Ecology of Algal Mats from Hypersaline Ponds in the British Virgin Islands

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Benthic sediment samples ranging from poorly aggregated sand to complex, stratified mats were collected from six hypersaline ponds from March and July 1995 in the British Virgin Islands. Assemblages were analyzed with respect to species composition and abundance within visibly distinct layers in each mat sample. In individual ponds there was no apparent association between changing depth and the development of the benthic mats. Some species were present in all samples (e.g. Oscillatoria sp.) while others were restricted to single sites (e.g. Johannesbaptistia pellucida). Primary species included Microcoleus chthonoplastes, Phormidium spp., Coccolithus stagnina, and purple sulfur bacteria. Quantitative analysis of community structure included cluster and principal component analysis. Samples from individual ponds were often clustered; however, this was subject to seasonal variation. Mats collected in March were generally thicker and contained more layers than those in July. Variation among sites was not explained by the measured variation in environmental factors such as average pond salinity, depth, and oxygen concentration (mg/L). This study provides a detailed analysis of mat communities in hypersaline ponds and compares them with similar mat communities from other areas.

Key Words: algal mats, British Virgin Islands, Caribbean Sea, Cyanobacteria, hypersaline ponds

INTRODUCTION

Microbial mats consist of layers of microorganisms. Each layer has a unique species composition due to sediment chemistry and the resulting gradients of oxygen, light and nutrients found in the mats (Bauld 1984; Cohen and Rosenberg 1989; Des Marais et al. 1992). They are of special importance because they are among the few communities where prokaryotes form conspicuous assemblages and often occur in extreme environments (Paerl et al. 2000). Microbial mats occur in a variety of environments, including intertidal zones (Potts and Whitton 1980; Stal et al. 1985), hot springs (Ward et al. 1987), sulfur springs (Oren 1987), deep-sea vents (Belkin and Jannasch 1987), and hypersaline ponds (D’Amelio et al. 1987; Stal 1994). Where light is available these microbial mats are commonly dominated by cyanobacteria (Sage and Sullivan 1978; Potts and Whitton 1980; Shilo 1989). Here we provide a description of the flora and community ecology of microbial mats from hypersaline ponds in the British Virgin Islands.

Previous accounts of cyanobacterial mats from the Caribbean emphasized mat physiology and biochemistry in the Bahamas (e.g., Paerl et al. 1993; Pinckney and Paerl 1997). Our study provides an extensive floristic account of this community type, and we present a quantitative study of the biota in a series of hypersaline ponds (i.e., with salinity > 50 psu; Hammer 1986) from the British Virgin Islands. Hydrology and salinity in BVI ponds is highly variable (Jarecki and Walkey 2006), and these ponds include habitats with and without strongly developed mats (Jarecki 2004).

Six BVI salt ponds were sampled during spring and summer of 1995 and, in two of the sites, along a depth gradient. We examined the following hypotheses: 1) that biodiversity and species composition are related to environmental variables, 2) that water depth will influence the community structure of the mats, and 3) that mat community structure will vary seasonally.

MATERIALS AND METHODS

The benthic community was sampled on March 19th and 26th, 1995 and again between July 26th to July 31st, 1995, from six hypersaline ponds: Banana Wharf Pond, Banana
Beef Island (29 samples, 5 from July), Belmont Pond, southwest Tortola (5 samples; all in March), Bones Bight Pond, Anegada Island (17 samples; 5 from July), Point Peter Pond, Anegada Island (6 samples, 5 from July), Guana Island Pond, Guana Island (5 samples, all in March), and Witches Brew Pond, east end of Tortola (10 samples, 5 from March) (Fig. 1). Hereafter sites will be referred to by name without appending ‘pond’. Of the five samples from Guana Island, organisms were only found in two. Consequently, the maximum abundance for all five samples was entered into the data matrix as one number. This same method was used for the five samples from Belmont and the five samples collected in July from Witches Brew. At Banana Wharf and Bones Bight, samples were taken along a depth gradient. These samples were 1 to 6 m from the shore, covering a depth range from 4 to 18 cm at Banana Wharf and 10 to 26 cm at Bones Bight.

The principal chemical and physical differences between ponds are shown in Table 1. Chemical characteristics of pond water were measured in situ on a monthly basis from January to December 1995 and are given as mean values (Jarecki 2004). Nitrate concentrations were measured with a Milton Roy Spectronic Mini-20 field spectrophotometer after chemical reaction using Nitraver 5 Nitrate Reagent (Hach Co., Loveland, Colorado). Dissolved oxygen was measured by titration using an azide modification of the Winkler method (reagents from Hach Co.). Water samples for O2 testing were collected in a turbulence-free sampler designed to hold a 60 mL BOD bottle (Hach Co.). Time of day was not standardized in these measurements.

A sample consisted of a 10 x 10 cm mat, 1-5 cm thick. Each was wrapped in newspaper and placed in a plastic bag for shipment to Nova Scotia by courier (24-48 h). On arrival in Nova Scotia mats were divided into three: 1) 1/3 was preserved in 5% formalin in seawater, 2) 1/3 was refrigerated at 5°C for subsequent microscopic analysis, and 3) 1/3 was frozen at -70°C for subsequent pigment analysis (discarded after a freezer malfunction). The formalin-preserved samples were used as a control to ensure that major population changes had not occurred in the refrigerated samples prior to microscopic analysis.

Using the refrigerated material, a small sample from each of the visibly distinct layers was extracted using a razor blade and mounted in 30% clear corn syrup in distilled water, and ringed with nail polish. Equivalent samples from each layer of each mat were taken to provide similar smears on the slide suitable for observation. Slides were examined at 1000x magnification. The cyanobacteria were identified using Desikachary (1959), Bird and McLachlan (1977) and Humm and Wicks (1980). Non-cyanobacterial prokaryotes are referred to as bacteria. Relative abundance of organisms in each slide was recorded using a scale from zero to five, with zero representing absence and five representing dominance (see Table 2 for complete scale). Mat thickness was recorded.

Quantitative data were analyzed using cluster analysis (Euclidean distance and complete linkage analysis) and principle component analysis using SYSTAT v. 5.2.1 (SYSTAT 1992). The mats from the various ponds consisted of varying numbers of macroscopically distinct layers. To compare abundance of species among mats, mat structure was simplified by using the maximum
abundance of each species in any one layer as the value for that sample. The species composition for each site was determined by averaging the species abundances from each mat (Table 2).

**RESULTS**

**Macroscopic morphology of mats and environment**

The mats and benthic communities from the various ponds were macroscopically distinct. Strongly developed mats were not formed in Guana Island, Belmont, or Point Peter (in March), though thick mats were observed in Belmont at other times of the year (Jarecki 2004). Each of these was composed of loose or hardened sand with no obvious microbial community. The mats from Bones Bight varied in consistency and were also composed largely of sand. Mats from this pond often had a layer of blackened, preserved leaves near the bottom of the mat. Witches Brew mats had a dark green, undulating surface and layers of dark brown material. These mats tended to be thinner than the mats from the remaining pond and no granular layers were present. Banana Wharf mats had an orange-pink surface and a purple layer in the middle of the mat. Other layers (up to 15 could be distinguished macroscopically) were varying shades of green and brown, and the lower portion of the mat was particulate and often black.

The differences in mat structure and biodiversity
observed among ponds were not correlated with environmental variables (Table 1). The data provide an environmental overview of the habitat diversity of ponds in the British Virgin Islands. In the absence of additional seasonal characterization of the mat biotas, we did not attempt to infer biological composition based on the environmental data.

**Biota**

Seventeen species (or species groups), eleven of which were cyanobacteria, were identified from these mats. However, since several groups were not identified to the species level, the actual number of species present was probably greater. Of these species, some (e.g., *Oscillatoria* spp.) were ubiquitous, while others (e.g., *Johannesbaptistia pellucida*) were very rare. Three of the mat species (a chromophyte, a dinoflagellate and a foraminiferan) were not previously described in the literature as occurring in microbial mats. The distribution of each species is described below, and the average abundance in each pond is shown in Table 2.

*Aphanothece pallida* (Kützing) Rabenhorst was most prominent at Banana Wharf where it was occasionally dominant in layer 2 along with *Oscillatoria, Phormidium, Coccochloris* and bacteria. At Bones Bight it was present in the upper layer where it co-occurred with a wide range of species, whereas at Point Peter it was associated with foraminifera and *Oscillatoria*.

*Chroococcus minutus* (Kützing) Nageli was abundant at Banana Wharf and it was the dominant organism of layer 4. Here it was typically mixed with *Oscillatoria, Phormidium, Microcoleus* and bacteria. At Belmont it was the most abundant organism in some samples, but in others it occurred only sporadically. It was found singly, in twos or in fours and occasionally in large colonies.

*Coccochloris stagnina* Drouet et Daily was abundant in the upper layer of Banana Wharf, and it occurred in all communities with conspicuous mats. The cells were 4-6 µm wide and about three times as long as wide. *C. stagnina* was typically associated as a co-dominant with *Oscillatoria* spp. at Banana Wharf and in lower abundance with *Microcoleus* and the chromophyte at the other sites.

*Gomphosphaeria aponina* Kützing was found only in Bones Bight where it was often associated with *Oscillatoria, Phormidium* and the chromophyte. Although this species was never common, the spherical colonies were distinctive.

*Johannesbaptistia pellucida* Taylor et Drouet was present only at Witches Brew. It was found only in the spring samples when it was rare. This species is easily identified based on filament morphology. It was found as single filaments mixed with *Microcoleus, Phormidium* and the dinoflagellate.

Two species of *Phormidium* appeared to be present - one had a diameter of 1-2 µm, whereas the other was 3-5 µm diameter. Cells were longer than wide, and some had spherical granules adjacent to the cross-walls. *Phormidium* spp. often co-occurred with *Microcoleus*, the dinoflagellate, and *Oscillatoria* spp. at Fat Hogs Bay, but at Banana Wharf it was present deep in the mats with the chromophyte, *Oscillatoria* spp. and *Aphanothece pallida*.

*Spirulina subsalsa* Oersted was a common constituent of the mats. This species occurred as single filaments in the upper layers of the mats, although occasionally several filaments were intertwined. It tended to occur in assemblages where *Coccochloris stagnina, Oscillatoria* spp. and bacteria were dominant.

Filaments of *Symplaca cf. laete-viridis* Gomont were 1-5 µm diameter. This species was found only in mats from Banana Wharf where it was usually associated with *Phormidium, Chroococcus, Oscillatoria*, and the chromophyte.

*Microcoleus chthonoplastes* Thuret was abundant at Banana Wharf, and at Witches Brew it was a dominant organism in the upper layers. It was present at all sites where distinct mats were formed. It co-occurred with virtually all other taxa in these mats.

*Oscillatoria* spp. were dominant in Banana Wharf. Aggregations of these filaments (ca. 1 µm diameter) were found throughout most mats. Several species may have been present, but it was difficult to differentiate between species since the filaments were so narrow that morphological details were difficult to resolve.

*Schizothrix mexicana* Gomont was relatively abundant in mats from Bones Bight and was also found at Witches Brew. It was typically present in layer 2. It was found with *Oscillatoria* spp. and the chromophyte at Bones Bight, and with *Microcoleus chthonoplastes*, and species of *Oscillatoria* and *Phormidium* at Witches Brew.

Purple sulfur bacteria were present in the mats where they occurred as purple or gray cells. One or both of these forms were likely *Chromatium* species (Caumette 1987). The purple bacteria formed a distinct purple layer in the middle portion of the mats from Banana Wharf. The gray bacteria were more abundant near the top of the mats but were not confined to one layer. This species
was found not only in Banana Wharf, where it was most abundant, but also in the upper layers of many of the mats; it was not observed at Guana Island, where mats were not well developed.

Yellowish brown, multicellular aggregations (tentatively identified as a chromophytic alga) were present as three-dimensional spherical colonies that often aggregated together. The spherical colonies varied in size, but were commonly 15-30 µm diameter. These were the dominant organisms from Bones Bight Pond. At Banana Wharf and Witches Brew, abundance increased toward the bottom of the mats. At the latter two sites this organism typically co-occurred with Oscillatoria (both sites), Phormidium (only Banana Wharf), and Microcoleus (only Witches Brew).

Diatoms were observed at all sites except Point Peter; they occurred only in the top layers and were never abundant. Only cells with obvious cytoplasmic contents were counted, not empty frustules. Several genera of pennate diatoms were present including species of Navicula, Gomphipora, Surirella, and Nitzschia; however, it was beyond the scope of this work to identify them beyond the genus level.

Living dinoflagellates were found only in Witches Brew. Cells were about 50 µm diameter with conspicuous walls and flagellar grooves. More precise identification should be undertaken based on living material.

A foraminiferan species occurred in one mat from Banana Wharf, all five mats from Point Peter, all five mats from Bones Bight and one mat from Witches Brew. Foraminifera were extremely rare in the spring samples; however, they were a conspicuous element in the summer mats except those from Banana Wharf.

Nematodes were conspicuous at Banana Wharf Pond and Bones Bight Pond where they occurred as scattered individuals in the upper layers of the mats. Nematodes comprised the only conspicuous animal in the mat community.

**Cluster and principal component analysis**

Relative similarity among all of the mats based on the average species abundance is shown in Fig. 2. The mat samples from a given site collected in a given season tended to cluster together. There were two main groups of mats. One group contained primarily the spring mats from Banana Wharf and the majority of mats collected from Bones Bight. The other group contained the summer mats from Banana Wharf, the mats from Belmont and Guana Island, as well as the spring mats from Witches Brew, all mats from Point Peter and the rest of the mats from Bones Bight. In the cluster analysis there was no suggestion that samples clustered according to water depth.

In the PCA analysis the first two and three PCA axes
accounted for ca. 50% and 60% of the variance, respectively. This suggests a strong correlation among species distributions and coordinated patterns of distribution of the mat communities. The separation of the March mats from Banana Wharf and mats from Bones Bight from the remaining mats is evident in the PCA analysis (Fig. 3). The mats from Banana Wharf are distinguished from the other mats along PCA axis 1 by abundance of *Coccochloris*, *Oscillatoria*, bacteria, and nematodes, all of which have high positive correlations with PCA axis 1. PCA axis 2 separates the mats from Witches Brew from the rest of the mats based on taxa with high negative correlations with PCA axis 2: diatoms, *Johannesbaptistia*, *Schizothrix*, and dinoflagellates. The mats were further separated according to the season in which they were collected by PCA axis 3. The components with high positive correlations with PCA axis 3 were *Spirulina*, *Aphanothece*, mat thickness, and number of layers in the mats (Table 3).

Neither cluster analysis nor principal component analysis revealed a separation of the mats from Banana Wharf or Bones Bight based on water depth. This is not surprising, given the water clarity, and the fact that even the deepest ponds did not provide a particularly large depth gradient (max depth of 44 cm).

**DISCUSSION**

Previous comparisons of mats from different geographic areas were based largely on chemical or morphological characteristics. In addition, algal mats were placed in categories based on structure or

### Table 3. Factor scores for axes 1-3 from principal component analyses

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<tr>
<th>Taxon</th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
</tr>
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<tr>
<td><em>Aphanothece</em></td>
<td>0.242</td>
<td>0.089</td>
<td>0.681</td>
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<tr>
<td><em>Chroococcus</em></td>
<td>0.598</td>
<td>0.219</td>
<td>0.111</td>
</tr>
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<td><em>Coccochloris</em></td>
<td>0.670</td>
<td>0.010</td>
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<td>0.150</td>
<td>0.088</td>
</tr>
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<td><em>Johannesbaptistia</em></td>
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<td>-0.747</td>
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<tr>
<td><em>Microcoleus</em></td>
<td>0.592</td>
<td>-0.544</td>
<td>-0.127</td>
</tr>
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<td><em>Oscillatoria</em></td>
<td>0.843</td>
<td>0.053</td>
<td>0.216</td>
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<td><em>Phormidium</em></td>
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<tr>
<td><em>Spirulina</em></td>
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<td>0.055</td>
<td>0.735</td>
</tr>
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<td><em>Symploca</em></td>
<td>0.275</td>
<td>0.058</td>
<td>0.009</td>
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<tr>
<td>nematodes</td>
<td>0.796</td>
<td>0.001</td>
<td>0.070</td>
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<tr>
<td>sulfur bacteria</td>
<td>0.855</td>
<td>0.219</td>
<td>0.169</td>
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<td>chromophyte</td>
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<td>0.083</td>
<td>0.483</td>
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<td>diatoms</td>
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<td>-0.662</td>
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<td>dinoflagellates</td>
<td>0.017</td>
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<tr>
<td>foraminifera</td>
<td>-0.198</td>
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<td>0.755</td>
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<tr>
<td>no. of layers</td>
<td>0.205</td>
<td>-0.073</td>
<td>0.691</td>
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<table>
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<tr>
<th></th>
<th>percent variance</th>
<th>cum. % variance</th>
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<td>Percent variance</td>
<td>18.2%</td>
<td>61.6%</td>
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microenvironmental conditions (Ward et al. 1989; Bauld et al. 1992). Of the seven mat types distinguished by Bauld et al. (1992), the mats discussed here are basically of the “smooth/stratiform” type.

The well developed mats from Banana Wharf, Bones Bight and Witches Brew had an extremely gelatinous consistency. This is likely due to the production of exopolysaccharides (EPS) by the prokarotes, and especially the cyanobacteria in these mat communities. Richert et al. (2005) cultured cyanobacteria from Polynesian mats and found a diversity of EPS components. The function of these materials in situ is problematic; however, Richert et al. (2005) concluded that the EPS may be of considerable interest because of their biological activities.

The mat algae from the British Virgin Islands (except the chromophyte and dinoflagellate species) were mostly recorded previously in microbial mats from other sites (Sage and Sullivan 1978; Potts and Whitton 1980; Wharton et al. 1983; Stal et al. 1985; Stal and Krumbein 1985; D’Amelio et al. 1989; Oren 1989; Bauld et al. 1992). In spite of considerable overlap in species composition, the community structure of individual mats from the British Virgin Islands differed substantially from other areas. The mat communities from Aldabra may be the closest to the well-developed mats described here (Potts and Whitton 1980).

Like the mats from Mellum Island (North Sea) (Stal and Krumbein 1985), the mats we studied did not contain any heterocystous cyanobacteria. The mats from Banana Wharf and the Mellum Island were dominated by M. chthonoplastes and Oscillatoria spp. and contained Chromatium. M. chthonoplastes was also the most abundant species in the mats from Witches Brew. Mellum Island mats occurred on intertidal flats and were characterized by a green cyanobacterial layer with a red layer of sulfur bacteria and a black zone of sulfate reducing bacteria (Stal and Krumbein 1985; Stal et al. 1985). The mats from Banana Wharf similarly had the green and red layers, but the top layer was orange-pink. The bottom layer at Banana Wharf was also black; however, no conspicuous bacteria were evident. Thus despite similarities, there were large differences in the mat communities from Mellum Island and the most comparable ones from the British Virgin Islands. Sheridan (2001) described heterocystous cyanobacterial mats epiphytic on mangroves at many sites in the Caribbean Archipelago; however, we did not sample the mangrove vegetation. The high nitrate levels (Table 1) suggest that nitrogen is not limiting in the ponds from the British Virgin Islands, although high salinity may have artificially elevated the measurements (Jarecki 2004).

Mats in salt marshes from the Mississippi Gulf coast, though they were not in a hypersaline environment, contained common cyanobacteria that were also present in the mats from the British Virgin Islands (Sage and Sullivan 1978). Most notably, the cyanobacteria in the Mississippi mats included: Schizothrix mexicana (at Banana Wharf and Bones Bight); Gomphosphaeria aponina (at Bones Bight); Coccocloris stagnina, (at many sites) and Johannesbaptista pellucida (at Fat Hog’s Bay).

Hypersaline submerged mats from Guerrero Negro, Mexico, were from a habitat similar to the mats from the British Virgin Islands (D’Amelio et al. 1989). The species composition of these Mexican mats paralleled samples from Banana Wharf. In the upper portion of mats from both of these sites, diatoms and Spirulina spp. were present while Oscillatoria spp. and Microcoleus spp. were abundant in lower layers of both. However, bacterial taxa conspicuous at Guerrero Negro (Beggialot spp. and Chloroflexus spp.) were not apparent in mats from the British Virgin Islands.

The cluster and principal component analyses indicated significant seasonal differences in species composition. Hence mats from Banana Wharf and Bones Bight formed two groups based on month of collection. The mats collected in the two seasons also differed in thickness and number of layers. Cyanobacterial communities at a Mississippi salt marsh also varied according to season, although the abundance of the dominant species remained relatively constant (Sage and Sullivan 1978).

Our analysis of the benthic mats from the British Virgin Islands shows these communities to be highly diverse and dynamic. Extensive variation occurs within ponds at a given time, seasonally in the same pond and among ponds. Differences among benthic mats were not related to chemical differences or to hydrological differences described by Jarecki and Walkey (2006). The extent to which the biota of a given mat reflects purely stochastic events (e.g., random colonization from terrestrial, aerial or marine sources) and subsequent competitive interactions remain to be established. Our study demonstrates that it is difficult to make predictions of the mat communities from hypersaline ponds on observation of only a few ponds from a single sample period. This finding is particularly significant in light of
the overall importance of benthic mat development to salt pond ecosystems, including their ability to seal the bottom of ponds and thereby prevent groundwater exchange (Davis 1978) and their role as producers in the salt pond community (Jarecki 2004).

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