruby, E. G., and M. J. McFall-Ngai. 1996a. Bioluminescence in the symbiotic squid *Euprymna scolopes* is controlled by a daily biological rhythm. *Journal of Comparative Physiology* 179:65–73.
- Boettcher, K. J., Ruby, E. G., and M. J. McFall-Ngai. 1996b. Bioluminescence in the symbiotic squid *Euprymna scolopes* is controlled by a daily biological rhythm. *Journal of Comparative Physiology A* 179:65–73.
- Bose, J. L., Kim, U., Bartkowski, W., Gunsalus, R. P., Overley, A. M., Lyell, N. L., Visick, K. L., and E. V. Stabb. 2007. Bioluminescence in *Vibrio fischeri* is controlled by the redox-responsive regulator ArcA. *Molecular Microbiology* 65:538–553.
- Bose, J. L., Rosenberg, C. S., and E. V. Stabb. 2008. Effects of *luxCDABEG* induction in *Vibrio fischeri*: Enhancement of symbiotic colonization and conditional attenuation of growth in culture. *Archives of Microbiology* 190:169–183.
- Claes, M. F. and P. V. Dunlap. 2000. Aposymbiotic culture of the sepiolid squid *Euprymna scolopes*: Role of the symbiotic bacterium *Vibrio fischeri* in host animal growth, development, and light organ morphogenesis. *Journal of Experimental Botany* 286:280–296.
- Clarke, W. D. 1963. Function of bioluminescence in mesopelagic organisms. *Nature* 198:1244–1246.
- Crookes, W. J., Ding, L. L., Huang, Q. L., Kimbell, J. R., Horwitz, J., and M. J. McFall-Ngai. 2004. Reflectins: The unusual proteins of squid reflective tissues. *Science* 303:235–238.
- Dahlgren, U. 1916. Production of light by animals. *Journal of the Franklin Institute* 181:525–556.
- Dilly, P. N. and P. Herring. 1978. The light organ and ink sac of *Heteroteuthis dispar*. *Journal of Zoology, London* 186:47–59.
- Dunn, A. K., Millikan, D. S., Adin, D. M., Bose, J. L., and E. V. Stabb. 2006. New *rfp+* and pES213-derived tools for analyzing symbiotic *Vibrio fischeri* reveal patterns of infection and *lux* expression *in situ*. *Applied Environmental Microbiology* 72:802–810.
- Fidopiastis, P. M., von Boletzky, S., and E. G. Ruby. 1998. A new niche for *Vibrio logei*, the predominant light organ symbiont of squids in the genus *Sepiolo*. *Journal of Bacteriology* 180:59–64.
- Fitzgerald, J. M. 1977. Classification of luminous bacteria from the light organ of the Australian pinecone fish, *Cleidopus glotamaris*. *Archives of Microbiology* 112:153–156.
- Geszvain, K. and K. L. Visick. 2006. Roles of bacterial regulators in the symbiosis between *Vibrio fischeri* and *Euprymna scolopes*. *Progress in Molecular and Subcellular Biology* 41:277–290.
- Goodman-Lowe, G. D. 1998. Diet of the Hawaiian monk seal (*Monachus schauinslandi*) from the northwestern Hawaiian islands during 1991 to 1994. *Marine Biology* 132:535–546.
- Graf, J., Dunlap, P. V., and E. G. Ruby. 1994. Effect of transposon-induced motility mutations on colonization of the host light organ by *Vibrio fischeri*. *Journal of Bacteriology* 176:6986–6991.
- Hanton, R. T., Claes, M. F., Ashcraft, S. E., and P. V. Dunlap. 1997. Laboratory culture of the sepiolid squid *Euprymna scolopes*: A model system for bacterium-animal symbiosis. *Biological Bulletin* 192:364–374.
- Harper, R. D. and J. F. Case. 1999. Counterillumination and its antipredatory value in the plainfin midshipman fish *Porichthys notatus*. *Marine Biology* 134:529–540.

- Harvey, E. N. 1952. *Bioluminescence*. Academic Press, New York.
- Hastings, J. W. and E. P. Greenberg. 1999. Quorum sensing: The explanation of a curious phenomenon reveals a common characteristic of bacteria. *Journal of Bacteriology* 181:2667-2668.
- Herring, P. 1977. Luminescence in cephalopods and fish. *Symposia of the Zoological Society of London* 38:127-159.
- Johnsen, S., Widder, E. A., and C. D. Mobley. 2004. Propagation and perception of bioluminescence: Factors affecting counterillumination as a cryptic strategy. *Biological Bulletin* 207:1-16.
- Jones, B. W. and M. K. Nishiguchi. 2004. Counterillumination in the Hawaiian bobtail squid, *Euprymna scolopes* Berry (Mollusca: Cephalopoda). *Marine Biology* 144:1151-1155.
- Latz, M. I. 1996. Physiological mechanisms in the control of bioluminescent countershading in a midwater shrimp. In *Zooplankton: Sensory Ecology and Physiology*, eds. P. H. Lenz, D. K. Hartline, J. Purcell, and MacMillan, D. L., pp. 163-174. Amsterdam: Gordon and Breach.
- Latz, M. I. and J. F. Case. 1992. Slow photonic and chemical induction of bioluminescence in the midwater shrimp, *Sergestes similis* Hansen. *Biological Bulletin* 182:391-400.
- Lee, K.-H. and E. G. Ruby. 1994a. Competition between *Vibrio fischeri* strains during initiation and maintenance of a light organ symbiosis. *Journal of Bacteriology* 176:1985-1991.
- Lee, K.-H. and E. G. Ruby. 1994b. Effect of the squid host on the abundance and distribution of symbiotic *Vibrio fischeri* in nature. *Applied Environmental Microbiology* 60:1565-1571.
- Makemson, J. C. and G. V. Hermosa, Jr. 1999. Luminous bacteria cultured from fish guts in the Gulf of Oman. *Luminescence* 14:161-168.
- McCann, J., Stabb, E. V., Millikan, D. S., and E. G. Ruby. 2003. Population dynamics of *Vibrio fischeri* during infection of *Euprymna scolopes*. *Applied Environmental Microbiology* 69:5928-5934.
- McFall-Ngai, M. and J. G. Morin. 1991. Camouflage by disruptive illumination in Leignathids, a family of shallow-water, bioluminescent fishes. *Journal of Experimental Biology* 156:119-137.
- McFall-Ngai, M. J. 2000. Negotiations between animals and bacteria: The "diplomacy" of the squid-*Vibrio* symbiosis. *Comparative Biochemistry and Physiology, Part A, Molecular and Integrative Physiology* 126:471-480.
- McFall-Ngai, M. J. and M. K. Montgomery. 1990. The anatomy and morphology of the adult bacterial light organ of *Euprymna scolopes* Berry (Cephalopoda: Sepiolidae). *Biological Bulletin* 179:332-339.
- Millikan, D. S. and E. G. Ruby. 2002. Alterations in *Vibrio fischeri* motility correlate with a delay in symbiosis initiation and are associated with additional symbiotic colonization defects. *Applied Environmental Microbiology* 68:2519-2528.
- Millikan, D. S. and E. G. Ruby. 2004. *Vibrio fischeri* flagellin A is essential for normal motility and for symbiotic competence during initial squid light organ colonization. *Journal of Bacteriology* 186:4315-4325.
- Montgomery, M. K. and M. J. McFall-Ngai. 1992. The muscle-derived lens of a squid bioluminescent organ is biochemically convergent with the ocular lens. *The Journal of Biological Chemistry* 267:20999-21003.
- Montgomery, M. K. and M. J. McFall-Ngai. 1995. The inductive role of bacterial symbionts in the morphogenesis of a squid light organ. *American Zoologist* 35:372-380.
- Moynihan, M. 1983. Notes on the behavior of *Euprymna scolopes* (Cephalopoda: Sepiolidae). *Behavior* 85:25-41.
- Nishiguchi, M. K. 2002. Host-symbiont recognition in the environmentally transmitted sepiolid squid-*Vibrio* mutualism. *Microbial Ecology* 44:10-18.
- Nishiguchi, M. K., Ruby, E. G., and M. J. McFall-Ngai. 1998. Competitive dominance among strains of luminous bacteria provides an unusual form of evidence for parallel evolution in Sepiolid squid-*Vibrio* symbioses. *Applied Environmental Microbiology* 64:3209-3213.
- Nyholm, S. V., Deplancke, B., Gaskins, H. R., Apicella, M. A., and M. J. McFall-Ngai. 2002. Roles of *Vibrio fischeri* and nonsymbiotic bacteria in the dynamics of mucus secretion during symbiont colonization of the *Euprymna scolopes* light organ. *Applied Environmental Microbiology* 68:5113-5122.
- Nyholm, S. V. and M. J. McFall-Ngai. 2004. The winnowing: Establishing the squid-*Vibrio* symbiosis. *Nature Reviews Microbiology* 2:632-642.
- Nyholm, S. V., Stabb, E. V., Ruby, E. G., and M. J. McFall-Ngai. 2000. Establishment of an animal-bacterial association: Recruiting symbiotic vibrios from the environment. *Proceedings of Natural Academy of Sciences USA* 97:10231-10235.
- Ochman, H. and N. A. Moran. 2001. Genes lost and genes found: Evolution of bacterial pathogenesis and symbiosis. *Science* 292:1096-1099.

- Ruby, E. G. 1999. The *Euprymna scolopes* *Vibrio fischeri* symbiosis: A biomedical model for the study of bacterial colonization of animal tissue. *Journal of Molecular Microbiology and Biotechnology* 1:13–21.
- Ruby, E. G. and L. M. Asato. 1993. Growth and flagellation of *Vibrio fischeri* during initiation of the sepiolid squid light organ symbiosis. *Archives of Microbiology* 159:160–167.
- Ruby, E. G., Greenberg, E. P., and J. W. Hastings. 1980. Planktonic marine luminous bacteria: Species distribution in the water column. *Applied Environmental Microbiology* 39:302–306.
- Ruby, E. G. and M. J. McFall-Ngai. 1991. Symbiont recognition and subsequent morphogenesis as early events in an animal-bacterial mutualism. *Science* 254:1491–1494.
- Ruby, E. G. and M. J. McFall-Ngai. 1999. Oxygen-utilizing reactions and symbiotic colonization of the squid light organ by *Vibrio fischeri*. *Trends in Microbiology* 7:414–420.
- Ruby, E. G. and J. G. Morin. 1979. Luminous enteric bacteria of marine fishes: A study of their distribution, densities, and dispersion. *Applied Environmental Microbiology* 38:406–411.
- Ruby, E. G. and K. H. Nealon. 1976. Symbiotic association of *Photobacterium fischeri* with the marine luminous fish *Monocentris japonica*: A model of symbiosis based on bacterial studies. *Biological Bulletin* 151:574–586.
- Ruby, E. G., Urbanowski, M., Campbell, J., Dunn, A., Faini, M., Gunsalus, R., Lohstroh, P., Lupp, C., McCann, J., Millikan, D., Schaefer, A., Stabb, E., Stevens, A., Visick, K., Whistler, C., and E. P. Greenberg. 2005. Complete genome sequence of *Vibrio fischeri*: A symbiotic bacterium with pathogenic congeners. *Proceedings of Natural Academy of Sciences USA* 102:3004–3009.
- Shears, J. S. 1988. The use of a sand-coat in relation to feeding and diel activity in the sepiolid squid *Euprymna scolopes*. *Malacologia* 29:121–133.
- Singley, C. T. 1983. *Euprymna scolopes*. In *Cephalopod Life Cycles*, ed. P. R. Boyle. London: Academic Press.
- Stabb, E. V. 2005. Shedding light on the bioluminescence “paradox.” *ASM News* 71:223–229.
- Stabb, E. V. 2006. The *Vibrio fischeri*-*Euprymna scolopes* light organ symbiosis. In *The Biology of Vibrios*, eds. F. L. Thompson, B. Austin, and Swings, J., pp. 204–218. Washington: ASM Press.
- Sugita, H. and Y. Ito. 2006. Identification of intestinal bacteria from Japanese flounder (*Paralichthys olivaceus*) and their ability to digest chitin. *Letters Applied Microbiology* 43:336–342.
- Sycuro, L. K., Ruby, E. G., and M. McFall-Ngai. 2006. Confocal microscopy of the light organ crypts in juvenile *Euprymna scolopes* reveals their morphological complexity and dynamic function in symbiosis. *Journal of Morphology* 267:555–568.
- Visick, K. L. 2005. Layers of signaling in a bacterium-host association. *Journal of Bacteriology* 187:3603–3606.
- Visick, K. L., Foster, J., Doino, J., McFall-Ngai, M., and E. G. Ruby. 2000. *Vibrio fischeri* lux genes play an important role in colonization and development of the host light organ. *Journal of Bacteriology* 182:4578–4586.
- Visick, K. L. and M. J. McFall-Ngai. 2000. An exclusive contract: Specificity in the *Vibrio fischeri*-*Euprymna scolopes* partnership. *Journal of Bacteriology* 182:1779–1787.
- Visick, K. L. and E. G. Ruby. 2006. *Vibrio fischeri* and its host: It takes two to tango. *Current Opinion in Microbiology* 9:632–638.
- Warner, J. A., Latz, M. L., and J. F. Case. 1979. Cryptic bioluminescence in a midwater shrimp. *Science* 203:1109–1110.
- Wei, S. L. and R. F. Young. 1989. Development of symbiotic bacterial bioluminescence in a nearshore cephalopod, *Euprymna scolopes*. *Marine Biology* 103:541–546.
- Widder, E. A. 1998. A predatory use of counterillumination by the squaloid shark, *Isistius brasiliensis*. *Environmental Biology of Fishes* 53:267–273.
- Young, R. E. 1977. Ventral bioluminescent countershading in midwater cephalopods. *Symposia of the Zoological Society of London* 38:127–159.
- Young, R. E. 1995. Aspects of the natural history of pelagic cephalopods of the Hawaiian mesopelagic-boundary region. *Pacific Science* 49:143–155.
- Young, R. E. and C. F. Roper. 1976. Bioluminescent countershading in midwater animals: Evidence from living squid. *Science* 191:1046–1048.
- Zhou, J., Clark, C., and J. Huissoon. 2007. SIFT approach used in fish tracking for autonomous underwater vehicle. *Proceedings of the 2007 International Symposium on Unmanned Untethered Submersible Technology (UUST)*. <http://www.ausi.org/events/uust/proceedingsForm.pdf>