Benthic microalgae community response to flooding in a tropical salt flat

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Abstract
This research evaluated the effect of flooding on the microphytobenthos community structure in a microbial mat from a tropical salt flat. Field samples were collected during four consecutive days: on the first three days the salt flat was dry, on the fourth day it was flooded by rain. In order to evaluate the community maintained in flood conditions, samples from this area were collected and kept in the laboratory for 10 days with sea water. The results of total abundance of microphytobenthos varied from $4.2 \times 10^8$ to $2.9 \times 10^9$ organisms L$^{-1}$, total density increased one order of magnitude under the effect of water for both situations of precipitation in the salt flat and in experimental conditions, an increase due to the high abundance of Microcoleus spp. Shannon index ($H'$) was higher during the desiccation period. Our data suggest that changes in the abundance of organisms were due to the effect of water. The dominance of the most abundant taxa remained the same under conditions of desiccation and influence of water, and there is probably a consortium of microorganisms in the microbial mat that helps to maintain these dominances.

Keywords: microphytobenthos, cyanobacteria, Microcoleus spp., hypersaline environment.

Resposta da comunidade de microalgas bentônicas à inundação em uma planície hipersalina tropical

Resumo
Esse trabalho avaliou os efeitos da inundação na estrutura da comunidade microfitobentônica de um tapete microbiano em uma planície hipersalina tropical. As amostragens foram realizadas no campo durante quatro dias consecutivos: nos três primeiros dias o local estava seco e no quarto dia foi inundando com chuva. Para avaliar a comunidade mantida em condições de inundação, foram coletadas amostras dessa região, sendo mantidas em laboratório por 10 dias com água do mar. Os resultados mostraram que a abundância total do microfitobentos variou de $4.2 \times 10^8$ a $2.9 \times 10^9$ organismos L$^{-1}$, a densidade aumentou em uma ordem de grandeza com a influência da água, tanto na planície hipersalina como nas condições experimentais, um aumento que foi devido às maiores abundâncias de Microcoleus spp. O índice de Shannon ($H'$) foi mais elevado durante o período de dessecação. Nossos dados sugerem que as mudanças na abundância dos organismos foram devidas ao efeito da água, a dominância dos táxons mais abundantes permaneceu a mesma durante as condições de dessecação e inundação, e possivelmente existe um consórcio entre os microrganismos do tapete microbiano no qual eles ajudam a manter essa dominância.

Palavras-chave: microfitobentos, cianobactérias, Microcoleus spp., ambientes hipersalinos.

1. Introduction
Benthic microalgae are the main primary producers in tropical salt flats (Stal, 2001; Adame et al., 2012), where hypersaline conditions preclude the presence of most plants. Salt flat benthic communities are usually established in the superficial layers of microbial mats, mostly composed by cyanobacteria and sometimes by a film of diatoms (Stal, 2001; 2012). Many species that form a cyanobacteria mat in salt flats are well adapted for diazotrophic growth, because this environment is usually N limited (Adame et al., 2012; Stal, 2012).

Microphytobenthic or benthic microalgae from microbial mats are commonly enveloped in exopolimmeric substances (EPS), like proteins, nucleic acids, polysaccharides, and other substances, that protect these organisms from desiccation.
and other stress factors in hypersaline environments (Stal, 1995, 2001; Abed and Garcia-Pichel, 2001; Paerl and Yannarell, 2010; Scott and Marcarelli, 2012).

The regulatory salinity effect on microbial mat structure and function has been well established (Pinckney et al., 1995; Stal, 1995, 2001; Paerl and Yannarell, 2010; Hagemann, 2011; Scott and Marcarelli, 2012). Physiological adaptations of cyanobacteria to survive in hypersaline environments are related with specific mechanisms that regulate the osmotic stress internally (Hagemann, 2011). Some of these adaptations are the extrusion of toxic inorganic ions, e.g. high concentration of Na\(^+\), and accumulation of organic solutes that protect macromolecules against denaturation and retain hydration. (Hagemann, 2011).

Salt flats are plains where cyanobacteria mats are commonly found and pennate diatoms dominate the sediment surface; these microalgae are adapted to high salinity (Oren, 2012). This environment is submitted to high and fast changes in salinity, owing to the large accumulation of salt, which is a consequence of dry periods after spring tide and high evaporation rates, including salinity reducing rainfall (Zack and Roman-Mas, 1988; Adame et al., 2012). Water is an essential variable related with salinity and it bears direct influence on the microbial mat community, especially in natural events of desiccation (Paerl and Yannarell, 2010; Scott and Marcarelli, 2012). Effects of desiccation and rewetting were tested in benthic communities, and the results demonstrated changes in terms of functions and the composition of bacteria communities. Some studies show that rewetting increases microbial activity (McKew et al., 2011; Conrad et al., 2014). The goal of this study was to contribute to a better understanding of the desiccation and flooding effects on the cyanobacteria community structure in terms of abundance, diversity and evenness indices in a tropical salt flat environment.

2. Material and Methods

2.1. Study area

Samples were collected from a salt flat area (23° 00’ S and 43° 36’ W) in the Guaratiba mangrove (Rio de Janeiro, Brazil, Figure 1). This area is a part of the Sepetiba bay estuary system, and the total Guaratiba mangrove area is 28.3 Km\(^2\), of which 20.9 Km\(^2\) comprise mangrove forests and 7.4 Km\(^2\) comprise salt flats (Estrada et al., 2013). This area is characterized by a small variation in topography and a tidal range below 2 m (Estrada et al., 2013).

Pellegrini (2000) analyzed the salinity along a transect perpendicular to the sea during the years 1996 to 1998. The average registered in the salt flat varied between 55 and 103, a variation that was due to the local topography and climate. This salt flat area is usually flooded in the high spring tides and in the regular meteorological tides, while desiccations were associated with low rainfall levels, low tides and high evaporation rates.

According to the Marambaia Meteorological Station, during 2010 and 2011, temperatures ranged from 22 to 35 °C in the summer, and 17 to 30 °C in the winter; rainfall was more frequent in the summer than in the winter, with maximum values of 245 and 70 mm, respectively.

Figure 1. Sampling site location in the Guaratiba salt flat (light gray) surrounded by mangrove forest (dark gray), in Sepetiba Bay, Rio de Janeiro State – Brazil.
2.2. Sampling and experimental design

Single daily samples were collected from the salt flat between July 25 (day 1) and 28 (day 4) 2010, from 08:00am to 08:30am. The sediment was dry from day 1 to day 3, but it rained on day 4 and the microbial mat was covered with rainwater at the time of sampling.

In order to evaluate how the community would react to the maintenance of flood conditions, two sediment samples were collected on July 18, 2011, from the same dry sampling site with plexiglass cores and transported to the lab. In the experiment conditions, the microbial mats were kept under oligotrophic marine water in plastic tanks, with added NaCl to reach a final salinity value of 6 g L$^{-1}$, similar to the salt flat sampling area. The Flooded Experiment (FE) was kept outdoor for 10 days, and these samples were identified as 10-d FE (A) and (B).

In all samples, a plastic core was used to collect the microphytobenthic community. The first 1 cm of the cores were fixed with a 2% formaldehyde solution neutralized with hexamethylenetetramine. The community was identified and quantified in Palmer-Malloney chambers (0.1 mL), using an optical microscope with phase contrast (Olympus BX51). Morphotypes were identified to the lowest level possible according to Anagnostidis and Komárek (1988). A cyanobacteria trichome was considered as one organism; and in the case of diatoms and dinoflagellates, each cell was counted as one organism. Diversity was calculated using the Shannon index ($H'$), and evenness was calculated by Pielou’s index ($J'$) (Magurran, 2004).

3. Results

July 2010 was marked by low rainfall levels according to the Marambaia Meteorological Station. Prior to the sampling day, rainfall was recorded on July 18, seven days before the beginning of this study, with 4 mm to this day; during the sampling period on day 4 (July 28th) accumulated precipitation reached 38 mm (from 03:00am to 06:30am).

Microbial mat color changes were observed in the Guaratiba salt flat; during the days that preceded the rain, the microbial mat appearance was that of a dead community, with a dark color which was simultaneously whitened by the salt. Nevertheless, the mat surface changed completely to a greenish color on the rainy day.

The total density of the sediment microalgae community ranged from $4.2 \times 10^8$ to $2.9 \times 10^9$ organisms L$^{-1}$. It increased one order of magnitude under the effect of water for both the situation of rainfall in the salt flat and in experimental conditions (Figure 2).

![Figure 2](image-url)

**Figure 2.** Abundance in organisms L$^{-1}$, Shannon Index ($H'$) and Evenness Index ($J'$), and, relative abundance (%) of the most abundant taxa in the microbial mat during desiccation and flood periods. Day 1 to day 4 = salt flat sampling, 10 d-FE = 10 days of Flooding Experiment. Others (cyano = cyanobacteria) and Others (diatoms and dinof. = dinoflagellates) were described on Table 1.
Cyanobacteria from the Oscillatoriales order were the dominant group, especially Microcoleus spp. This taxa varied from $9.9 \times 10^8$ (day 1) to $3.7 \times 10^9$ (day 2) organisms L$^{-1}$ during the desiccation period on the salt flat, and 1.2 $\times 10^8$ (day 4) to 1.7 $\times 10^9$ (10d-FE-B) organisms L$^{-1}$ under water effect. The Lyngbya spp. contribution to total density was higher in the salt flat, especially on day 1 with 1.1 $\times 10^9$ organisms L$^{-1}$. Leptolyngbya spp. (Order Pseudanabaenales) ranged from 6.4 $\times 10^7$ (day 2) to 6.3 $\times 10^8$ (10d-FE-A) organisms L$^{-1}$ (Figure 2). These three taxa together represented 68% to 80% of all benthic microalgae (Figure 2).

An experimental flooding water effect caused some changes to this community, compared with the salt flat, i.e. Phormidiaceae Class (represented mainly by Microcoleus spp. and Phormidium spp., whose identification on specific level was not possible on the optical microscopic analysis) and filamentous cyanobacteria n.i. (not identified) presented relative abundance between 4% and 12% (Figure 2), while in the salt flat were not recorded. Moreover Navicula spp. and pennate diatoms n.i. were no reported in the experiment (Table 1), but in the salt flat their relative abundances reached 14% and 12%, respectively. Less abundant taxa were grouped in ‘Others’. More details on Table 1.

Shannon index ($H'$) varied between 1.62 (day 4) to 2.49 (day 1), and evenness index ($J'$) ranged from 0.51 (day 4) to 0.68 (day 2, Figure 2). $H'$ and $J'$ indices of the experiment were similar to the salt flat, except for day 4, highlighting the effect of rainfall water on the community. Species richness was greater on Day 1 with 15 taxa, whereas the other days ranged between 8 and 10 taxa (Table 1).

4. Discussion

Benthic microalgae from salt flat microbial mats were dominated by Microcoleus spp., Leptolyngbya spp. and Lyngbya sp. This pattern of dominance was reported by many authors in similar conditions (Paerl et al., 2000; Jonkers et al., 2003; Casillas-Martinez et al., 2005; Eriksson et al., 2010; Adame et al., 2012). Lyngbya, Microcoleus and Phormidium dominances are common in microbial mats classified as polygonal mats; such mats are not consolidated and, during dry periods, they develop polygon shaped wide cracks with upturned and overgrown margins (Eriksson et al., 2010). This was the microbial mat sampled in the Guaratiba salt flat.

Dominance of the most abundant taxa as Microcoleus and Leptolyngbya remained in all analyses, possibly due to the existence of a consortium of microorganisms in the microbial mat, a dominance which was maintained in desiccation conditions and under the influence of water. This consortium is very important for the survival of microalgae in extreme conditions, i.e. desiccation, through the exchange of nutrients and organic compounds among microorganisms such as heterotrophic bacteria and these dominant cyanobacteria. The microbial mat consortium was discussed by other authors as being crucial to community diversification, and enables the co-existence of different organisms with different metabolisms (aerobic and anaerobic) due to physical-chemical gradient, which is accentuated with increased depth (Van Gemerden, 1993; Des Marais, 2010; Stal, 2012). Functional groups are distributed according to

Table 1. Presence (+) and absence (-) of the taxa identified during the desiccation and flood periods of sampling. Day 1 to day 4 = salt flat sampling, 10-d FE = 10 days of Flooding Experiment.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Salt Flat</th>
<th>Experiment</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td>Leptolyngbya spp.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lyngbya sp.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Microcoleus sp.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phormidium sp1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phormidium spp.</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phormidales</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cyano n.i. 2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cyano n.i.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amphora sp1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Diadeneis sp.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Navicula sp1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Navicula sp2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Navicula spp.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitzschia spp.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fragilariaceae/ Rhaphoneidaceae</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pennate n.i.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coscinodiscus spp.</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Centric n.i.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Thecate n.i.</td>
<td>+</td>
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</tr>
</tbody>
</table>

* Others (cyano).  ** Others (diatoms and dinoflagellates).
the need to obtain energy for their vital functions, which in turn is limited by gradients of oxygen, light and sulfide (Van Gemerden, 1993; Des Marais, 2010; Stal, 2012).

Further studies confirmed the importance of the consortium for microalgae, i.e. carbon and/or nitrogen deficient experiments with *Chlamydomonas reinhardtii* and the nitrogen-fixing bacteria *Azobacter* revealed decreased chlorophyll content and cell death with 2 months of *C. reinhardtii* monoculture, whereas the *Azobacter-Chlamydomonas* consortium lasted at least two years (Gyurffân et al., 1986). Watanabe et al. (2005) demonstrated that chlorophyll content from *Chlorella sorokiniana* decreased after the fifth month, when growth occurred in monoculture; nevertheless, when there was growth in association with the presence of bacteria and fungi, that content remained constant. Those authors discussed the benefits of the consortium based on the availability and consumption of CO₂ and O₂, just as the fact that bacteria and fungi attached microalgae released mucilage, thereby decreasing the distance of diffusion and increasing the efficiency of nutrient exchange. The prevailing cyanobacteria in this study were species with a mucilage sheath; furthermore, cyanobacteria and diatoms produce a lot of organic matter, which can serve as an energy source to microorganisms. The fact that all these organisms grew in a mat could encouraging exchange of nutrients between them, due to its proximity.

The influence of water was observed in terms of increased density and changes in the composition of less dominant species on the rainy day on the salt flat during the experiment. In dry conditions, water may be a relief to organisms, leading to an increase of populations density. Cyanobacteria migration in the salt flat was observed by changes to sediment color and high density of *Microcoleus* spp., which resulted in reduced diversity and evenness indices. Typically, the $H'$ index and the richness are inversely correlated with the environmental extremity as dehydration (Rothrock Junior and Garcia-Pichel, 2005).

Some studies showed that cyanobacteria are stimulated by water, as they migrate in an opposite direction to desiccation (Garcia-Pichel and Pringault, 2001; Pringault and Garcia-Pichel, 2004). These authors repeatedly tested migration with or without light and without chemical variations, and concluded that the migration response was caused by the water effect. Paerl et al. (2000) also demonstrated the effect of water on desiccated microbial mats; the cyanobacteria community response was fast after hydration, and they start photosynthesis after 30 minutes and N fixation after few hours.

Salinity changes are a consequence of the influence of water in salt flats. It has been widely discussed in the literature as one of the main factors in structuring the benthic community in microbial mats. Adame et al. (2012) recorded community structure differences across the intertidal zone of the cyanobacteria mat on the salt flat of Giralia Bay (Australia). Pinckney et al. (1995) and Paerl et al. (2000) have concluded that salinity is an influential factor for the composition and functioning of a community. Pinckney et al. (1995) worked with hypersaline ponds in the Bahamas, and revealed different productivity and N fixation response to osmotic stress, with *Scytonema* and *Microcoleus*. Paerl et al. (2000) reviewed three environments, with different physical and stress features, including desiccation, to show differences in the composition and functioning of a community in microbial mats in distinct extreme environments. They suggested that successful reproduction and survival in extreme conditions of radiation, temperature and periodic desiccation occurs as a result of mutualistic relationships among populations within the consortium.

Our data suggest that changes in the abundance of organisms, including changes in the composition of some species, were due to the effect of water, inasmuch as changes occurred after one day on the salt flat and remained for 10 days into the experiment.

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