

Motility patterns of *Beggiatoa* and *Phormidium corallyticum* in black band disease

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Abstract Black band disease of corals is composed of a microbial consortium that generates and maintains an active sulfur cycle. The consortium includes the cyanobacterium *Phormidium corallyticum*, the sulfide-oxidizing bacterium *Beggiatoa* spp., sulfate-reducing bacteria, and other microbes. Together the metabolic processes of these bacteria produce vertical gradients of oxygen and sulfide, within the 1 mm thick band, to form a migrating oxygen/sulfide interface. *Beggiatoa* are typically located at oxygen/sulfide interface environments as a result of negative photo-, sulfido-, and aerotaxes. However, *Beggiatoa* are commonly found on the surface of black band in an environment that is aerobic and highly illuminated. Vertical migrations of black band *Beggiatoa* and *Phormidium* were investigated *in situ* under varying light intensities. *Phormidium* dominated the surface of the band only under low light levels, whereas *Beggiatoa* spp. was present on the surface along with *Phormidium* during high light. This unusual pattern differs from other *Beggiatoa*/cyanobacterial mat systems.

Key words Coral disease, Black band disease, Microbial migrations, *Phormidium corallyticum*, *Beggiatoa*

Introduction

Black band disease is comprised of a complex and distinct microbial consortium (Antonius 1981; Garrett and Ducklow, 1975; Richardson et al. 1997) consisting of the cyanobacterium *Phormidium corallyticum* (Rützler et al. 1983; Taylor 1983), the sulfide-oxidizing bacterium *Beggiatoa* spp. together with sulfate-reducing bacteria (Garrett and Ducklow 1975), marine fungi (Ramos-Flores 1983), and other microbes (Ducklow and Mitchell 1979). The dominant microorganism is the cyanobacterium, which

lends a dark reddish/brown color to the band community due to the cyanobacterial light harvesting pigment phycoerythrin. Black band disease is characterized by both horizontal migration of the entire band community across corals, killing coral tissue, and vertical migrations of the microorganisms within the band (Richardson 1996).

The black band microbial community generates and maintains an active sulfur cycle analogous to those found in other microbial mat communities (Carlton and Richardson 1995). Oxygen produced by photosynthesis of *Phormidium corallyticum* occurs simultaneously with sulfide production by sulfate reducers. Together these form an interface of oxygen and sulfide with steep concentration gradients of each. It is precisely at such an oxygen/sulfide interface that populations of *Beggiatoa* are typically found in nature (Jørgensen 1982). In microbial mat communities, *Beggiatoa* spp. normally migrate vertically following the oxygen/sulfide interface in a pattern in which *Beggiatoa* are present at depth within mats during daylight hours (when oxygenic photosynthesis drives the oxygen/sulfide interface downwards) and move to the mat surface during darkness as sulfide concentrations accumulate (Jørgensen 1982; Garcia-Pichel et al. 1994).

There have been a number of studies of environmental cues and factors controlling the vertical migration patterns of natural populations of *Beggiatoa*. In microbial mats in Hunter's Hot Springs, Oregon, *Beggiatoa* moved downwards into the mat during the day and returned to the surface at night. Isolates from these mats revealed a negative photo-response to full spectrum light, an aversion to blue light, and growth inhibition by full sunlight (Nelson and Castenholz 1982). Negative chemotaxis to oxygen was shown in culture by *Beggiatoa* filaments isolated from Kala

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Lagoon, Denmark (Møller et al. 1985). These combined responses may help *Beggiatoa* locate their optimum chemical environment at the oxygen/sulfide interface (Nelson 1992).

Along with the vertical migrations of *Beggiatoa*, gliding filamentous cyanobacteria perform photo- and chemotactic dependent motility patterns. Microbial mats are often dominated by oscillatorian cyanobacteria (a group that includes the genus *Phormidium*) that carry out diel migration patterns. Many species of cyanobacteria move downward into naturally occurring microbial mats in mid-morning (a negative photic response to high light) and return to the surface at dusk where they remain throughout the night (Castenholz 1968; Richardson and Castenholz 1987; Castenholz and Jørgensen 1991; Garcia-Pichel and Castenholz 1994; Bebout and Garcia-Pichel 1995; Kruschel and Castenholz 1998).

In black band disease, there appears to be a unique microbial migration pattern of *Beggiatoa* and *Phormidium coralliticum*. *Beggiatoa* is found on the surface of the mat during high light intensities but not during lower light levels. The *Phormidium* population is thus present below *Beggiatoa* during daylight hours, a very unusual pattern in that, in other microbial mat systems, both *Beggiatoa* and migrating cyanobacteria move downward into the mat and neither is exposed to the surface environment of high light. Here, we document the unusual vertical migrations within the black band disease community in the natural environment under both naturally occurring and manipulated variations in light intensity.

Methods

Field Studies

Vertical motility patterns associated with *Phormidium coralliticum* and *Beggiatoa* spp. of 20 different black band disease infections were examined at four reefs in the Florida Keys National Marine Sanctuary (Algae, Horseshoe, Little Grecian and Looe Key) and one reef (Picture Reef) in Biscayne National Park. Depths ranged from <1 (Little Grecian) to 10 m. Black band on six species of corals were studied. These were *Montastraea cavernosa*, *M. annularis* (species complex), *Colpophyllia natans*, *Diploria strigosa*, *D. clivosa*, and *Meandrina meandrites*. All observations were conducted underwater using SCUBA.

Light Measurement

Light levels (photosynthetically active radiation from 400-700nm) were measured *in situ* using a diver operated Biospherical Instruments, Inc. (San Diego, California) QSI-140 quantum averaging meter and recorded. The instrument is periodically calibrated at Biospherical Instruments, Inc. Natural (ambient) light levels were measured in conjunction with observations of the surface bacterial populations (n=17). Light levels were also experimentally manipulated (and measured) on the reef using neutral density screen to cover the band for up to 2 hours (n=3). Four layers of screen were held in place approximately 1 cm above the coral (and band) surface using nails that were inserted into dead coral skeleton adjacent to the band. This resulted in a reduction in light level of approximately 60%.

Photodocumentation

The surface of each black band was examined for the relative presence of white clumps of *Beggiatoa* spp. (the white is due to sulfur granules) and the dark (highly pigmented) populations of *Phormidium*. The bands were photographed *in situ* using a Sea and Sea Motormarine II equipped with a 2:1 macro lens.

Statistics

Light intensity versus relative position of the two populations was analyzed using a student's t-test (equal variances not assumed) and a non-parametric Mann-Whitney U test.

Results

Band migrations with manipulated light intensity

There was a distinctive pattern present in the vertical position and migration of *Phormidium* and *Beggiatoa* with varying light intensity (Table 1). At relatively high (for the underwater reef environment) light levels of 191-308 $\mu\text{E}/\text{m}^2\cdot\text{sec}$, both *Beggiatoa* spp. and *Phormidium* were present on the surface of the band (n=15). At lower light levels (68-234 $\mu\text{E}/\text{m}^2\cdot\text{sec}$), *Phormidium* was present on the surface of the band and there was no observable *Beggiatoa* (n=5). There was a slight overlap of light intensities where members of both populations were visible or where only *Phormidium* was present (Table 1). Results of the two statistical tests that compared relative position vs. light intensity were significant in both the student's t-test ($t = 3.053$, $df = 4.628$, $p < 0.05$) and the Mann-Whitney U test ($z = -2.837$, $p = 0.005$).

Vertical migrations could easily be controlled by manipulating light intensity with neutral density filters. Figure 1 shows the surface of an *in situ*

Table 1 *In situ* light intensity and black band bacterial population visible on the band surface. Light data and corresponding observations of black band are presented with light intensity listed from high (top) to low (bottom). Data are included from the 20 bands investigated.

Light level ($\mu\text{E}/\text{m}^2\cdot\text{sec}$)	Surface population	
	<i>Beggiatoa</i>	<i>Phormidium</i>
310	Both visible	
301	Both visible	
291	Both visible	
285	Both visible	
279	Both visible	
277	Both visible	
272	Both visible	
271	Both visible	
251	Both visible	
250	Both visible	
238	Both visible	
234	Not visible	Visible
232	Both visible	
231	Both visible	
231	Both visible	
224	Not visible	Visible
191	Both visible	
155	Not visible	Visible
138	Not visible	Visible
68	Not visible	Visible

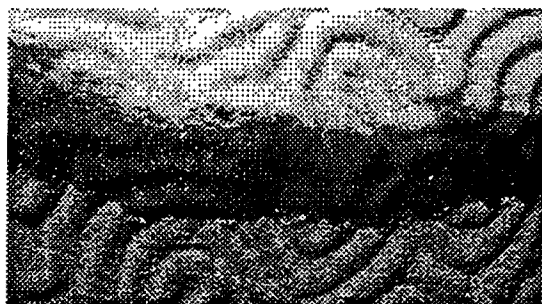


Fig 1 Vertical migration of *Phormidium coralyticum* and *Beggiatoa* in black band disease during experimental manipulation of *in situ* light intensity.

black band immediately after experimentally deployed neutral density screens were removed. The (control) left side of the band was not shaded during the experiment. It can be seen that white clumps of *Beggiatoa* are visible on the band surface. In contrast, the right side of the band (experimentally shaded) exhibits only the presence of *Phormidium coralyticum*, revealed by a band surface that was noticeably darker. The photograph was taken after the filters had been in place for approximately one hour. In all experiments of this type, black band populations responded consistently with only *Phormidium* present on the surface under low light and *Beggiatoa* and *Phormidium* on the surface under high light.

Band migrations with naturally varying light intensity

Migrations of the two black band microorganisms occurred in response to naturally occurring variations in light as well as experimentally manipulated ones. For example, an early morning measurement was made in which one area of a band was blocked from direct sunlight as a result of shading by another part of the same coral colony. The light intensity illuminating the band surface under the shaded condition was $68 \mu\text{E}/\text{m}^2\cdot\text{sec}$. Within the shadow, *Phormidium* covered the surface of the band, while an unshaded section of the band directly adjacent exhibited white *Beggiatoa* clumps on the surface along with the *Phormidium*. Light intensity at this point was $285 \mu\text{E}/\text{m}^2\cdot\text{sec}$. When the angle of the sun changed to fully illuminate the previously shaded section, white clumps of *Beggiatoa* began to appear immediately (within seconds).

Combined measurements of initial ambient light levels followed by experimental manipulations of light resulted in microbial migrations that were always consistent (Table 2).

Table 2 Manipulation of *in situ* light levels and observations of the resulting vertical migration of *Phormidium coralyticum* and *Beggiatoa*. High light levels consisted of ambient light, low light levels were obtained using screens.

<i>In situ</i> light manipulation	Observed vertical migrations
High to low light intensity	
From 231 to $155 \mu\text{E}/\text{m}^2\cdot\text{sec}$	<i>Beggiatoa</i> migrated down (viewed after 1 hr interval)
From 238 to $138 \mu\text{E}/\text{m}^2\cdot\text{sec}$	<i>Beggiatoa</i> migrated down (viewed after 2 hr interval)
Low to high intensity	
From 67 to $285 \mu\text{E}/\text{m}^2\cdot\text{sec}$	<i>Beggiatoa</i> migrated up (within seconds)

Discussion

The natural light variation in the coral reef environment can be dramatic due to the clarity and shallow depth of the water. As discussed previously, such variations in light are important migration cues for microorganisms. In particular, black band disease is exposed to wide ranges of light intensity, as this disease commonly occurs at very shallow depths. *In situ* photosynthetic photon flux densities (photosynthetically active radiation) near the surface of black band disease have been measured to range from <100 (cloudy days, early and late hours, etc.) to >2200 $\mu\text{E m}^{-2} \text{s}^{-1}$ on the brightest days on shallow reefs (Carlton and Richardson 1995). *Beggiatoa* have been observed to be present on the band surface under the highest light conditions (Carlton and Richardson, unpublished observation), a highly unusual pattern that inspired this research.

As in other microbial mat systems, the microbial vertical migration patterns found in black band disease appear to be controlled by light intensity. *Phormidium corallyticum* dominates the surface microbial community only when light intensity is very low. As light intensity increases, either *P. corallyticum* moves down into the band, or *Beggiatoa* spp. moves up to the surface. The transition occurs when light intensities are in the range of 190–234 $\mu\text{E/m}^2\text{-sec}$. The vertical movement of *Phormidium* into the band as light intensity increases could be a combination of step-up photophobotaxis, in which a gliding microorganism reverses direction upon experiencing a sudden increase in light intensity, and negative phototaxis, movement away from a light source (Castenholz 1982). In this manner, individual filaments would move down as far as possible into the band to avoid the surface high light. As filaments move down, however, others become exposed to the high light, which in turn results in movement of these filaments into the band. Such behavior is termed “self-shading” and is well-documented for many naturally occurring populations of cyanobacteria (Castenholz 1982). In black band disease, while some *Phormidium* can remain (along with *Beggiatoa*) on the band surface under high light conditions, this may simply be due to the fact that there is nowhere for the cyanobacteria to migrate except for under the less dominant (in terms of biomass) *Beggiatoa*. Black band microbial communities are typically <1 mm thick, with hard calcium carbonate coral

skeleton present immediately below the band. While it is known that populations of *Desulfovibrio* spp. are present at the bottom of the band community, such populations have only been observed (using confocal laser microscopy) in co-occurrence with filaments of *Phormidium* (Schnell et al., manuscript in preparation). Thus there is no thick microbial layer for both black band *Phormidium* and *Beggiatoa* to “disappear” into as in the much thicker microbial mat communities studied elsewhere.

In situ observations of the position of *Phormidium corallyticum* in relation to light intensity are consistent with laboratory studies on the photosynthetic capabilities of this species. Kuta (2000) demonstrated that *P. corallyticum* has an extremely low threshold (approximately 29 $\mu\text{E/m}^2\text{-sec}$) for attaining maximum photosynthesis (P_{max}) in culture. This finding, coupled with the motility pattern of black band *Phormidium*, is consistent with other studies demonstrating that certain gliding filamentous cyanobacteria migrate down into microbial mats to attain a light level optimum for photosynthesis while avoiding photosynthesis-saturating intensities (Garcia-Pichel et al. 1994).

Whereas the *Phormidium* migration may be explained in terms of what is known about the physiology of this species and cyanobacterial motility responses in general, the underlying reasons for the migration of black band *Beggiatoa* are more elusive. *Beggiatoa* is often present on the surface of black band in an environment that is aerobic as well as highly illuminated, a microenvironment that is unusual for this genus. As mentioned previously, both negative photo- and aerotaxis are well documented for sediment-associated *Beggiatoa*. In addition to these responses, a co-occurring negative taxis to high sulfide concentrations has been proposed as a mechanism to help *Beggiatoa* position itself at the oxygen/sulfide interface in some mat systems (Jørgensen 1982; Møller et al. 1985). In the case of black band, however, it appears that a potential negative sulfidotaxis is only operative at night or under low light conditions. Such a response would aid the population in moving to the band surface at night (where O_2 is present) as sulfide accumulates, and to the oxygen/sulfide interface within the band under low light conditions during the day. Such an interpretation agrees with the results of studies on other migrating *Beggiatoa*

populations. During high light conditions, however, the situation is markedly different. *In situ* profiles of O₂ in black band when *Beggiatoa* is present on the surface have revealed that the band is oxygenated well below the *Beggiatoa* population, and that *Beggiatoa* is no longer at the O₂/sulfide interface (Carlton and Richardson, manuscript in preparation). At this time *Beggiatoa* could not be performing sulfide oxidation since no sulfide is present, and could not be exhibiting a negative tactic response to a substance that is not there. Microelectrode measurements have shown the band to be oxygenated to depths of 600 µm, with a subsurface O₂ maxima, when *Beggiatoa* is on the band surface. At these times, oxygenic photosynthesis is occurring within the band in accordance with the low P_{max} of *Phormidium corallyticum* (Carlton and Richardson, manuscript in preparation). The vertical profile of oxygen is very different when *Beggiatoa* is not on the band surface – in these cases there is a much steeper gradient of O₂, probably the result of sulfide-oxidation (which consumes O₂) at the interface by *Beggiatoa* when it is in the mat.

In summary, it appears that 1) the motility responses of *Phormidium* are much stronger than the "normal" negative aero- and phototaxes exhibited by *Beggiatoa* populations in other environments, and, as a result, 2) black band *Beggiatoa* must be performing a metabolic mode other than sulfide-oxidation under high light. An understanding of the *Beggiatoa* migration will require further study into the metabolic properties and tolerances of black band *Beggiatoa*. Additional studies will require experimental manipulations designed to separate the movements and cues of one population from the movement and cues of the other, using individual cultures of each. When we understand the motility cues and physiological capabilities of these microorganisms, only then can we understand how the black band microbial consortium can sustain such a highly structured microbial community and how this structure contributes to the etiology of this disease.

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