Modern stromatolite phototrophic communities: a comparative study of procaryote and eucaryote phototrophs using variable chlorophyll fluorescence

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Abstract
Stromatolites are laminated organosedimentary structures formed by microbial communities, principally cyanobacteria although eucaryote phototrophs may also be involved in the construction of modern stromatolites. In this study, productivity and photophysiology of communities from stromatolites (laminated) and thrombolites (nonlaminated) were analysed using fluorescence imaging. Sub-samples of mats were excised at Highborne Cay, Bahamas, and cross-sectioned to simultaneously analyse surface, near-surface (1–2 mm), and deeper (2–10 mm) communities. Rapid light curve parameters and nonphotochemical downregulation showed distinct differences between phototroph communities, consistent with the reported quasi-succession of classic stromatolite mat types. Greater productivity was shown by cyanobacteria in Type 1 and Type 3 mats (first and final stage of the succession, Schizothrix gebeleinii and Solentia sp. respectively) and lower productivity within Type 2 mats (intermediate mat type). Eucaryote mat types, dominated by stalked (Striatella sp. and Licmophora sp.) and tube-dwelling (e.g. Nitzschia and Navicula spp.) diatoms, showed greater productivity than cyanobacteria communities, with the exception of Striatella (low productivity) and an unidentified coccoid cyanobacterium (high productivity). Findings indicate comparative variability between photosynthetically active procaryote and eucaryote sub-communities within stromatolites, with a pattern logically following the succession of ‘classic’ mat types, and lower than the productivity of eucaryote dominated ‘nonclassic’ mat types.

Introduction
Modern stromatolites are living examples of laminated microbial structures that have been present on the planet for over 80% of Earth history (Reid et al., 2000; Riding, 2000; Visscher et al., 2000). The margins of Exuma Sound, Bahamas, host the only known examples of modern stromatolites growing in open marine conditions, similar to those of many Precambrian platforms (Reid et al., 1995, 1999). These living structures enable the study of interactions between phototrophic cyanobacteria and heterotrophic bacteria in the biostabilization of carbonate sand grains, biogenic precipitation of calcium carbonate, and resultant accretion of stromatolite lamina (Reid et al., 2000). Thrombolites are similar in many respects to stromatolites, but lack the lamina structure and are characterized by a macroscopic clotted fabric (Shapiro, 2000).

Early work by Reid et al. (2000), at Highborne Cay, Exuma Sound, described three distinct microbial communities forming modern marine stromatolites. These communities, referred to as Mat Types 1, 2 and 3, show a quasi-succession from a pioneer community of filamentous cyanobacteria, mainly Schizothrix gebeleinii, which form unlithified layers of trapped and bound sediment.
(Type 1), to biofilm mats dominated by sulphate reducing bacteria which form thin crusts of microcrystalline carbonate (Type 2), to a climax community dominated by the coccoid endolithic cyanobacterium Solentia sp. which create cemented layers of fused sand grains (Type 3). Stromatolite laminae form by a cycling of these communities with each subsurface layer representing a former surface mat. Stolz et al. (2009) expanded the study of ‘classic’ mat types described by Reid et al. (2000) to include the following trapping and binding communities: bacterial mats dominated by the filamentous cyanobacteria Phormidium sp., which form pudding-like mounds, and diatom mats dominated by either stalked diatoms, Striatella unipunctata and Licmophora spp., or tube-dwelling Naviculid diatoms.

Although a variety of microbial communities have been described on the surfaces of modern marine Bahamian stromatolites (e.g. Stolz et al., 2009), the relative importance of procaryotes vs. eucaryotes in stromatolite formation and the physiology of the photrophs are not well known. Two previous studies provide some initial data. Kromkamp et al. (2007) and Perkins et al. (2007) used pulse amplitude modulated (PAM) fluorimetry to investigate the photophysiology of the cyanobacterial communities in classic mat type stromatolite samples. Standard rapid light curve (RLC) analysis in Kromkamp et al. (2007) gave proxy measurements of productivity [relative electron transport rate (rETR)] and the mechanisms behind photoactivation and inactivation upon sand burial. Perkins et al. (2007) described the ability of the cyanobacteria communities to withstand periods of natural sand burial (a common and potentially prolonged occurrence in the dynamic near shore sites where the stromatolites form) with rapid photosynthetic reactivation postburial upon exposure to low light and oxygen. These previous studies (Kromkamp et al., 2007 and Perkins et al., 2007) both acknowledged methodological problems associated with fluorescence signal from subsurface communities. Effectively, subsurface phototrophic cells representing the separation of taxa into distinct layers (see Stolz et al., 2009 and also Table 1 and Fig. 1 in this study) result in data that are difficult to interpret because distances of cells from the fluorimeter and subsurface light environments are unknown (see Perkins et al., 2010a for full details of this issue). This is analogous to measurements made on soft sediment biofilms with vertical migratory diatom cells (Kromkamp et al., 1998; Serôdio and Catarino, 1999; Perkins et al., 2002; Mouget et al., 2008; Perkins et al., 2010a).

To overcome errors associated with the presence of subsurface phototrophic communities in determining stromatolite productivity, this study used a mini-version of a Walz Imaging-PAM (Mini-I-PAM) to image cross sections of mats. Using this method, fluorescence measurements were made on individual layers (surface, 0 –1 mm; shallow subsurface, 1–2 mm; or deep subsurface, 2–10 mm; see below), exposing each community or sub-community to the same light environment during measurements and also maintaining each community at

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Photosynthetic-community (cross-section layer)</th>
<th>Dominant phototropic taxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>Type 1 – caramel surface</td>
<td>Schizothrix gebelainii</td>
</tr>
<tr>
<td></td>
<td>Type 3 – green subsurface</td>
<td>S. gebeleinii, Solentia spp., Oscillatoria sp</td>
</tr>
<tr>
<td>Sample 2</td>
<td>Type 2 – bacterial biofilm with near-surface caramel cyanobacterial layer</td>
<td>Caramel layer: S. gebeleinii</td>
</tr>
<tr>
<td>Sample 3</td>
<td>Near-surface cyanobacteria (Type 3 mat)</td>
<td>Solentia spp. and Hyella spp., probably also Microcoleus and Oscillatoria</td>
</tr>
<tr>
<td>Sample 4</td>
<td>Pink Fuzz stalked diatoms</td>
<td>Striatella unipunctata</td>
</tr>
<tr>
<td>Sample 5</td>
<td>Yellow fuzz stalked diatoms</td>
<td>Licmophora remulus, Oscillatoria sp. (potentially including other Licmophora spp., S. unipunctata and Thalassionema sp.)</td>
</tr>
<tr>
<td>Sample 6 –</td>
<td>Surface diatom layer</td>
<td>Tube-dwelling diatoms</td>
</tr>
<tr>
<td>surface</td>
<td>tube-dwelling diatoms with subsurface cyanobacteria</td>
<td>(Nitzschia spp., Navicula spp. and Amphipleura rutilans)</td>
</tr>
<tr>
<td>Sample 7 –</td>
<td>Surface layer</td>
<td>Phormidium sp. and S. gebeleinii</td>
</tr>
<tr>
<td>cocoid pudding mats</td>
<td>Subsurface cyanobacteria – Type 3 mat</td>
<td>Coccoid cyanobacteria (Cyanotochea)</td>
</tr>
</tbody>
</table>
the same distance from the fluorimeter, rather than having communities overlaying each other as in previous work (Kromkamp et al., 2007; Perkins et al., 2007). Although there will still be some element of subsurface signal because of the 3-D structure of the phototrophic layers within the sample, this error will be greatly reduced in comparison with measurements taken directly above the natural surface in which cell positions are unknown and the light levels apply only to the stromatolite surface. The attenuation of light within the stromatolite matrix can also be partly corrected for using the productivity proxy developed by Sérodió (2003) as explained in the methodology. The resultant data are therefore a far more accurate comparative measurement of community productivity than in previous work.

This paper investigated the comparative productivity and photophysiology of the procaryotic and eucaryotic communities forming modern marine stromatolites at Highborne Cay. The overarching aim of the study was to determine the potential comparative photosynthetic activity of each procaryote phototrophic community in the different morphological mat types seen in the quasi-succession reported by Reid et al. (2000) and to compare these with the eucaryote communities described in Stolz et al. (2009). The study aimed to test the hypothesis that the cyanobacterial communities would have lower productivity and would be low light acclimated because of their subsurface locality within the stromatolite matrix, whereas surface eucaryote communities would have greater productivity and would be comparatively high light acclimated. In addition, we also tested the hypothesis that productivity would differ between stromatolite mat types defined within the ‘quasi-succession’ reported in literature (Reid et al., 2000; Stolz et al., 2009; Bowlin et al., 2011).
Materials and methods

Sample collection

Samples were excised from stromatolites formed in shallow water on the eastern beach (Stromatolite Beach) of Highborne Cay, Exuma Sound (76°49′W, 24°43′N; Reid et al., 1999) in July 2008. Samples were only collected during the morning (08:00–11:00). Samples were quickly placed onto a bed of site sand with overlying site seawater, inside a sealed opaque plastic container (and hence maintaining the sample in darkness). Samples were then quickly (within 20 min) returned to the laboratory onboard the R/V F.G. Walton Smith for fluorescence measurements. Samples were therefore effectively dark adapted for 20 min prior to measurements. On reaching the laboratory, samples were immediately cross-sectioned using a fine scalpel and fluorescence measurements were made (see below). Examination by light microscopy (e.g. Fig. 1) showed little to no damage to the 3-D structure of the stromatolite caused by cross sectioning. The study compared the productivity and photophysiology of the 13 procaryote and eucaryote phototrophic communities from seven sample types described in Table 1. Samples (n = 7, Table 1, column 1) were collected such that 10 individual sample replicates were obtained for each community (n = 13, Table 1, column 2).

Community structure

All samples collected were analysed for community type, sub-community type and dominant taxa (Table 1) reported in the concomitant studies by Stolz et al. (2009) and Bowlin et al. (2011). The same nomenclature was used to describe communities and sub-communities of both procaryotes and eucaryotes and the same taxa are reported as dominant in these groupings. Thus, the sub-communities referred to in this study are indicative of those defined within the mat types referred to in previous literature, including the classic mat types of Reid et al. (2000).

Fluorescence measurements

All fluorescence measurements were made using a Walz Mini-I-PAM fluorimeter and supporting Walz analysis software. Fluorimeter settings resulted in a low frequency nonactinic measuring beam and a 0.6 s saturating pulse of over 8000 μmol m⁻² s⁻¹ photosynthetically active radiation (PAR). Measurements of minimum or operational yield (F₀ in the dark and F in the light respectively) and maximum fluorescence yield (Fₘ in the dark and Fₘ in the light) were made, with calculation of PSII quantum efficiency following Genty et al. (1989):

\[
Fₙ/Fₘ = (Fₘ - F₀)/Fₘ \quad \text{or} \quad \Delta F/Fₘ = (Fₘ - F)/Fₘ
\]

for measurements in the dark and light, respectively.

Serôdio (2003) developed a chlorophyll fluorescence-based index to estimate productivity (where E is the applied photosynthetically active radiation, PAR in μmol m⁻² s⁻¹):

\[
P_{flu} = E\left[F₀ - \frac{Fₙ}{Fₘ - F₀}\right]
\]

where \(r\)ETR is the relative electron transport rate calculated as the product of ΔF/Fₘ and E (Sakshaug et al., 1997; Perkins et al., 2006, 2010a). This results in a proxy (P_{flu} in Serôdio, 2003) that corrects for background fluorescence from the sediment (effectively the autozero step in setting up the fluorometer prior to use), this simplifies to:

\[
P_{flu} = \frac{F₀}{Fₘ} \cdot r\text{ETR}
\]

where \(r\)ETR is the relative electron transport rate calculated as the product of ΔF/Fₘ and E (Sakshaug et al., 1997; Perkins et al., 2006, 2010a). This results in a proxy (P_{flu} in Serôdio, 2003) that corrects for background fluorescence from the sediment (effectively the autozero step in setting up the fluorometer prior to use), this simplifies to:

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\[
P_{flu} = \frac{F₀}{Fₘ} \cdot r\text{ETR}
\]
Images of the cross-sectioned samples were focussed using the Mini-I-PAMs live video mode before switching to either red light (samples dominated by Cyanobacteria) or blue light (samples dominated by diatoms) illumination under the fluorescence imaging mode. It was interesting to note that samples analysed using both blue and red light separately, whether diatom or cyanobacteria dominated, showed no significant difference between each set of measurements. This was also noted in previous work by the authors (R.G. Perkins and J.C. Kromkamp, pers. obs.). Each community [illustrated in Fig. 1: surface diatom/cyanobacteria mixed community, surface cyanobacteria (typically the caramel layer) and subsurface cyanobacteria (green layer)], was imaged using 10 replicate polygons, carefully selected to enclose the community of interest. Note that subsurface images were only taken within 10 mm of the surface of the stromatolite to analyse communities within oxygenated zones reported in Stolz et al. (2009). The Mini-I-PAM processes the fluorescence yields detected within the selected polygon regions, hence performing separate light curves on each area. These measurements were repeated on 10 separate samples giving 10 pseudoreplicates within 10 true replicates, thus accounting for variability within samples and between samples. The data presented are therefore effectively mean values from 100 RLCs or the calculated values from the Eilers & Peeters (1988) curve fitting process when applied to all data for the 100 RLCs. The initial values of minimum and maximum fluorescence yields at the start of each light curve were used as proxy values of \( F_0 \) and \( F_m \) [in all cases, these were the minimum and maximum yields, respectively, observed for each light curve set and indicated that chlororespiration (a form of oxygen-dependent electron transport) and state transitions had not depressed the \( F_m \) yield]. The initial value of \( F_0 \) was then used in the calculation of \( P_{\text{fluo}} \), following Seroôdio (2003) as described above (but retaining the nomenclature rETR), whilst \( F_m \) was used in the calculation of the downregulation parameter of nonphotochemical quenching (NPQ) calculated as (e.g. see Perkins et al., 2006, 2010a, b):

\[
NPQ = \frac{(F_m - F'_m)}{F''_m}
\]

### Results

**Stromatolite phototrophic communities**

Table 1 presents the stromatolite phototrophic communities and sub-communities observed in the seven sample types investigated in this study. Nomenclature, except where stated, and taxa are as reported in the concomitant work by Stolz et al. (2009) and Bowlin et al. (2011). Images of the phototrophic communities are presented in Fig. 1. Sample 1, 2 and 3 (Fig. 1a–c) refer to the three ‘classic’ mat types of Reid et al. (2000). Figure 1a shows the Type 1 mat with the upper S. gebeleinii community and underlying mixed cyanobacteria. Figure 1b shows the Type 2 mat with upper bacterial biofilm and underlying S. gebeleinii community. Figure 1c shows the Solentia spp. community and underlying mixed cyanobacteria community. Figure 1d, the Pink Fuzz stalked diatom community sample, Fig. 1e and h, the Yellow Fuzz stalked diatom community, Fig. 1f and i, tube-dwelling diatom community and Fig. 1g and j, the ‘Pudding mat’ community, are the phototrophic ‘nonclassic’ mat type communities described in Table 1 and Stolz et al. (2009).

**‘Classic’ mat types 1, 2 and 3**

Type 1 stromatolite mat communities (Sample 1) showed a large difference between the caramel layer of Schizothrix at the surface and the subsurface Type 3 cyanobacteria community (Fig. 2). Schizothrix had higher rETR throughout the light curve and hence a higher rETR_max
and higher $\alpha$ (Table 2) than the subsurface community. Light saturation was higher for Schizothrix compared with the subsurface community, with an $E_k$ of 180 compared with 40 $\mu$mol m$^{-2}$ s$^{-1}$ PAR, respectively. In addition, Schizothrix showed only slight evidence for photoinhibition (i.e. a decrease in rETR at light levels above that for rETR$_{\text{max}}$), but comparatively high NPQ (Fig. 2) compared with the subsurface community which had clear photoinhibition and lower NPQ induction.

In contrast to Type 1 communities, Type 2 mats consisting of Schizothrix communities underlying a surface biofilm (Sample 2) showed a flatter light response curve with clear photoinhibition above rETR$_{\text{max}}$ (Fig. 3). Light curve parameters rETR$_{\text{max}}$ and $\alpha$ were lower (Table 2) for Schizothrix in Sample 2 mats, with an $E_k$ value calculated at 350 $\mu$mol m$^{-2}$ s$^{-1}$ PAR, appearing falsely high as this exceeds the saturation light level of 200 $\mu$mol m$^{-2}$ s$^{-1}$. The level of NPQ induction (Fig. 3) was similar to the surface layer of Schizothrix in Type 1 mats (Fig. 2), reaching a value of 0.12 by the end of the light response curve (compared to 0.16 for Type 1 Schizothrix community).

In Type 3 mats (Fig. 4), surface and subsurface Solentia communities showed almost identical light response curves. Light curves were intermediate between Type 1 and Type 2 mats as communities showed a comparatively high rETR throughout the light curve but with clear photoinhibition. In addition, NPQ induction was similar to levels observed in Type 1 and 2 mats (Fig. 4). rETR$_{\text{max}}$, $\alpha$ and $E_k$ values (Table 2) were similar for Type 3 communities of Sample 3 and were also similar to the Type 1 surface Schizothrix community. Overall, productivity decreased going from Type 1 to Type 2, as indicated by a

<table>
<thead>
<tr>
<th>Mat type</th>
<th>Dominant taxa</th>
<th>rETR$_{\text{max}}$ (rel. units)</th>
<th>$\alpha$ (rel. units)</th>
<th>$E_k$ ($\mu$mol m$^{-2}$ s$^{-1}$ PAR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>Surf – Schizothrix gebeleinii</td>
<td>0.80</td>
<td>0.004</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>Sub-surf – Schizothrix, Solentia and Oscillatoria spp.</td>
<td>0.22</td>
<td>0.004</td>
<td>50</td>
</tr>
<tr>
<td>Type 2</td>
<td>Near Surf – S. gebeleinii</td>
<td>0.35</td>
<td>0.001</td>
<td>350</td>
</tr>
<tr>
<td>Type 3</td>
<td>Near Surf – Solentia and Hyella spp.</td>
<td>0.65</td>
<td>0.004</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Sub-surf – mixed cyanobacteria</td>
<td>0.70</td>
<td>0.005</td>
<td>140</td>
</tr>
<tr>
<td>Stalked diatom mat (Pink Fuzz)</td>
<td>Surf – Striatella unipunctata</td>
<td>0.34</td>
<td>0.005</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Sub-surf – S. gebeleinii</td>
<td>0.34</td>
<td>0.009</td>
<td>40</td>
</tr>
<tr>
<td>Thrombolite Pink Fuzz</td>
<td>Surf – probable Striatella unipunctata</td>
<td>0.40</td>
<td>0.004</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Sub-surf – probable S. gebeleinii</td>
<td>0.95</td>
<td>0.016</td>
<td>60</td>
</tr>
<tr>
<td>Stalked diatom mat (Yellow Fuzz)</td>
<td>Surf – Licmophora remulus</td>
<td>2.26</td>
<td>0.013</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>Sub-surf – S. gebeleinii</td>
<td>0.77</td>
<td>0.009</td>
<td>90</td>
</tr>
<tr>
<td>Tube diatom mats (Pustular blankets)</td>
<td>Surf – tube-dwelling diatoms</td>
<td>1.40</td>
<td>0.019</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Sub-surf – Solentia spp., S. gebeleinii</td>
<td>0.81</td>
<td>0.013</td>
<td>65</td>
</tr>
<tr>
<td>Coccoid pudding mats</td>
<td>Surf – Phormidium and Schizothrix</td>
<td>1.02</td>
<td>0.009</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Sub-surf – coccoid cyanobacteria</td>
<td>0.81</td>
<td>0.004</td>
<td>205</td>
</tr>
</tbody>
</table>

Surf, surface layer of cells; Sub-surf, sub surface layer of cells; Near Surf, cells just below stromatolite surface (see Stolz et al., 2009 for details). $\alpha$, light use coefficient; $E_k$, light saturation coefficient (rounded to nearest 5 units).
comparative reduction in both rETR\text{max}, and then increased again in Type 3 mats.

‘Nonclassic’ mat types with eucaryote communities

The two stalked diatom stromatolite mat types, Pink Fuzz (Sample 4) and Yellow Fuzz (Sample 5) dominated by Striatella \textit{sp.} or Licmophora \textit{sp.} respectively, and both overlaying \textit{Schizothrix}, showed distinctly different productivity and downregulation (Fig. 5). Surprisingly, the surface \textit{Striatella} community (Sample 4, Pink Fuzz) had a light response curve almost identical to the subsurface \textit{Schizothrix} of Sample 4 with similar rETR\text{max}, \(\alpha\) and \(E_k\) values (Table 2). In comparison, \textit{Licmophora} (Sample 5, Yellow Fuzz) had the highest values of rETR throughout the light response curve and hence the highest rETR\text{max} and \(\alpha\) of any phototrophic community. In addition, the subsurface \textit{Schizothrix} underlying \textit{Licmophora} had a higher rETR\text{max} and \(\alpha\) than the \textit{Schizothrix} community underlying \textit{Striatella}, as well as being higher than the \textit{Striatella} itself. The level of NPQ induction for both \textit{Striatella} and the subsurface \textit{Schizothrix} of the Pink Fuzz community (Sample 4) was greater than the two communities of Yellow Fuzz (Fig. 6) and also saturated at the end of the light curve. These were the only communities to show saturation of NPQ induction.

Pink Fuzz and Yellow Fuzz surface eucaryote communities on nearby Thrombolites were additionally compared with those on Stromatolites. Interestingly, Pink Fuzz on the nearby thrombolite samples showed a very different pattern to Pink Fuzz on stromatolite samples (Fig. 6) despite the hypothesis that these were the same community types (verified by light microscopy). Surface stalked diatoms on thrombolites, dominated by \textit{Striatella} \textit{sp.}, had a similar light curve and level of NPQ induction as the comparable layer on the stromatolite surface, hence showing similar rETR\text{max}, \(\alpha\) and \(E_k\) (Table 2). The thrombolite subsurface cyanobacteria dominated by \textit{Schizothrix}, underlying the stalked diatoms, had higher rETR\text{max}, \(\alpha\) and \(E_k\) than the diatoms (Fig. 6, Table 2), with values similar to those of the subsurface \textit{Schizothrix} layer in the stromatolite Yellow Fuzz community (Fig. 5, Table 2) despite having a similar level of NPQ induction to the surface stalked diatoms.

Tube-dwelling diatoms at the surface of stromatolite sample 6 were a highly productive community with high rETR, showing no downregulation in rETR above rETR\text{max}. The light curve for the subsurface Type 3 cyanobacterial community saturated with a comparatively high rETR\text{max} relative to the majority of cyanobacteria subsurface layers (Fig. 7 and Table 2). The subsurface Type 3 cyanobacteria community showed downregulation in rETR at high irradiance despite a similar level of NPQ induction as the surface diatoms, with neither community showing saturation of NPQ induction (Fig. 7). In general, with the exception of the \textit{Striatella} community in Pink Fuzz (Sample 4), the eucaryote diatom-dominated communities and the associated subsurface cyanobacteria showed high productivity.

The final community studied was the relatively soft coccolid pudding mats (Sample 7) formed on the surface of some stromatolites. This community is composed of a surface layer of \textit{Phormidium} and \textit{Schizothrix}, with a subsurface layer of coccolid cells tentatively identified as \textit{Cyanothece} (Stolz \textit{et al.}, 2009). The two layers showed similar productivity (Fig. 8), although surprisingly the subsurface coccolid layer had higher rETR\text{max} and \(\alpha\) than the surface layer (Table 2). Both layers had comparatively high productivity compared with the cyanobacterial communities in other samples and both communities showed typical patterns of NPQ induction which did not saturate by the end of the light curve (Fig. 8). Apart from the \textit{Striatella} Pink Fuzz community (see above), the coccolid community was the only sample in which the
subsurface cyanobacterial productivity exceeded the productivity of the surface community.

**Discussion**

Photosynthetic activity varied greatly between sub-communities of both procaryotic and eucaryotic autotrophs in the stromatolite samples collected at Highborne Cay. Productivity was measured for the sub-communities previously defining the succession of classical stromatolite mat types (Reid *et al.*, 2000; Stolz *et al.*, 2009; Bowlin *et al.*, 2011), and hence, the sub-communities referred to and used also to refer to the stage of this succession. With regard to the procaryotic cyanobacteria, comparative productivity was higher for Type 1 *Schizothrix* communities (Sample 1) than *Schizothrix* underlying Type 2 bacterial-dominated biofilms (Sample 2). Productivity was again higher in Type 3 mats (Sample 3). The data in this study therefore show a pattern in productivity which parallels the pattern in stromatolite sediment accretion suggested by Reid *et al.* (2000). It should be noted that these data for productivity are comparative and not quantitative. As samples were cross-sectioned, it could be argued that enhanced exposure to oxygen may change absolute values of productivity, and the authors do not dispute this. However, this would be highly unlikely to change the comparative patterns in data observed for either the productivity proxy or the data on photoacclimation.

**Comparative productivity**

Within Type 1 mats, the surface caramel layer of *S. gebeleinii* had a comparatively high rETR$_{\text{max}}$ of 0.80 compared with the subsurface Type 3 community of *Schizothrix, Solentia* and *Oscillatoria* spp. (rETR$_{\text{max}}$ of 0.22). This suggests greater primary productivity near the surface and with a mat type progression from Type 1 to Type 2 and thence to Type 3 as a form of climax community. This progression was thought to be dependent upon a pause in sediment accretion, presumably resulting from changes in hydrodynamic forces (wave and storm events) with corresponding changes in sediment supply (Reid *et al.*, 2000; Stolz *et al.*, 2009). The data in this study therefore show a pattern in productivity which parallels the pattern in stromatolite sediment accretion suggested by Reid *et al.* (2000).
hence a potential for a greater role in polymer production and a potential for sediment trapping. Type 1 mats are
typified by pioneer filamentous cyanobacteria, *Schizothrix*,
dominating during periods of rapid sedimentation (Reid et al., 2000; Visscher et al., 2000; Riding et al., 2000; Stolz et al., 2009); hence a high rETR indicating high productivity would be expected. Such a high level of productivity would also be required to produce the large amounts of extracellular polymeric substances (EPS) as reported for *Schizothrix* within Type 1 mats (Stolz et al., 2009) which is of high importance in defining its role in sediment binding.

In contrast to Type 1 mats, the Type 2 mat with a near-surface layer of *Schizothrix* showed a lower rETRmax of 0.35 rel. units. Type 2 biofilms (overlying the Type 1 community in Sample 2) are thought to form during a pause in sediment accretion and represent the stage at which bacterial mineralization of biofilm polymer leads to formation of a micritic crust (Reid et al., 2000). This period is not favourable for cyanobacterial photosynthetic activity, leading to lower productivity. It is possible that the bacterial activity within the biofilm may reduce the amount of oxygen available to the cyanobacteria potentially inhibiting oxygen-dependent electron transport; this is consistent with previous findings that oxygen is essential for cyanobacterial photosynthetic reactivation postburial (Perkins et al., 2007).

As the pause in sedimentation increases in duration, the cyanobacterial community evolves into climax Type 3 community dominated by *Solentia* (which bores into the sand grains increasing matrix stability) and *Hyella*, sometimes with a subsurface Type 3 cyanobacterial community (Reid et al., 2000; Stolz et al., 2009). Both layers in the Type 3 mats (Sample 3) had higher productivity than the Type 1 community underlying the Type 2 biofilms (Sample 2) with rETRmax of 0.65 and 0.70 rel. units for the surface and subsurface communities, respectively. This would be expected if the climax community had effectively moved through a less favourable period for photosynthetic activity into a period when cyanobacterial photosynthetic activity increased. Type 3 mats consist of a climax community comprising microboring of sediment
grains by Solentia and potentially an increase in Schizothrix biomass (Bowlin et al., 2011). However, analysis using oxygen microelectrodes by Stolz et al. (2009) indicated broadly similar oxygen profiles for the three ‘classic’ mat types but with an oxygen maximum for a Type 2 mat approximately double that for Type 1 and 3 mats. The higher oxygen in the Type 2 mat suggests greater photosynthetic oxygen evolution despite a concomitantly higher level of bacterial respiration which would be expected to reduce the oxygen detected in the Type 2 profile (Stolz et al., 2009). One explanation for the higher oxygen in Type 2 mats might be a lower oxygen diffusivity because of inhibition by the micritic layer leading to oxygen accumulation. Unfortunately, EPS data for Type 1 and 2 mats in the same study were pooled, although a higher percentage as well as absolute amount of colloidal low molecular weight polymer (cLMW) was observed in Type 3 mats compared with the pooled Type 1 and 2 mats. Comparatively greater cLMW production would support a higher rate of photosynthetic productivity (Smith & Underwood, 1998; Decho, 2000; Perkins et al., 2001; Underwood, 2001).

Bowlin et al. (2011) reported that as the period of low sedimentation persists, or in the absence of frequent burial, eucaryotic surface colonizers (stalked and tube-dwelling diatoms) develop on the stromatolite surface. In this study, there was a large difference in the productivity of the two stalked diatom communities with Licmophora of Yellow Fuzz having a higher rETR$_{\text{max}}$ than Striatella of Pink fuzz (2.26 compared with 0.34 rel. units). Subsurface layers of Schizothrix underlying the Pink and Yellow Fuzz also showed differences in productivity with rETR$_{\text{max}}$ of 0.77 rel. units under Licmophora compared with 0.34 rel. units under Striatella. The different surface communities therefore not only differ in their own productivity, but also appear to have different affects on the productivity of the subsurface cyanobacteria. It is likely that this would have implications on sub-community function regarding sediment binding and accretion.

When comparing thrombolite and stromatolite photosynthetic communities, differences in photophysiology were observed despite the communities being hypothesized to be comprised of the same taxa. Pink Fuzz on thrombolite samples had a low rETR$_{\text{max}}$ (0.40 rel. units) similar to the Pink Fuzz on the stromatolite but the subsurface layer of cyanobacteria, presumably Schizothrix, was potentially far more productive with an rETR$_{\text{max}}$ of 0.95. This difference for the subsurface Schizothrix suggests two possibilities: firstly, subtle differences in the depth of the subsurface cyanoabacterial layer in the stromatolite fabric result in different states of photoacclimation (high or low light states), or secondly, other unknown factors lead to higher rates of productivity, suggesting more favourable conditions for the Schizothrix subsurface compared to Licmophora and within the Thrombolite structure.

The potential productivity of the Stromatolite tube-dwelling diatom community was intermediate between the Licmophora and Striatella communities, with an rETR$_{\text{max}}$ of 1.4. This rETR$_{\text{max}}$ indicates higher productivity than the prokaryote autotrophs, but considerably lower than the Licmophora community. The subsurface layer of Schizothrix under the tube-dwelling diatoms showed a relatively high rETR$_{\text{max}}$ of 0.81. Thus, in two of three cases, the surface eucaryote potential productivity was significantly higher than that of the underlying cyanobacteria layer, but in all cases the cyanobacteria were photosynthetically active with reasonably high rETR$_{\text{max}}$.

The relevance of the coccoid pudding mat community in stromatolite formation is not known. However, this mat type was noted as an exception in that the subsurface coccoid layer (tentatively identified as Cyanothece) had a higher rETR$_{\text{max}}$ and $\alpha$ compared with the Schizothrix/Phormidium community (rETR$_{\text{max}}$ of 1.02 rel. units compared with 0.81, $\alpha$ of 0.009 compared to 0.004 rel. units).

![Fig. 8. Light response curves (upper panel) and NPQ induction during the same set of light curves (lower panel) for 'coccoid pudding mats' thought to be comprised of Phormidium sp. and Schizothrix gebeleinii overlying a coccoid cell layer possibly Cyanothoe sp. All data mean ± SE from 10 replicate samples.](image-url)
The high rETRmax suggests that the cocoid cells are potentially highly active with respect to productivity within the subsurface communities. This is in agreement with the high proportion of cLMW polymer, the high subsurface Chl a content and the penetration of oxygen to 24 mm (the deepest profile of all mat types) reported in Stolz et al. (2009).

In general, comparative productivity data based on the rETRmax proxy suggest cyanobacterial communities have comparatively lower productivity than surface eucaryotic communities dominated by stalked and tube-dwelling diatoms; an exception is the cocoid cyanobacteria community of the pudding mats, with rETRmax equivalent to the diatoms. These productivity patterns are supported by the oxygen profiles and the polymer data of Stolz et al. (2009).

**Photoacclimation and downregulation**

Comparison of the RLC parameters rETRmax, α and Ek enable states of photoacclimation, for example adaptation to high light or low light (in relative terms), to be compared between samples. High rETRmax and Ek with relatively low α indicates high light acclimation and vice versa for low light acclimation. However, in this study the product FαrETR was used to estimate productivity to reduce the influence of light attenuation in the cross-sectioned sample. As a result, any differences in photoacclimation need to be treated with caution. Procyrate sub-communities showed no consistent patterns in photoacclimation state. For example, surface caramel Schizothrix had a similar α to the subsurface Type 3 community, although the rETRmax and Ek were higher at the surface. Type 3 mat communities showed similar values of rETRmax, α and Eκ to the Schizothrix caramel layer, despite variation of the communities with depth. Type 3 mats near the stromatolite surface had low α and Eκ, but a relatively high rETRmax. In contrast the eucaryote diatom communities showed higher α and rETRmax compared with the cyanobacteria communities, despite having Eκ values of a similar magnitude. This would suggest a greater potential productivity of the eucaryotes but otherwise no difference in light acclimation state. Downregulation through photoprotection was suggested for the surface/near-surface Schizothrix communities with high carotenoid content being observed and a high sensitivity to light under a light microscope reported (Stolz et al., 2009). Also the tube-dwelling diatoms were reported by Stolz et al. (2009) as showing sensitivity to light, but in this study they showed comparatively high rETRmax, α, Eκ, and levels of NPQ which were similar to those of the stalked diatom communities.

Downregulation through induction of NPQ was observed to increase as the light level incrementally increased in RLCs for all samples, however the magnitude of this increase varied. For subsurface sub-communities of cyanobacteria the level of NPQ induction was similar reaching values of around 0.1–0.16 by the end of the RLC, except for the Type 1 mixed community which induced NPQ to a lower extent (approximately 0.04 by the end of the RLC) and the Schizothrix layer underlying the Pink Fuzz on both stromatolites and thrombolites which had higher levels of NPQ (0.3 and 0.4 for stromatolite and thrombolite sub-communities, respectively). This suggests a relatively constant level of photoacclimation indicated by the ability to induce NPQ. The relatively high level of NPQ for the Schizothrix layer under the two Pink Fuzz communities is surprising, but it was noted that the level of NPQ induction of the surface Pink Fuzz (Striatella for the stromatolite samples and presumably the same dominant taxa at least for the thrombolites) was also the same (0.4 by the end of the RLCs for both stromatolite and thrombolite sub-communities). Overall, the Pink Fuzz stalked diatom/Schizothrix community had lower productivity (see above) and higher levels of down-regulation when compared with all other sub-communities, suggesting low light acclimation. In all cases the more productive eucaryote communities dominated by stalked diatoms and tube-dwelling diatoms showed higher levels of NPQ induction compared with the cyanobacteria, suggesting a better ability to cope with the relatively higher light level at the stromatolite surface.

**Conclusions**

Overall, this study has shown differences in comparative productivity in photosynthetic sub-communities within stromatolite mat types. Broadly speaking, the cyanobacterial communities typical of ‘classic’ mat types had lower productivity, without showing clear patterns in photoacclimation in the form of high or low light acclimation. Productivity of the eucaryote, principally diatom dominated, communities was mostly higher, except in the stalked diatom community dominated by Striatella, probably resulting from the low Chl a content of this sub-community reported by Stolz et al. (2009). The patterns in comparative productivity correlate well with the quasi-succession between the morphological phenotypes (mat types) reported by Reid et al. (2000) and defined with respect to community by Stolz et al. (2009). A photosynthetically active cyanobacterial community in Type 1 mats is followed by a potentially less productive sub-community underlying Type 2 mats, when bacterial mineralization activity is high. This in turn is followed by a more productive mixed cyanobacteria climax community in Type 3 mats. In the longer term, surface eucaryote sub-communities, dominated by diatoms, form mixed communities which have compara-
tively higher productivity than the cyanobacterial subcommunities.

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