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# Effect of the Squid Host on the Abundance and Distribution of Symbiotic *Vibrio fischeri* in Nature†

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***Euprymna scolopes*, a Hawaiian species of bioluminescent squid, harbors *Vibrio fischeri* as its specific light organ symbiont. The population of symbionts grew inside the adult light organ with an average doubling time of about 5 h, which produced an excess of cells that were expelled into the surrounding seawater on a diurnal basis at the beginning of each period of daylight. These symbionts, when expelled into the ambient seawater, maintain or slightly increase their numbers for at least 24 h. Hence, locations inhabited by their hosts periodically receive a daily input of symbiotic *V. fischeri* cells and, as a result, become significantly enriched with these bacteria. As estimated by hybridization with a species-specific *luxA* gene probe, the typical number of *V. fischeri* CFU, both in the water column and in the sediments of *E. scolopes* habitats, was as much as 24 to 30 times that in similar locations where squids were not observed. In addition, the number of symbiotic *V. fischeri* CFU in seawater samples that were collected along a transect through Kaneohe Bay, Hawaii, decreased as a function of the distance from a location inhabited by *E. scolopes*. These findings constitute evidence for the first recognized instance of the abundance and distribution of a marine bacterium being driven primarily by its symbiotic association with an animal host.**

*Vibrio fischeri* is a marine luminous bacterium that is found both within the light-emitting organ of the Hawaiian sepiolid squid *Euprymna scolopes* (3, 26) and as one of several species of luminous bacteria that occur in the bacterioplankton of Kaneohe Bay, Hawaii (12). The symbiotic isolates, as well as most of the planktonic isolates, of Hawaiian *V. fischeri* are distinctive in that they do not produce visibly luminous colonies on laboratory media, although they become brightly luminous after colonizing the light organ of a host squid (3, 6). Thus, in a previous study, colony hybridization with specific gene probes was used to determine that these non-visibly luminous *V. fischeri* occur at a relatively high concentration in seawater collected from Kaneohe Bay (12). This study also put forth the hypothesis that the abundance of these non-visibly luminous *V. fischeri* isolates might be related to the cooccurrence of their host, *E. scolopes*.

A single adult *E. scolopes* squid (i.e., one with a mantle length greater than about 10 mm) contains a population of between $10^7$ and $10^9$ *V. fischeri* cells in its light organ (21). Because luminous bacteria typically occur at concentrations of only a few tens to hundreds of cells per 100 ml of coastal seawater (19, 22, 24), a single squid can contain a number of *V. fischeri* cells equivalent to that found free-living in 10,000 $m^3$ of seawater. The light organ of *E. scolopes* has pores (15, 16) through which it can communicate with the ambient environment, and laboratory experiments have shown that adult squids release a large number of their symbionts into seawater (11, 21). Thus, depending on the frequency of expulsion of symbionts and the extent of their subsequent proliferation, survival, and dispersal, these released symbiotic *V. fischeri* cells could greatly increase the number of luminous bacteria present in natural animal habitats.

In this study, we investigated the hypothesis that the abundance and distribution of at least one class of marine microorganisms, symbiotic *V. fischeri*, are controlled by the dynamics of their relationship with their host. This hypothesis was tested by determining the pattern and degree of symbiont release and by examining the fate of released symbionts in ambient seawater. In addition, the abundance of symbiotic *V. fischeri* was determined both at locations in Kaneohe Bay that either are or are not inhabited by *E. scolopes* and at points on a transect leading away from a known animal habitat. The patterns that we detected point to a direct correlation between the abundance of non-visibly luminous *V. fischeri* in seawater and sediments and the presence and behavior of its animal host.

## MATERIALS AND METHODS

**Symbiont expulsion experiments.** To determine the natural pattern of their symbiont expulsion, adult squids were collected in Kaneohe Bay and placed in aquaria, for 1 day, typically without food to decrease the number of extraneous bacteria expelled into the seawater from their enteric tracts. Each squid was then incubated under natural daylight conditions in 2 liters of Kaneohe Bay seawater that had been sterilized by passage through 0.22-μm-pore-size filters. Every 2 h, the number of *V. fischeri* CFU present in the incubation seawater was determined by spreading an aliquot on a nutrient-rich agar medium (SWT agar) containing 0.5% tryptone, 0.3% yeast extract, and 0.3% glycerol in 70% seawater (17). At the same time, the luminescence of 10-ml aliquots of this incubation seawater was measured with a sensitive photometer (Luminescence Photometer, model 3000; Biospherical Instruments, Inc.).

Almost all of the colonies arising from the squids during incubation in sterile seawater were *V. fischeri*, as determined by the criteria of colony morphology and DNA-DNA hybridization of colony lifts with a *V. fischeri*-specific *luxA* gene probe (12). About 14 h after the incubation began, a few new colony types typically appeared in the water samples; however, even then the *V. fischeri* colonies remained numerically dominant. The number of symbiotic *V. fischeri* CFU present in adult *E.*

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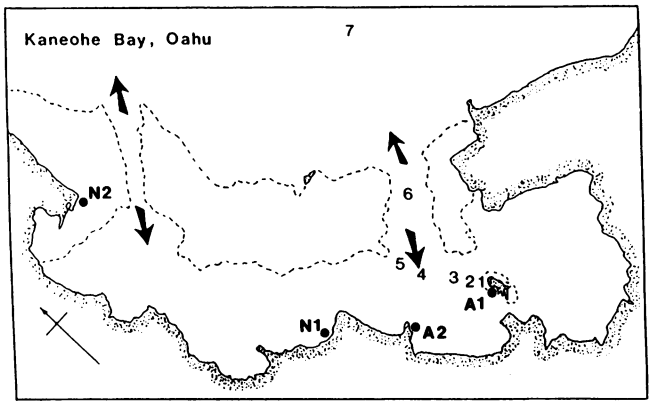


FIG. 1. Map of sampling sites in Kaneohe Bay. Four coastal locations are indicated, including two (A1 and A2) at which *E. scolopes* was often encountered, and two (N1 and N2) where the animals have never been observed. Seven transect locations (between site 1 and site 7) lie within the southeast channel connecting the bay to offshore waters. The arrows represent the main water flows into and out of the bay in each tidal cycle.

*scolopes* light organs was determined from light organ homogenates as previously described (21).

In a few instances, we used animals that had been fed a normal diet of shrimp before the experiment. Only slightly more symbionts were found in the light organs and in the seawater in which such animals were maintained; therefore, the 1-day starvation did not appear to significantly affect the quantity of symbionts expelled.

**Proliferation and survival of symbiotic *V. fischeri* in seawater.** *V. fischeri* ES114, isolated from an adult *E. scolopes* light organ (3), was used to examine the symbiont's potential for proliferation and survival (i.e., maintenance of culturability) during incubation in seawater. Colonies of ES114 freshly grown on SWT agar were resuspended in filter-sterilized seawater to an optical density (at 600 nm) of approximately 0.5. Dilutions of this suspension were inoculated into seawater that had been collected from site 1 in Kaneohe Bay (Fig. 1) and had been passed through a 0.2-μm-pore-size Nuclepore filter under a mild vacuum (less than 250 mm Hg [1 mm Hg = 133.322 Pa]). Changes in the number of CFU of ES114 were monitored by spreading dilutions of the suspensions on SWT agar and incubating them for 24 h at 24 to 26°C.

A rifampin-resistant derivative of strain ES114, designated ESR1 (5), was inoculated into 500 ml of either 0.2-μm-filtered, 5 μm-filtered, or unfiltered seawater collected from either site 1 or the offshore location, site 7 (Fig. 1). These ESR1 cells were practically the only ones to form colonies on SWT agar containing rifampin (100 μg/ml), and thus they were easily counted on plates spread with aliquots of seawater that contained natural assemblages of microorganisms (i.e., unfiltered and 5-μm-filtered seawater).

To obtain symbionts that had been freshly expelled, an adult squid was incubated in 500 ml of filter-sterilized seawater for a 12.5-h period beginning just prior to daybreak. Two milliliters of this seawater was then inoculated into 200 ml of filter-sterilized seawater, and the numbers of *V. fischeri* CFU present were determined later in three independent experiments.

**Sampling and processing of seawater and sediment samples.** Between 1989 and 1992, numerous water samples were collected at depths of between 20 and 30 cm from several locations in Kaneohe Bay (Fig. 1), including (i) nearshore areas inside the bay (A1, A2, N1, and N2) and (ii) transect points in the southeast channel of the bay between sites 1 and 7. Surface (upper 0.5 cm) sediment samples were also collected from the four nearshore locations (A1, A2, N1, and N2) and were mixed with an equal volume of sterile seawater, disrupted for 15 s by isothermal sonication (W-370 Sonicator; Heat System-Ultrasonics, Inc.), and cleared of large particles by low-speed centrifugation for 10 s.

Different volumes of these seawater and sediment samples were filtered through 0.45-μm-pore-size membranes (Millipore Corp., Bedford, Mass.) and incubated on the surface of SWT agar plates. Luminous colonies were enumerated, and individual ones were picked and taxonomically assigned by using the criterion of growth on minimal medium agar plates containing either lactate, maltose, mannitol, gluconate, or cellobiose as the sole carbon source (17). Colonies arising on other filters were also processed for hybridization as described previously (12). A *V. fischeri*-specific *luxA* gene sequence amplified from the genomic DNA of strain ES114 by the PCR was used as the probe (27). Such isolates from Hawaiian seawater have all been shown to be symbiosis-competent *V. fischeri* (12) and to be most closely related to strains directly isolated from *E. scolopes* light organs (11).

## RESULTS

**Expulsion of symbiotic bacteria from the host.** When individual adult *E. scolopes* squids were maintained under conditions of cyclic illumination (12 h of light and 12 h of darkness), a rapid increase in the concentration of symbiotic *V. fischeri* cells in the surrounding seawater was observed at the beginning of each light period (Fig. 2A). The appearance of bacteria occurred at the same time (relative to the light-dark cycle) regardless of when the squid was placed in the incubation chamber, suggesting that the phenomenon was on a diurnal cycle. Because the abundance of the bacteria rose by a factor of $10^4$ (from <5 to >50,000 CFU/ml) in less than 2 h, the increase had to have been due to expulsion of symbionts from the squid light organ rather than due to growth of bacteria already present in the water. This expulsion event was also easily detected as a punctuated, yet transitory, increase in the luminescence of the seawater that occurred once each day, coinciding with the time of symbiont expulsion (Fig. 2B). Because the level of luminescence of expelled *V. fischeri* symbionts has been reported to decrease by a factor of over 100 within 3 h of release from the light organ (3), the luminescence of the incubation seawater was due primarily to recently expelled symbionts and is therefore a convenient indication of their release. Thus, squids appear to actively expel excess symbiotic *V. fischeri* cells in a discrete episode that is coincident with daybreak and lasts for no more than an hour or two.

In four separate experiments, we quantified the total number of symbionts released during an expulsion event, using 13 medium-sized adult squids (i.e., those with mantle lengths of between 8 and 12 mm) collected over a period of 2 years. The average number of *V. fischeri* CFU appearing in the surrounding seawater during each 24-h period (from one morning to the next) was $5.1 \times 10^8$ CFU per squid per day (Table 1). To obtain an estimate of the number of symbionts remaining in the light organ after an expulsion event, some of these squids were dissected, and dilutions of the light organ homogenates were spread on SWT agar plates. The number of symbionts inhabiting the light organ of one of these medium-sized squids averages about $10^7$ CFU (21), which suggests that over 95% of the symbiotic cells were expelled each morning. These values

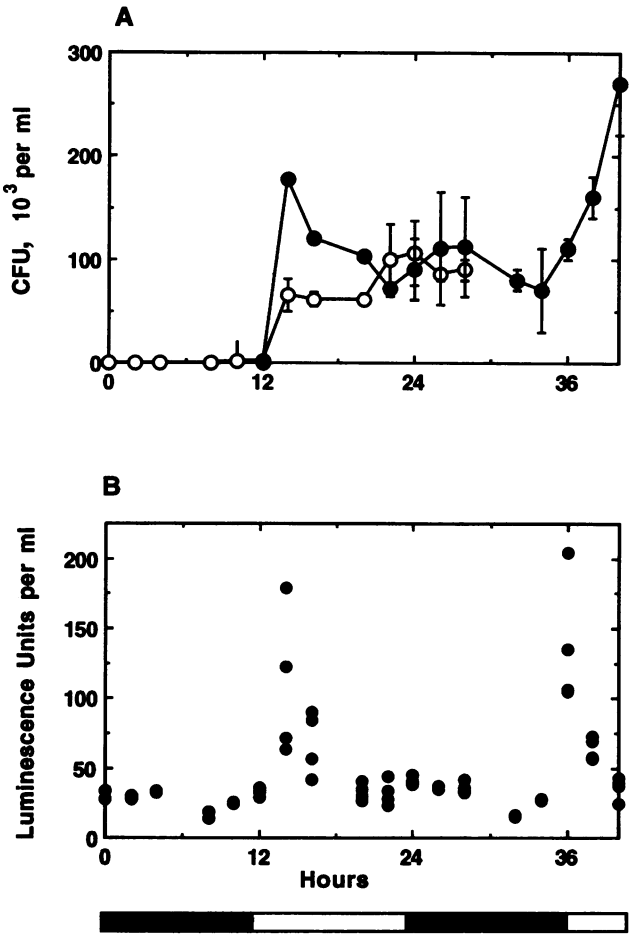


FIG. 2. (A) Changes in the numbers of *V. fischeri* CFU expelled from a squid placed in an incubation chamber at the beginning of the light period (closed circles) and a squid placed in the incubation chamber at the beginning of the dark period (open circles). Error bars indicate standard deviations. (B) Luminescence of the freshly expelled symbiotic bacteria. One luminescence unit is equal to $10^3$ quanta per s, and the photometer background reading (dark current) was between 20 and 50 luminescence units. The horizontal bar at the bottom of the figure indicates the periods of darkness (closed portions) and light (open portions) during the incubation.

could also be used to calculate the average generation time of symbionts within the light organs as described by the equations $\mu = (\ln N - \ln N_0)/(t - t_0)$ and $g = \ln 2/\mu$ (where $\mu$ is the growth rate, $N$ is the number of cells at time $t$, $N_0$ is the number of cells at time $t_0$, and $g$ is the generation time [or doubling time]). Thus, inside the light organs of its hosts, symbiotic *V. fischeri* appears to double its numbers at an average rate of once every 4.8 h (Table 1).

**Survival of expelled symbionts.** When diluted into filter-sterilized seawater collected from Kaneohe Bay, laboratory-grown cells of *V. fischeri* ES114 increased to a maximum density of about $10^5$ CFU/ml (Fig. 3). The number of CFU per milliliter rose rapidly before reaching this value and then stabilized for at least the next 60 h. When the viable cell density was measured 19 days later, 10 to 15% of these ES114 cells were still present as CFU (data not shown). To determine whether *V. fischeri* cells that were freshly expelled from the light organ exhibited the same behavior as laboratory-cultured

TABLE 1. Expulsion of symbiotic *V. fischeri* by adult *E. scolopes* squids[a]

| Expt[b] | No. of squids tested | Expelled symbionts ($10^8$ CFU)/squid[c] | Net doubling time (h)[d] |
|---|---|---|---|
| I | 2 | 1.8 | 6.2 |
| II | 5 | 10.5 | 3.6 |
| III | 2 | 2.2 | 5.2 |
| IV | 4 | 6.0 | 4.1 |
| Mean ± SD | | 5.1 ± 3.5 | 4.8 ± 1.0 |

[a] Adult squids tested ranged between 8 and 12 mm in mantle length.
[b] Each experiment was done with squids collected at different sampling times during 1991 and 1992.
[c] CFU of symbiotic *V. fischeri* in filter-sterilized seawater containing a squid for at least 24 h were measured in the early morning (see Materials and Methods).
[d] Doubling times were calculated on the basis of an average remaining population of $10^7$ CFU per light organ (see Results).

*V. fischeri*, we diluted 2 ml of seawater containing about $10^6$ CFU of newly expelled symbionts per ml into 200 ml of filter-sterilized seawater. Again, the density showed an initial rapid increase to about $10^5$ CFU/ml, and these cells remained culturable for at least 60 h (Fig. 3). This result suggested that naturally expelled symbionts are neither more nor less suited to survive in seawater than laboratory-cultured cells.

Although symbiotic *V. fischeri* cells can proliferate and maintain their numbers in filter-sterilized seawater from nearshore squid habitats, a different response was seen when cells were diluted instead into unsterilized seawater, which contained a natural mixture of indigenous heterotrophic bacteria and predatory protists. In these experiments, strain ESR1, a rifampin-resistant derivative of strain ES114, was used as the *V. fischeri* inoculum because it was easier to select and enumerate CFU of ESR1 when they were present in aliquots of natural seawater that were spread on SWT agar supplemented with rifampin. While, as with strain ES114 (Fig. 3), dramatic growth and proliferation followed dilution of ESR1 cells into seawater that had been filter sterilized by passage through a 0.2-μm-pore-size membrane, a different pattern occurred after dilution of these cells into either unfiltered seawater or 5-μm-filtered seawater (Fig. 4). Although cells in all of the inocula proliferated after dilution, the apparent net doubling times during the first 24 to 36 h were approximately three times longer in both the unfiltered and the 5-μm-filtered seawater than in the 0.2-μm-filtered (filter-sterilized) seawater. In addition, the maximum density of ESR1 reached only about 150 CFU/ml under the first two conditions, compared with a concentration of almost $10^5$ CFU/ml in the filter-sterilized seawater (Fig. 4).

After an incubation of 77 h, there were 57 and 29% decreases from the maximum density of CFU reached in the unfiltered and 5-μm-filtered seawater, respectively, while no such decrease was observed in the filter-sterilized seawater. Interestingly, there was an even more dramatic decrease in CFU of ESR1 diluted into seawater collected from site 7 (Fig. 4), an offshore location (Fig. 1).

**Distribution of *V. fischeri* cells in squid habitats.** The results of the experiments described above suggested that significant expulsion and subsequent proliferation and maintenance of symbionts occur in seawater containing symbiotic squids. Thus, one might predict a high abundance of symbiotic *V. fischeri* in those natural environments like Kaneohe Bay that are inhabited by *E. scolopes*. Therefore, we enumerated *V. fischeri* CFU

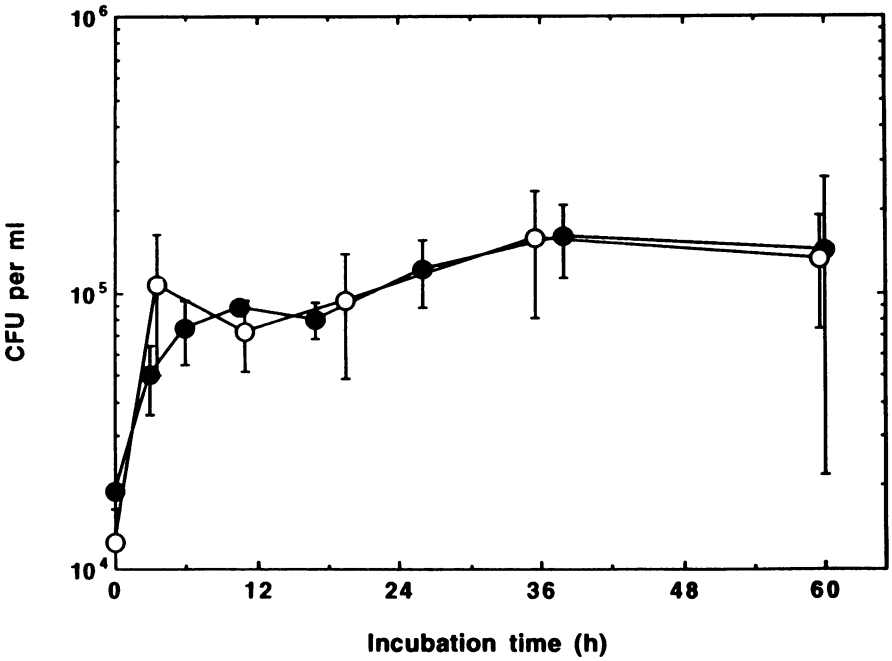


FIG. 3. Changes in concentrations of *V. fischeri* ES114 (closed circles) and of freshly expelled symbionts (open circles) incubated in 0.22-μm-filtered seawater. Error bars indicate standard deviations.

in water and sediment samples collected from four locations: two sites (A1 and A2) at which *E. scolopes* was frequently encountered ("animal habitats") and two others (N1 and N2) at which we have never found *E. scolopes* ("no-animal habitats") during our 3-year study. All of the sites were located about 10 m from shore in water about 1 m deep overlying mixed coral sand and mud sediments.

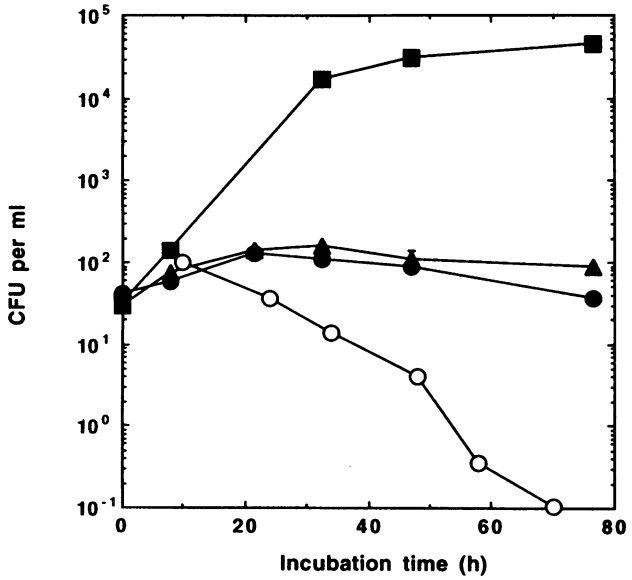


FIG. 4. Changes in concentrations of *V. fischeri* ESR1 incubated in 0.2-μm-filtered (squares), 5-μm-filtered (triangles), and unfiltered (closed circles) seawater collected from an animal habitat, site 1, and changes in CFU of *V. fischeri* ESR1 incubated in natural seawater collected from outside Kaneohe Bay at site 7 (open circles). The locations of these sites are indicated in Fig. 1. Error bars (when larger than the symbol) indicate standard deviations.

Both seawater and sediments from animal habitats were enriched with non-visibly luminous *V. fischeri* (Table 2). Specifically, *V. fischeri* was present at average densities of 2.1 and 0.46 CFU/ml of water and 148 and 31 CFU/ml of sediment at locations A1 and A2, respectively, while only 0.07 and <0.33 *V. fischeri* CFU/ml of water and <14 and <6 CFU/ml of sediment were estimated to be present at locations N1 and N2, respectively. Thus, in both water samples and sediment samples, the average abundance of *V. fischeri* in animal habitats was greater than in no-animal habitats. In contrast, the patterns of abundance of other luminous bacteria (identified as the closely related species *Vibrio harveyi* and *Photobacterium leiognathi*) showed no such differences between the two pairs of sites

TABLE 2. Abundance and distribution of luminous bacteria in samples taken at different locations in Kaneohe Bay[a]

| Location | Mean CFU/ml of sample ± SE (*n*) | | | |
|---|---|---|---|---|
| | Water column | | Sediment | |
| | Luminous[b] | Non-visibly luminous[c] | Luminous | Non-visibly luminous |
| A1 | 0.33 ± 0.04 (21) | 2.1 ± 0.62 (15) | 15 ± 2.5 (4) | 148 ± 73.5 (4) |
| A2 | 0.34 ± 0.10 (6) | 0.46 ± 0.10 (2) | 52 ± 19.6 (3) | 31 ± 19.8 (2) |
| N1 | 0.23 ± 0.02 (3) | 0.07 ± 0.02 (3) | 30 ± 14.1 (2) | <14 (3) |
| N2 | 1.00 (1) | <0.33 (2) | 15 ± 3.5 (2) | <6 (2) |

[a] While large populations of squids were found to inhabit locations A1 and A2, no squids were ever seen at locations N1 or N2 between 1990 and 1992. Samples of seawater and sediment were collected between 1989 and 1992 and processed as described in Materials and Methods.

[b] Luminous colonies were identified as arising primarily from cells of *V. harveyi* and *P. leiognathi*.

[c] Several values were below the detection limit of the plating. Data refer to non-visibly luminous *V. fischeri*.

TABLE 3. Effect of proximity to an *E. scolopes* habitat on the abundance of luminous bacteria in seawater

| Site | Distance (km)[a] | Mean CFU/100 ml of seawater sample ± SE (*n*) | |
|---|---|---|---|
| | | NVL *V. fischeri*[b] | Visibly luminous[c] |
| 1 | 0 | 177 ± 48.2 (21) | 27 ± 4.2 (20) |
| 2 | 0.09 | 40 ± 10.6 (7) | 43 ± 18.5 (7) |
| 3 | 0.14 | 22 ± 6.3 (5) | 39 ± 9.8 (7) |
| 4 | 0.86 | 9.1 ± 4.6 (3) | 56 ± 15.5 (4) |
| 5 | 0.98 | 8.0 ± 2.0 (4) | 43 ± 9.0 (9) |
| 6 | 1.60 | 5.6 ± 2.1 (2) | 67 ± 2.8 (2) |
| 7 | 3.11 | 3.2 ± 2.3 (3) | 10 ± 3.1 (5) |

[a] Distance from site 1, a squid-containing location, on the path indicated in Fig. 1.

[b] Symbiotic-type, non-visibly luminous (NVL) *V. fischeri*. Values that were below the limit of detection were not included in the calculations.

[c] Luminous CFU were identified as arising from cells of *V. harveyi* and *P. leiognathi*.

(Table 2). Thus, there was no evidence that whatever factors led to an increased abundance of *V. fischeri* in animal habitats were reflected in a higher concentration of nonsymbiotic luminous bacteria as well.

Symbiotic bacteria expelled into animal habitats are subject to subsequent dispersion to other locations by water movement. Seawater enters and leaves Kaneohe Bay tidally through two major channels (Fig. 1) located at the northeast and southeast basins of the bay (2). We examined whether there was a gradient of *V. fischeri* CFU extending from a well-characterized animal habitat (site 1) along a transect passing through the southeast channel to a location outside the bay (site 7). The average abundance of nonsymbiotic luminous bacteria in seawater samples varied less than sevenfold among the seven sites examined, with the lowest concentration (10 CFU/100 ml) occurring offshore at site 7 (Table 3). In contrast, the average density of *V. fischeri* CFU decreased exponentially as a function of the distance from site 1 to a value that was over 50-fold lower at site 7. The fact that the average density of *V. fischeri* CFU within the southeast channel (site 6) was three times higher in an outgoing tide (8.4 CFU/100 ml) than in an incoming tide (2.8 CFU/100 ml) further suggested that the primary source of these *V. fischeri* CFU was inside the bay.

## DISCUSSION

A fundamental, yet often poorly understood, factor in the evolution of microbial associations is the extent to which the host controls the abundance and population genetics of its symbionts. For example, ecological studies of bacterial root nodule symbionts (e.g., *Rhizobium* spp.) occurring free in soil have suggested that several factors affect their abundance and distribution. Among these are temperature, acidity, water potential, and the nature of other indigenous microorganisms (1). However, a major factor that determines long-term patterns of *Rhizobium* distribution is the cooccurrence of their specific legume hosts (28); i.e., the introduction of the appropriate host species eventually results in an elevated abundance of the corresponding *Rhizobium* species in the surrounding soil, especially within the host's rhizosphere, presumably due to release of viable cells from the root nodules (9) and/or stimulation of growth of bacteria in the rhizosphere.

There have been fewer studies of the effects that hosts exert on the ecology of their bacterial symbionts in aquatic environments. While some suggestions of a link between the abundance of the host and that of the symbiont have been made for other associations (4), the most extensive studies have focused on luminous bacteria and their light-organ-bearing hosts. Ruby et al. (22) documented a depth-related distribution of *Photobacterium phosphoreum*, the specific symbiont in the light organs of bathypelagic fishes, and suggested that the symbiotic associations between this bacterium and mid-water luminous fishes contribute to the specific abundance of *P. phosphoreum* at mid-water depths. However, evidence for this hypothesis remains absent because no experimental approach was possible with deep-sea host species. A subsequent study has observed a continuous release of symbiotic luminous bacteria from the light organs of shallow-water species of monocentrid and anomalopid fishes maintained in a laboratory (7). The number of symbionts released into the water ($10^6$ to $10^8$ cells per h per animal) led Nealson et al. (18) to suggest that symbiotically bioluminescent fishes could be a major source of planktonic luminous bacteria in seawater. Unfortunately, the irregular pattern of this release allowed the authors to estimate only that in the light organ the symbiont population doubled in number somewhere between once every 7 h and once every 135 h.

Adult *E. scolopes* squids also release their luminous symbionts, but in a much more predictable pattern. The single expulsion event that occurred each dawn (Fig. 2) suggests a dynamic and highly regulated modulation of the level of bacterial colonization in the host light organ. Because it apparently uses bioluminescence only at night (23), *E. scolopes* maintains its bacterial symbionts when they are needed and, as daylight approaches, expels a large portion of its population (perhaps to reduce the nutritional demands of the symbionts), probably by contracting muscle fibers of the lens overlying the light organ (8). During the day, the bacterial population of the adult host is restored by subsequent growth with a mean doubling time of about 5 h. Interestingly, this doubling time is three to four times longer than the apparent growth rate of the luminous bacterial population within the light organs of juvenile *E. scolopes* squids (21), suggesting that there is a significant difference in the ways in which the host controls symbiont colonization at different stages in its development.

Because a single adult *E. scolopes* squid expels about $5 \times 10^8$ bacterial cells into the ambient environment each day (Table 1), the habitats of this squid species must receive a significant input of cells of symbiotic *V. fischeri*. Once expelled, these cells can subsequently proliferate over a period of hours. When placed in either natural (unfiltered) or 5-μm-filtered seawater, *V. fischeri* cells increased in number with a net doubling time of between 10 and 13 h. This is similar to the 9- to 13-h doubling time that was previously estimated for the total bacterial population in Kaneohe Bay seawater by a variety of methods (10). In contrast, the net amount of *V. fischeri* symbiont accumulation was much greater, and the rate of accumulation was much higher, in filter-sterilized seawater, where during the initial 30 h the cell density increased to about $5 \times 10^4$ CFU/ml, with a net doubling time of about 4 h (Fig. 4).

Symbiotic *V. fischeri* cells also maintain their numbers in Kaneohe Bay seawater after this initial period of proliferation. Continued incubation in natural and 5-μm-filtered seawater resulted in only a gradual decrease in CFU density, while in filter-sterilized seawater the density of CFU of *V. fischeri* continued to increase. These cells sustained their maximal number ($10^5$ CFU/ml) for as long as 70 h and remained detectable even after 19 days. The different kinetics of growth and persistence of *V. fischeri* CFU in the three Kaneohe Bay seawater experiments (Fig. 4) might be attributed to differences both in competition for nutrients with other heterotro-

phic bacteria and in the degree of predation by small flagellates (25), which were present in the natural seawater and were not removed by the 5-μm-pore-size filter.

It is not known why, in general, sediment samples from the animal habitats contained about 100 times more *V. fischeri* CFU per milliliter than the overlying seawater samples. It is possible that this enrichment resulted from the behavior of *E. scolopes*: these squids are typically buried in the sandy sediment in the early morning, when they expel their symbionts (Fig. 2). Alternatively, it may reflect the tendency of some *Vibrio* spp. to attach to particles (14). It should also be noted that all of the bacterial dynamics described here reflect only the presence of cells that are capable of forming colonies on the isolation medium used. The actual abundance of *V. fischeri* cells in seawater might well be different from the number of CFU per milliliter if they entered a physiological state different from that in the laboratory culture and did not produce colonies on nutrient-rich media. The possibility of this so-called "viable but nonculturable" state (20) of *V. fischeri* in natural seawater is being studied. The preliminary results have suggested that many more nonculturable symbionts were present at site 1 than at site 7 (13).

If the expulsion and subsequent proliferation of expelled symbionts are a significant source of *V. fischeri* cells, one would predict that (i) locations inhabited by *E. scolopes* would have higher *V. fischeri* densities than locations with no squids and (ii) water samples taken further away from a squid habitat would have fewer *V. fischeri* cells (12). Both of these conditions were observed in a survey of several sites in Kaneohe Bay (Fig. 1). While relatively high concentrations of *V. fischeri* were found in water column and sediment samples collected from animal habitats, the concentrations of other, nonsymbiotic luminous bacteria did not differ as much between animal habitats and non-animal habitats (Table 2). The two nonsymbiotic luminous species are typical heterotrophic bacteria whose abundances might be simply functions of chemical and physical environmental conditions (e.g., nutrient concentration or temperature) that have been shown to affect luminous bacterial distribution (19, 22, 24).

In summary, the data support the hypothesis previously put forth (12) that the relatively high abundance of non-visibly luminous *V. fischeri* in locations inhabited by *E. scolopes* is due to a periodic and numerically significant expulsion of symbionts by host animals followed by the proliferation and survival of these bacteria in the ambient seawater and sediments. Thus, the abundance and distribution of a common and widespread species of marine bacteria apparently are, at least in some locations, largely functions of the presence of their symbiotic host.

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