ABSTRACT

Title of Thesis:	MICROCOSM STUDIES OF NUTRIENT CYCLING IN BAHAMIAN STROMATOLITES
	Nicholas Berman Jabro, Master of Science, 2008
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I report results of field observations and experiments that examine the oxygen and nutrient fluxes for stromatolites in Highborne Cay in the Exumas, Bahamas. The aim of this study is to determine whether nutrients play a role in the transition of the community structure within the mats that is thought to be responsible for lithification and, ultimately, mat growth and structure. The research includes nutrient monitoring of the sediment and water column, and measures of rates of oxygen and inorganic nutrient exchange from stirred microcosm chamber incubations of mats with varied community structure. On the basis of mat community composition, I hypothesized that different mat types would have different fluxes, and that Highborne mats would be limited by one or more nutrients that efficient recycling within the mats might otherwise help supply. Samples of the four major mat types were sealed in stirred microcosm flux chambers, incubated in a circulating water bath, and sampled for oxygen, NH_4, NO_3, PO_4, and Silicate. Nutrient

addition, treatments of PO_4 and Si were employed to investigate whether they stimulate primary productivity, signaling that mats are limited in these solutes. Nutrients in Highborne Cay were high in nitrogen relative to P, with N:P as high as 30. There was no difference in nutrient flux or productivity among mat types, and the addition of nutrients did not change mat productivity. These observations suggest that mat development in Highborne Cay is not limited by nutrients, but more likely structured by external physical factors such as the rate of turbulent flow which may limit the recruitment of competitors such as macroalgae and benthic branching diatoms.

MICROCOSM STUDIES OF NUTRIENT CYCLING IN BAHAMIAN STROMATOLITES

BY

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What goes into a thesis pales compared to what doesn't … and is nothing without it.

This stack of pages rests on a pedestal not only of background work and experiments both failed and fruitful – but also of constant support by an amazing community. I'd like to thank my extended Solomons family for all their love, support and encouragement throughout this process; it's changed my life in no small way.

I owe a debt to my advisors Roberta Marinelli and Rodger Harvey for taking a chance on a wayward English major who wanted to learn a completely different field, and then patiently waiting through all the growing pains such a change includes.

I'd like to thank my mom for being on this journey with me every step of the way. I'd also like to thank George Waldbusser, who taught me a great deal of what I know about science.

As I write this final piece, I have to acknowledge what publications and dissertations never include: For several years in Solomons the right group collected at the right time. Great work came from the collaboration of talented minds – though more than that, relationships came about. And weddings. And children. Death and heartache. Happiness, friendship and support. In other words: community, and life made rich because of it. I'd like to acknowledge everyone who was a part of this. You will never leave me.

This thesis is simply what happened in the meantime.

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INTRODUCTION

Stromatolites are layered, laminated deposits of calcium carbonate that are formed by a combination of microbial and mineral processes that include lithification of the mat structure. They have been found in the fossil record as far back as 3.5 billion years B.P. in the Precambrian, and are the oldest macroscopic evidence of life on earth (Grotzinger and Knoll 1999). Modern mats are thought to be analogs of those first extant biotic communities (Monty 1976), therefore the study of modern mats could provide a window to the past (Reid, Visscher et al. 2000). At one time, stromatolites were ubiquitous; however, 500-600 million years ago they began to decline, perhaps due to factors such as a shift in ocean chemistry brought on by the increase in oxygen, and the rise of competing organisms (Riding 1982; Semikhatov and Raaben 1996).

While today soft microbial mats are still commonly observed, laminated stromatolites are relatively rare (Cohen, Castenholz et al. 1984) and found in environments that are physically dynamic, geochemically unusual, and/or nutrient-poor (Paerl, Joye et al. 1993). These environments include hypersaline lakes in the continental United States, inter- and sub-tidal regions of the Bahamas, and a hypersaline embayment on the western shore of Australia. Interestingly, such environments may also limit competitors. While these mats are geologically defined as stromatolites, the microbial communities that constitute any given mat population seem as diverse as the environments in which they're found (Visscher and Stolz 2005). One of these environments is Highborne Cay in the Exumas, Bahamas (76°49'W; 24°43'N) – the study site for this project.

Bahamian stromatolites form in an open marine environment of normal salinity (Reid, Visscher et al. 2000). In Highborne Cay they grow on the landward side of an algal-ridge fringing reef (Reid, MacIntyre et al. 1999) characterized by crashing waves and high turbulence and kinetic energy. Their formation is microbially-mediated – and is thought to include trapping and binding of suspended sand grains (ooids) facilitated by the production of copious and sticky exopolysacchride (EPS) on the mat surface (Decho, Visscher et al. 2005).

The microbial communities that constitute Highborne mats are composed of four main functional groups: cyanobacterial photoautotrophs, sulfate reducers (SRBs), sulfide oxidizers (SOBs), and aerobic heterotrophs (Visscher, Reid et al. 1998; Visscher and Stolz 2005). It was previously thought that all microbial mat communities exist in a steep gradient of oxygen, sulfate, light, and redox potential on the order of a few mm and that the trophic groups (primary producers, consumers, and decomposers) are layered and interact across that gradient (Stolz, Botkin et al. 1988). However, recent studies suggest that most activity is found in the surface layer (Visscher and Stolz 2005). The microbial consortia in Highborne mats carry out specific metabolic processes that lead to the trapping and binding of sand grains and, ultimately, to $CaCO_3$ production and lithification which allows stromatolite growth and accretion (Visscher and Stolz 2005).

This surface community is the "living" component of the mat, and is a dynamic area, where the populations undergo changes in relative abundance (Reid, Visscher et al. 2000). As the mat accretes, its microbial assemblages migrate in order to remain in the photic zone, leaving behind a record of alternating layers of lithification that reflect previous fluctuations in the relative composition of the microbial communities.

Consequently, the diagenetic features of these sub-living layers can be linked to the communities that formed them, allowing a chronology to be developed (Reid, Visscher et al. 2000). Highborne mats have been classified according to the state of the microbial assemblages in this outermost "living" layer (Reid, Visscher et al. 2000), yet the names and characteristics of these classifications have varied with increasing study. For the purpose of this investigation, we outline four main types, as described below.

Cyanobacterial mats (CYN) consist primarily of the filamentous cyanobacterium *Schizothrix gebeleinii* (Golubic 1991), which entrains itself among the sand grains and helps reinforce the mat's structure. These cyanobacteria are diazatrophs and may be a necessary starting point for the community establishment and growth in oligotrophic environments.. These mats have three sources of N available to them: (1) N recycled within an existing mat (both organic and inorganic), (2) DIN from external sources such as the water column and sediments, and (3) N fixation. Prior investigation has calculated that N-fixation and uptake of N from the water column provides only 20% of the mat's N requirements, and that remineralized N is the stromatolite's greatest N source in Highborne Cay (Steppe, Pinckney et al. 2001). Uptake and biomass production is countered by losses of N from within the mat via denitrification, grazing, and transport by way of diffusion, erosion, advection, and/or burial (Joye and Paerl 1994). Schizothrix may be involved in generation of fixed nitrogen through N fixation.

Diatom-dominated mats (DIA) are formed via diatom colonization of CYN mats. They include benthic branching diatoms that (along with macroalgae of the genera *Batophora* and *Chondria* (Andres and Reid 2006)) colonize the exterior of the mat, likely

because of the hard substrate the mats provide, and possibly because of the nitrogen generated by the underlying diazotrophs.

EPS mats (EPS) are characterized by a relatively thick biofilm of exopolysacchride that is secreted by the cyanobacteria and covers the mat surface, filling the spaces between the grains. A thicker, more developed layer of *Schizothrix* often underlies the EPS, and embedded in it are aragonite crystals the precipitation of which is facilitated by heterotrophs and are the most conspicuous evidence of heterotrophic presence within the mat (Stolz 2003). At this point – though present – heterotrophs are sparse and less developed.

EPS may aid in mat development in three main ways. First, it may stabilize and protect cells and sediment against resuspension from the high-energy wave environment in which the mats persist (Decho, Visscher et al. 2005). Second, it may provide a chemically protective microenvironment by binding and concentrating Ca^{2+} and Mg^{2+} ions from the surrounding water, both of which have been shown to facilitate aragonite formation in seawater (Kawaguchi and Decho 2002). Finally, heterotrophs degrade the EPS , releasing Ca^{2+} ions and influencing $CaCO_3$ precipitation, and subsequently mat lithification since heterotrophs are also responsible for $CaCO_3$ precipitation. This process is more prevalent in the more developed mat type described below.

The final type of Highborne mat is a mature mat (MAT), because at this stage, the four microbial populations (cyanobacteria, SOBs, SRBs and aerobic heterotrophs) are all present, and form an efficient consortia. Figure 1 (Visscher and Stolz 2005) illustrates the chemical balance between the mat's consortia, which heavily relies on suphur cycling by SRB's and SOB's in order to complete the geochemical processes that ultimately

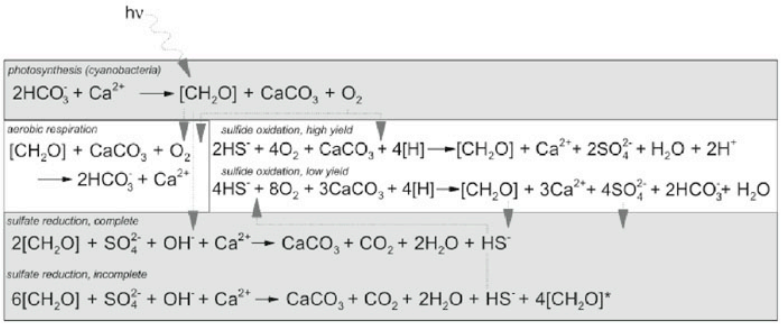

Figure 1 : A diagram of the major groups of microbes in a Highborne stromatolite and their associated chemical pathways. Taken from Visscher and Stolz 2005.

generate carbonate and lithify the mat. MAT mats are characterized by abundant coccoid *Solentia* sp. of cyanobacterium that bore into sand grains, allowing the grains to be more effectively fused by the abiotic precipitation of aragonite crystals caused by an abundance of CO_2 and Ca^{2+} due to heterotrophic activity (Decho, Visscher et al. 2005). At this stage, the stromatolites are thought to require little more than light to function (Visscher and Stolz 2005).

The transition between CYN, DIA, EPS, and MAT mat types is a complex dynamic, most likely essential to mat growth and survival because community fluctuations facilitate lithification and therefore provide structure (Golet and Ward 2001). However, the causes of such transitions among mat types are poorly understood; equally unclear is the extent to which internal or external factors influence stromatolite growth and development and, if so, how. Wave energy, eukaryotic competition, burial (Perkins, Underwood et al. 2001; Perkins, Kromkamp et al. 2007) and nutrient supply may all play roles in mat development.

Wave energy provides flow that is thought to be essential for stromatolite development in at least two ways. First, it suspends sediment, providing sand grains that may settle and "stick" to the exopolysaccharide layer. Without sediment supply to the mat surface, little accretion and growth is thought to be able to occur(Andres and Reid 2006). Suspension is intrinsically tied to wave energy since below a certain threshold velocity, no sediment is suspended in the water column (Eckman, Andres et al. 2008). Second, wave energy is thought to play a possible role in preventing the establishment and growth of eukaryotes that colonize the mat (Reid, Visscher et al. 2000), utilizing its

surface as a suitable substrate. Such colonization has the potential to subsequently outcompete the mat for nutrients, and perhaps preventing lithification.

Eukaryotic competition likely influences stromatolite development since diatoms, chlorophytes and others may colonize the surface of mats. These eukaryotes have been found in large enough quantities that some have hypothesized that Highborne mats are constructed chiefly by them (Riding, Awramik et al. 1991), an assertion that is not universally accepted (Pinckney and Reid 1997). Nevertheless, the relative abundances of these constituents in relation to the microbial populations within the mat must be taken into account in any calculations of chemical cycling, as eukaryotes may compete for nutrients, light, or micronutrients.

Competition for resources may be very important in mat development, since stromatolites may be limited by one or more nutrients, such as N, P, Fe, and other trace constituents (Paerl, Steppe et al. 2001). As is typical of some marine environments, N is the nutrient most commonly found to limit growth (Joye and Paerl 1993; Pinckney, Paerl et al. 1995). Previous studies performed flux measurements on the Highborne stromatolites (Pinckney and Reid 1997) and nutrient addition experiments on nearby Bahamian mats (Pinckney, Paerl et al. 1995), and concluded that additions of a combination of P and reduced organic carbon (glucose) significantly increased rates of N-fixation by the mats. These findings suggest P-limitation, however more research is needed to determine whether this is the case in Highborne Cay.

The goal of this thesis is to better characterize the chemical cycling between the mats and their environment, by investigating possible differences in nutrient fluxes among stromatolite types. To these ends, the following questions were addressed:

Q1: What external sources of nutrients are available to Highborne stromatolites?

Q2: Are Highborne mats limited by one or more nutrients?

Q3: Do productivity and nutrient fluxes differ across mat types due to the differing activity and requirements of their microbial constituents?

This study found that the waters around Highborne Cay are not oligotrophic, but can contain high concentrations of inorganic nutrients, as high as 20-30 μM of nitrogen, and that nutrient regeneration in sediments may contribute to the elevated water column nutrient concentrations. It is therefore unlikely that nutrient supply (both in magnitude or N:P ratio strongly affects on N-fixing cyanobacteria. Furthermore, nutrient concentrations in the study site do not differ significantly from those on the western (opposite) side of the cay – an area in which no stromatolites occur.

Flux experiments showed that Highborne mats exchange solutes with the environment, and may not operate as a "closed system". Experiments observed no difference in productivity or solute fluxes by mat type, and the addition of P and Si elicited no consistent change in productivity, suggesting that mats are not limited by these nutrients despite their low concentrations relative to N in the system. In light of these findings, stromatolite formation in Highborne Cay is likely a function of an internal response to external, physical mechanisms such as water flow and burial that limit the growth of competitors such as diatoms and macroalgae. It is unlikely that the nutrient

regime in Highborne Cay is the factor responsible for making this site a suitable environment for stromatolite persistence by virtue of oligotrophy.

CHAPTER 1: Sediment Nutrients

Introduction

Most stromatolites exist in niche environments such as highly saline ponds and embayments (Paerl, Joye et al. 1993) that are sheltered from high tidal flows or wave energy (Monty 1976; Cohen, Castenholz et al. 1984). The stromatolites of Highborne Cay are some of the only known to exist in a normal marine environment (Reid, Visscher et al. 2000) that has high wave energy, significant sediment transport (Eckman, Andres et al. 2008) and relatively low amounts of benthic flora and fauna (Andres and Reid 2006).

Nutrient availability has been thought to play a significant role in stromatolite persistence in surrounding areas (Pinckney, Paerl et al. 1995). Low nutrient concentrations may limit the growth of microbes within the stromatolite layers (Steppe, Pinckney et al. 2001), as well as the growth of epibionts that might otherwise overgrow and outcompete the resident microbial populations within each stromatolite head (Reid, Visscher et al. 2000). As part of a larger study to examine the physical, chemical, and biological dynamics that promote stromatolite persistence in this environment, nutrient concentrations were measured episodically in the water column and sediments in the vicinity of stromatolite reefs.

Sediment core incubations were performed to investigate the extent to which nutrient regeneration might contribute to measured nutrient concentrations at the study site in Highborne Cay. The data obtained form core incubations was used to help determine if sediments impact nutrient availability in the water column. For example, if sediments are a source of phosphate and uptake from sediments is rapid, the signal may not be detected in the water column data (chapter 2). This would suggest that phosphate

is being removed by the biomass resident in the mats or by abiotic processes. If, however, sediments are not a potential source of nutrients, allochthonous processes may control nutrient levels in the water column.

Methods

July 2005 a 6-cm core ~4cm in diameter was collected from the intertidal zone of the study site (76º49'10"W; 24º42'45"N) as part of ongoing studies. Sampling was done using an acrylic corer fitted with sampling ports at vertical intervals of 1cm, with careful attention to the retention of porewater (figure 2). The core was returned to the laboratory and incubated in a running water bath in a natural light/dark cycle. Porewater samples (< 1 ml) were taken at 2 cm intervals at 4 depths: 0, 2, 4, and 6 cm, at two different time points: T=0 (immediately upon return to the laboratory) and T=24h, using a 3 ml syringe and 18 gauge needle. Sample were passed through a 0.45 µm Acrodisc syringe filter (Pall Corporation), stored in a sterile 5-ml vial (Evergreen Scientific), and frozen at –20°C. These measures provide an indication of whether sediments are a source or sink for nutrients and the extent to which they may play a role in the nutrient dynamics of the system and supply to the stromatolites.

In December 2005, a more detailed time series study of sediment nutrient regeneration was conducted. Two 20-cm sediment cores were obtained on two different days– (8 Dec. and 9 Dec.) – approximately 10m shoreward of actively growing stromatolite and coral reef complexes (figure 2). Samples were taken using a PVC pipe 25 cm in length with a diameter of 2.54 cm. Both cores were taken at low tide, approximately ~30cm above the waterline. Upon recovery, the porewater was allowed to

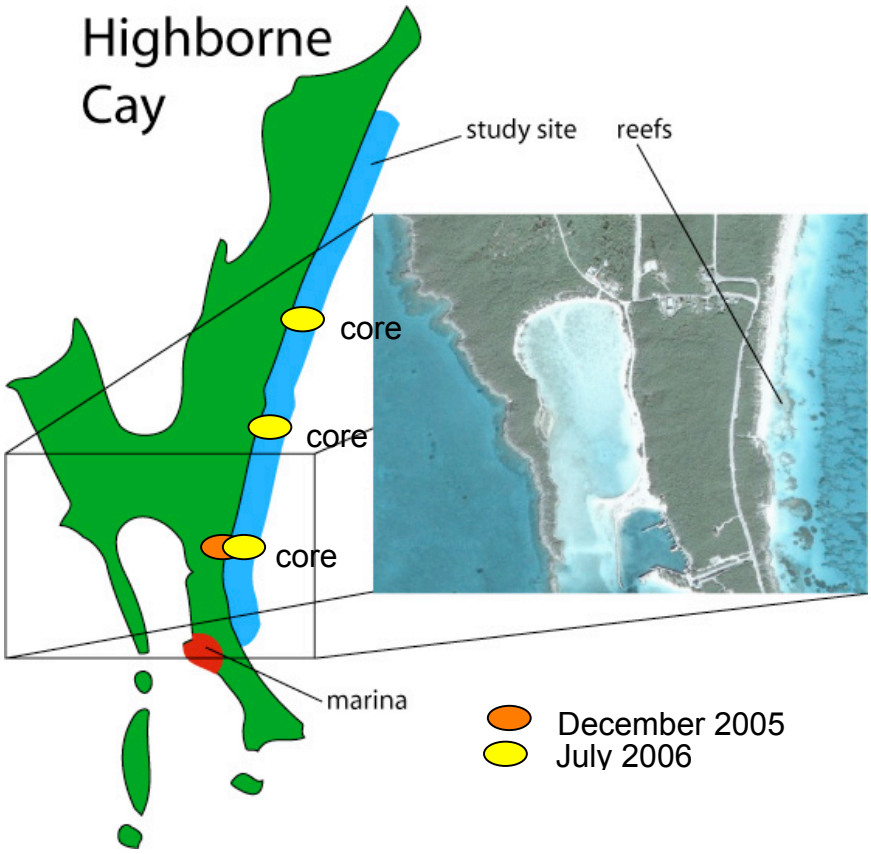

Figure 2 : A map of Highborne Cay and study sites. In red is the marina and site of the incubations and microcosms on the west side of the island. In blue along the eastern shore is the study site – a 2-km stretch of beach with stromatolites occuring mostly near the southern end.

drain from the core, with a portion collected during drainage. 2-cm subsections of the cores were sampled at three depths: 0-2 cm, 9-11 cm, and 18-20 cm. Each sediment subsection was homogenized on a plate, divided into five equal aliquots (each approx. 0.5 ml), deposited into five pre-weighed 5-ml snapcap vials, and weighed. Each vial was filled with porewater retained from the core until no air space remained, then sealed and reweighed. The overlying water salinity was recorded for later volume calculation from weight. The vials were incubated in the dark and shaken intermittently (every 30 minutes) to ensure the samples were well mixed. Samples were sacrificed every 2h for a total of 10h (n=1 for each time point) by using an 18G needle attached to a syringe to extract the porewater from within the sediments. Porewater was then passed through a 0.45 μm Acrodisc syringe filter, placed into a sterile 5-ml vial (Evergreen Scientific), and frozen.

On 25 July 2006 three additional cores were collected at 1200 from three different sites (figure 2). Core 1 was taken from the same site as the prior December samples (76º49'10"W; 24º42'45"N), core 2 was taken from a second site (76º49'9"W; 24º42'54"N) also ~10 shoreward of actively growing reefs, and core 3 was taken from a site farther northward on the beach (76º49'6"W; 24º43'9"N) containing no active mats. The same PVC corer was used to collect the samples, with cores taken at low tide ~30 cm above the tide line. Porewater was allowed to drain from each with none retained, the sediments were extruded, and 2-cm subsections were taken at 0-2, 6-8, and 12-14 cm. Each section was homogenized on a plate, and equally divided (~0.5 ml portions) into five pre-weighed 5-ml snapcap vials. Vials were each filled with 2.5 ml of overlying water that was collected from the study site and passed through a 0.2 μml capsule cartridge filter so that airspace remained. Samples were incubated in a natural light

regime on the deck of the study's research vessel docked in the marina at the west side of the cay. Vials were destructively sampled every 12 hours for 48h by porewater extraction using an 18G needle attached to a syringe, passed through a 0.45 µm Acrodisc syringe filter, into a sterile 5-ml vial (Evergreen Scientific), and frozen.

July 2005, December 2005 and July 2006 samples were analyzed for NH_4, NO_3, and PO_4, and Si. Analyses were performed on a Westco SmartChem Discrete Analyzer (Westco Scientific Instruments, Inc.). Analytes included NH_4 according to a modification of the phenyl-hypochlorite method (Koroleff 1976), NO_3 using the cadmium reduction method (Strickland and Parsons 1972), PO_4 according to a modification of EPA standard 365.2 and Eton et al. 1995, and silicate according to (Strickland and Parsons 1972). Concentrations were normalized to sediment wet weight and scaled to surface area of core by assuming 50% porosity. Porosity was calculated based on wet vs. dry weight using 1.02 g/ml as the density of 37.5 psu seawater and 2.83 as the density of carbonate sands.

Data Analysis

For December 2005 and July 2006 samples, the change in concentration over time ($\Delta C/\Delta t$) was determined for each depth using standard linear regression techniques to assess overall changes in nutrient concentrations. Each time point had one sample from each depth. Because core sections were incubated separately, they provide independent (but isolated) measures. All slopes of concentration changes over time were examined by setting a benchmark r-square value > 0.30 for acceptable trends. Slopes with r-square < 0.30 were visually inspected, and those that show consistent trends indicating low

variance and little change over time, i.e. no net flux, were also included. These net rates (nmoles g^{-1} hr^{-1}) were normalized to surface area (nmoles $cm^{-2}hr^{-1}$), and integrated over the depth of the core to be plotted as whole core net regeneration rates (nmoles cm^{-3} h^{-1}).

Results and Discussion

Results of core sample measurements taken in July 2005 show measurable concentrations of nitrogen species (particularly NO_3) with concentrations as high as 17 μM NH_4 and 19 μM NO_3, suggesting that the sediments are a potential a source of nutrients to the water column (figure 3). NH_4:NO_3 ratios in the T=0h sediment porewater averaged 0.56, whereas water column averages of the same ratios were 2.75 (chapter 2) with much more NH_4 relative to NO_3 Silicate data for all cores were below detection limits of 0.01 μM, and therefore are not further discussed.

Total ammonium concentration declined over the 24-hour incubation, while nitrate rose, suggesting net nitrification. PO_4 in sediment porewaters did not differ from water column concentrations (see chapter 2), and remained consistently low throughout the incubation. The nutrient concentrations are highly variable at depth, likely due to the significant advective flow that occurs within these carbonate sands, as well as sediment resuspension (Eckman, Andres et al. 2008). High flow through the sediments may lead to a more vertically homogeneous microbial population and therefore no changes in rates across depths would be expected.

Figure 4 shows a time series plot of NO_3 for all depths of core 1 of the December 2005 experiments. Time series such as this one, and the regressions obtained from them

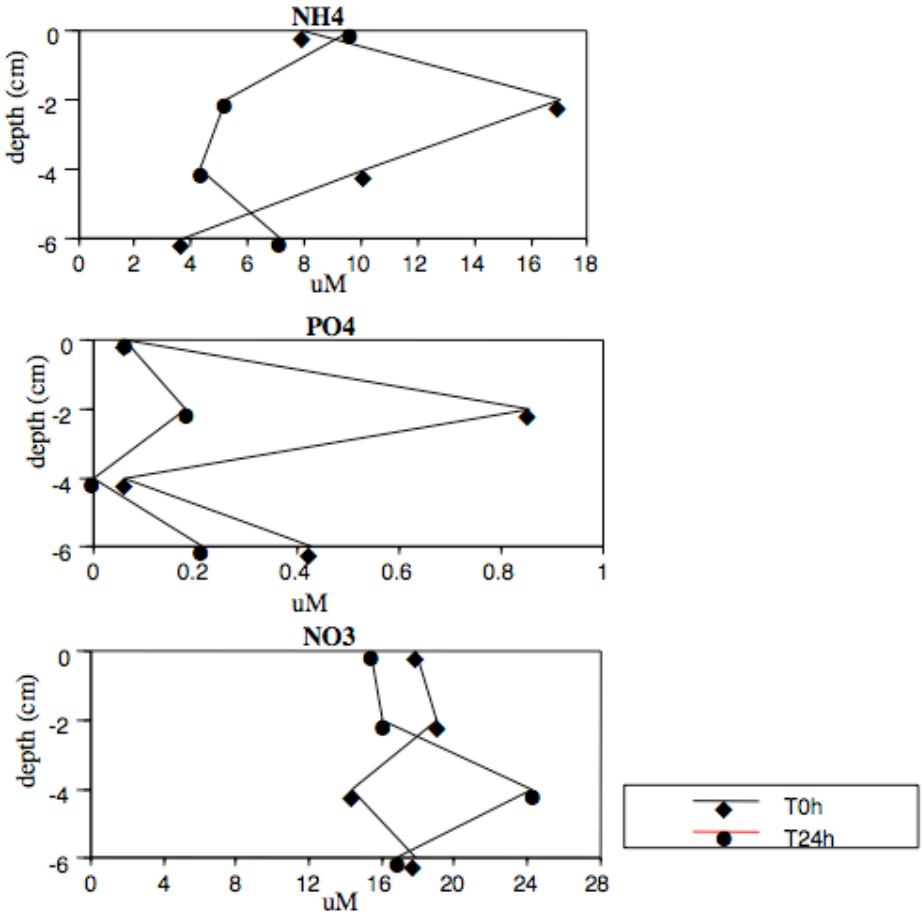

Figure 3: Porewater concentrations from July 2005. Porewater samples were taken at T=0 and T=24 and the core was incubated in a natural light/dark cycle. The bottom right plot is change in solute over time, showing that NO_3 increased and NH_4 decreased, suggesting possible net nitrification.

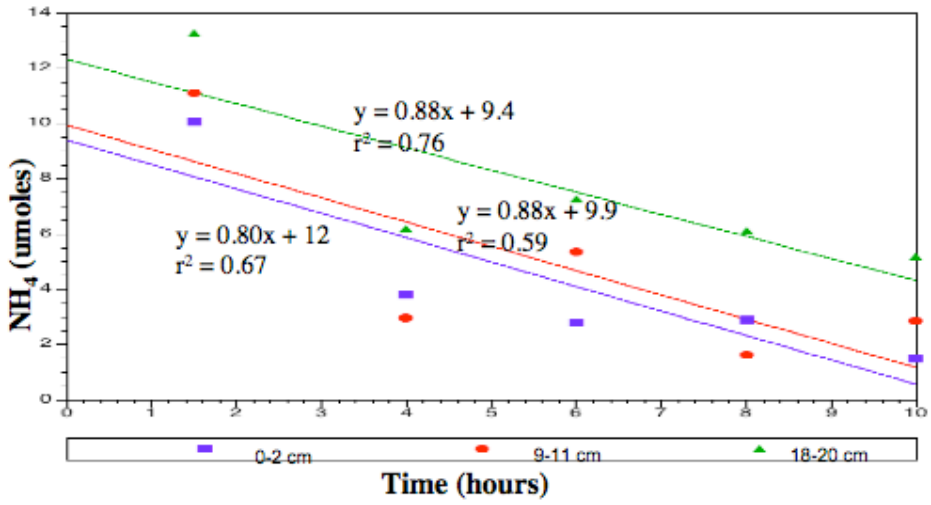

Figure 4: An example of a time series plot of NO_3 for all depths of core 1 during July 2006 experiments.

were used to estimate removal and formation rates for the December 2005 and July 2006 experiments. Table 1 lists r-square values, slopes, and *p* values for all solutes, cores, and both research periods. Of the nitrogen species, NH_4 concentrations were the most variable and therefore yielded fewer significant trends, most likely because this species is highly reactive (Wild, Woyt et al. 2005) and also undergoes adsorption/desorption (Mackin and Aller 1984). In contrast to NH_4, NO_3 yielded more significant trends, perhaps due to lower rates of NO_3 production, and preferential uptake of ammonium. However, these NO_3 trends show neither consistent uptake nor release.

Overall, net rates from the December 2005 sediments (figure 5) differ greatly among the replicate cores, with a net nitrogen uptake in core 1and net production in core 2. All incubations showed a lack of change in PO_4. This inconsistency between the two cores suggests that Highborne sediments are spatially and temporally heterogeneous. This might be the resilt of the heterogeneous growth of reef structures – both buried and unburied – at the study site and perhaps also episodic injection of organic matter into sediments, via advective flows.

July 2006 cores yielded clearer PO_4 trends with much higher r-square values. This may be because the processes that liberate PO_4 may happen over longer timescales than the relatively quick recycling of Highborne's nitrogen species that may lead to less viable rates (table 1). Nitrogen species (particularly NH_4) are highly bioavailble and thus quickly recycled, while PO_4 is bound by adsorption and competes with carbonate during the production of $CaCO_3$ to form $CaPO_4$ (Entsch, Boto et al. 1983). Once complexed with Ca^+ ions, PO_4 requires a large shift in overall water column oxygen or pH to be released (Krom and Berner 1980; Stumm and Morgan 1996). This is a change that may only be

bold=significant

season	core	depth	adj r2 nh4	slope nh4	p value nh4	adj r2 no3	slope no3	p value no3	adj r2 po4	slope po4	p value po4
dec	1	0-2	0.1186	-1.4	0.572	**0.7961**	-0.8845	0.046	0.4288	0.0018	0.139
dec	1	9-11	**0.5013**	-6.64	0.183	**0.5941**	-0.8781	0.127	**0.586**	-0.0282	0.081
dec	1	18-20	**0.5596**	-3.1226	0.146	**0.6661**	-0.8002	0.087	0.033	-0.0184	0.365
dec	2	0-2	**0.5299**	9.2959	0.178	**0.9332**	0.636	0.025	0.013	0.0004	0.381
dec	2	9-11	**0.5722**	-3.7946	0.823	0.0646	-0.2661	0.753	-0.318	-0.0024	0.8653
dec	2	18-20	0.1245	-4.0474	0.603	**0.795**	0.1278	0.341	-0.135	0.005	0.521
july	1	0-2	**0.237**	-0.0326	0.405	**0.0005**	0.0001	0.858	**-0.222**	0.0005	0.638
july	1	6-8	0.129	-0.2793	0.296	-0.1	0.0157	0.488	-0.308	-0.0046	0.826
july	1	12-14	-0.33	0.0047	0.979	**0.825**	0.0445	0.06	-0.26	0.0024	0.705
july	2	0-2	-0.132	-0.0681	0.518	**0.601**	0.0164	0.077	**0.172**	0.0016	0.269
july	2	6-8	-0.457	-0.0288	0.831	**0.431**	-0.0009	0.212	**-0.178**	0.0007	0.574
july	2	12-14	-0.169	0.0134	0.562	**0.669**	-0.0103	0.057	**-0.315**	0.0042	0.85
july	3	0-2	-0.333	0.0011	0.986	0.013	0.0022	0.38	**0.426**	0.0018	0.14
july	3	6-8	**-0.145**	-0.0017	0.534	**-0.328**	-0.0139	0.923	**0.434**	0.0017	0.137
july	3	12-14	0.081	-0.0028	0.329	**0.442**	0.0022	0.134	**0.766**	0.0013	0.033

Table 1: r-square values and slopes (nmoles*g^{-1}*h^{-1}) of rates of each solute for each core depth. The values in bold are significant [either r-square > 0.3 or a visible (though flat) trend below that threshold]

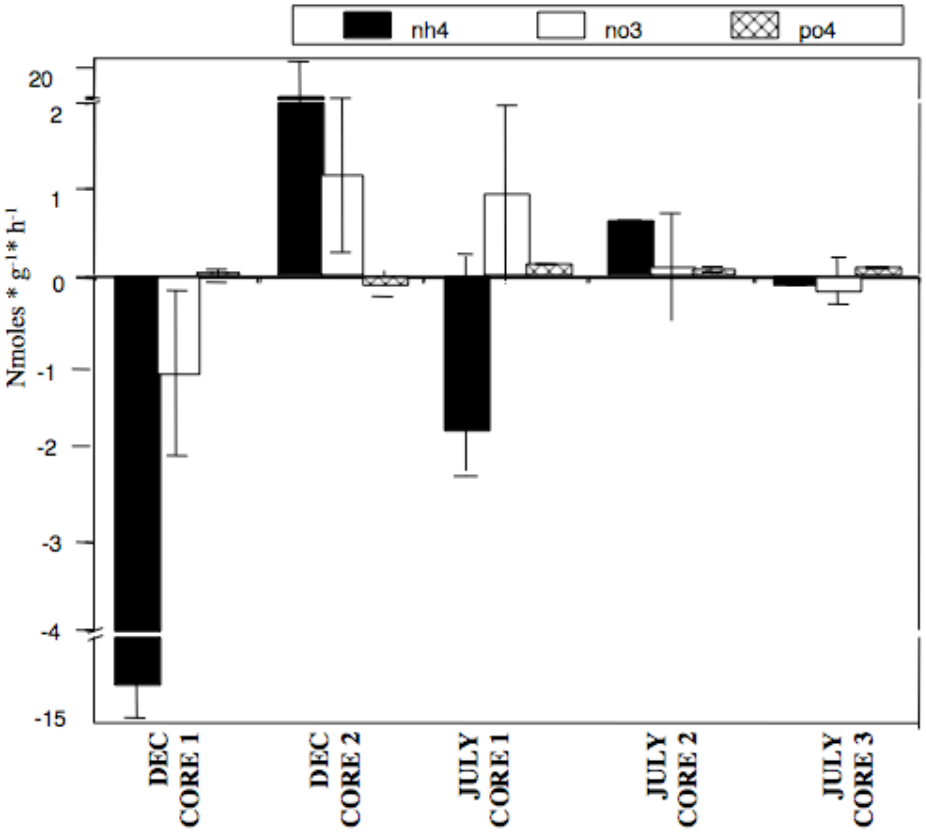

Figure 5: Totals of net potential rates per core for both December and july. Notice that December fluxes are an order of magnitude higher most likely due shorter incubation times. Rates are averaged over all depths.

possible on timescales on the order of the July 2.5d incubation, since this incubation length along with light/dark cycles may have promoted phototrophs whose photosynthesis/respiration may have sufficiently altered the oxygen levels, pH, or both, possibly liberating PO_4. Slopes of PO_4 for July (Table 1) are significant and positive, indicating a net flux out of the sediments and suggesting that phosphate may be stored within the sediments and released either abiotically by desorption, or biotically via organic matter remineralization.

July 2006 time series data yielded fewer changes in net concentration than Dec 2005. Net rates were also of a smaller magnitude than December rates (figure 5). Possible explanations include 1) the normal diel (light/dark) conditions in which the samples were incubated, and 2) much longer incubation times. Changing the light regime over a long time period may have promoted the dominance of oxygenic and anoxygenic phototrophs to take up available nutrients and incorporate them into biomass. These daytime reactions may mask the anaerobic (typically dark) signal of biomass remineralization and nutrient diagenesis, negating any large fluxes and rendering the supply expected from these net rates (figure 5) similar to the ambient water column concentrations. This incubation methodology, rather than any seasonal changes in magnitude, likely explains the difference in magnitude between December 2005 and July 2006 net rates within the sediments. However, the December 2005 incubation method is likely more reliable because it better mimics *in situ* conditions both in dark regime, as well as length of incubation.

Conclusion

The main goal of these sediment measurements and rate experiments was to discover whether the sediments contribute to the elevated concentrations of inorganic nutrients measured in water column samples (chapter 2). December incubations yielded net rates that suggest high variability within the sediments. In particular, December cores yielded rates with higher r-square values and viable trends for nitrogen species, with positive net rates of production possible.

Such results, while highly variable, do suggest the sediments can be a potential source of nutrients found in the July 2005 opportunistic core data (figure 3) and contribute to the elevated water column nutrient concentrations in Highborne Cay (chapter 2). In this case, stromatolite growth might be enhanced through nutrients supplied close to the sediment surface.

One possible source within the sediments of high inorganic nitrogen porewater concentrations and the elevated ambient nitrogen supply in Highborne Cay is the remineralization of buried organic matter beneath the sediments in the form of buried reef and mat material. Coral reef areas have been shown to have primary productivity 1 to 2 orders of magnitude higher than surrounding oligotrophic waters (d'Elia and Wiebe 1990; Adey 1998) and therefore a large amount of biomass in the system is available for remineralization. The Highborne study site is one of high wave energy and, therefore, high sediment resuspension and transport. It is not uncommon for entire reefs to become buried or uncovered in small timeframes on the order of 0.5d. The length of the Highborne Cay eastward-facing beach on which stromatolites are found is filled with a complex of stromatolitic, thrombolitic, algal and coral reefs (Andres and Reid 2006) and

therefore a great deal of biomass exists at the study site. At any one given time, a large portion of the site's biomass is buried beneath the sediment, and thus potentially available for remineralization. The site's high turbulance and resuspension might further increase remineralization rates (Stahlberg, Bastviken et al. 2006). This process has been observed in the Red Sea, where porewater nutrient concentrations exceed those of the overlying water by factors of 15-80 (Rasheed, Badran et al. 2002), and shown to be supplied by elevated nutrient levels in the regenerative spaces of the reef framework and coral sand.

CHAPTER 2: Water Column Nutrients

Introduction

As part of a larger study investigating the physical, chemical and biological dynamics that control stromatolite persistence in Highborne Cay, inorganic nutrients in the water column were measured episodically in both reef and non-reef areas on either side of the Cay. Nutrients may be an important factor in stromatolite persistence either as a source for microbes (if present) or by limiting epibionts that might otherwise outcompete the mats for resources and therefore afford the microbes within the mats a competitive advantage (if absent).

If Highborne Cay has low ambient inorganic nutrient concentrations, the microbial consortia that comprise the living portion of the mats may have a competitive advantage (Visscher and Stolz 2005), since stromatolite microbial populations may take advantage of cyanobacterial nitrogen fixation (Steppe, Pinckney et al. 2001). If, however, abundant nutrients exist in the Highborne system, the mats' internal recycling and nitrogen fixation may not afford them as much of an advantage, and therefore nutrients would play little or no role in aiding the existence of mats at the site, and the reason for stromatolite persistence at Highborne Cay may lie elsewhere.

Methods

In order to capture potential changes in nutrient concentrations at the study site, samples were taken opportunistically at different tidal heights and times of day. To explore why mats appear presently restricted to the east side of the cay, samples were taken from both the east study site, as well as the west side of Highborne where a marina

is located, wave activity is reduced relative to the east side of the cay, and no stromatolites are found.

July 2005 samples were taken from the west side of Highborne Cay (76º49'22.24"W; 24º42'31.62"N), as part of a pilot study to test flexible flux chambers in the field. These samples were taken near where the larger research group (of which this study is a part) normally set up sample incubations involving stromatolite heads. Samples were taken opportunistically according to the times in table 2. Seawater was collected by clean, acid-washed 5-ml syringe, then passed through a 0.45 µm Acrodisc syringe filter, placed into a sterile 5-ml vial (Evergreen Scientific), and frozen until analysis.

December 2005, triplicate water samples were collected twice daily at 1030 and 1730 on 5-11 December from a site (76º49'10"W; 24º42'45"N) on the east side of the Cay that contained abundant stromatolitic and coral reef structures. The static timing of sample collection allowed comparison of water column nutrients over different tidal heights in a diurnal tidal cycle, as measured by a DOBIE wave gauge (NIWA Instrument systems) which records wave and water height via pressure. Samples were processed as described above.

In July 2006 additional samples were collected daily at 1030 and 1630 on 24-31 July from another study area at the eastern study site (76º49'10.91"W; 24º42'47.63"N). In addition, triplicate samples were collected every two hours on 28 July from 1030-1830 to investigate possible variability over shorter timescales. For this time period, all samples were collected ~1m from a DOBIE wave gauge which records pressure (tidal height). Samples were processed as described above.

sample	date	time	sample	date	time
1	21-Jul	1230	10	23-Jul	1313
2	21-Jul	1430	11	23-Jul	1526
3	21-Jul	1630	12	23-Jul	1535
4	21-Jul	1900	13	24-Jul	1730
5	22-Jul	1100	14	25-Jul	830
6	23-Jul	730	15	25-Jul	1010
7	23-Jul	925	16	25-Jul	1210
8	23-Jul	954	17	25-Jul	1410
9	23-Jul	1122	18	25-Jul	1620

Table 2: Sampling scheme of overlying water of Marina for July 2005 as part of a larger flux study from that period.

Samples were analyzed for alkalinity, NH_4, NO_3, and PO_4, and silicate on a Westco SmartChem Discrete Analyzer (Westco Scientific Instruments, Inc.) – alkalinity according to a methyl orange method (EPA 310.2), NH_4 according to a modification of the phenyl-hypochlorite method (Koroleff 1976), NO_3 using the cadmium reduction method (Strickland and Parsons 1972), PO_4 according to a modification of EPA standard 365.2 and Eton et al. 1995, and silicate according to Strickland and Parsons 1972.

Solute concentrations for the two sample periods were analyzed using two separate one-way ANOVAs with concentration as the dependent variable and season and location (beach vs. marina) as independent variables. A natural log transform was used to meet assumptions of normality for the ANOVA. The potential relationship between tide and nutrient concentrations was examined using regression analysis (α=0.05) first with tide (height above mean low water) vs. nutrient concentration, and second with tide vs. the coefficient of variation (CV) of each time point's triplicate sample. All calculations were done using SAS 9.1. Tide height was included to investigate whether solute concentrations may be tidally driven either by advective pumping of solutes out of the sediments, or by other tidal transport and supply of allochthonous nutrients.

Results and Discussion

July 2005 nutrient concentrations over all samples taken on the west side of the cay (figure 6) averaged 6.13 µM ±7.94 (mean ± 1 standard deviation) for NH_4, 2.14 µM ±1.61 for NO_3, and 0.32 µM ±0.82 for PO_4. December 2005 averages for the east side of the cay were 5.00 µM ±6.46, 2.1 µM ±1.06, and 0.40 µM ±0.10 for NH_4, NO_3, and PO_4 respectively. July 2006 averages for the east side of the cay were 5.09 µM ±6.73, 0.76

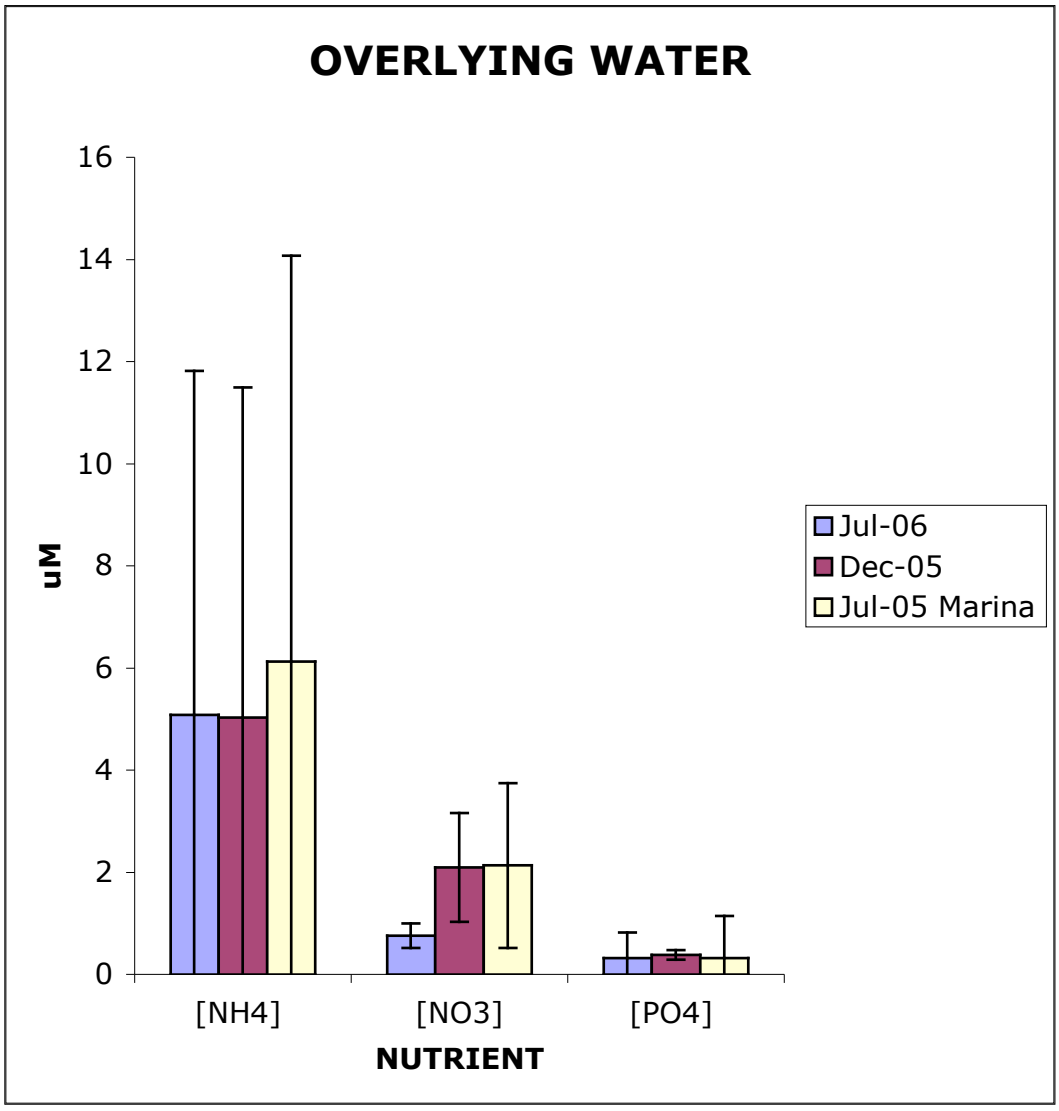

Figure 6: Total averages of all locations and entire study periods of water column nutrient concentrations for two sites (marina and study site) over three sampling periods. The only statistical difference between sites was NO_3 concentrations from July 2006 and December 2005 (ANOVA $F_{2,62}=36.55$ $p<0.001$).

μM ±0.24, and 0.33 μM ±0.49 for NH_4, NO_3, and PO_4 respectively. Silicate measurements were all below detection limits (0.01 μM) and therefore not further discussed.

Previous studies have found DIN and PO_4 concentrations at the study site below 2 μM and 1 μM respectively (Steppe, Pinckney et al. 2001). The 2005 and 2006 data measured here suggest that nutrients in the system are higher than those measured previously, particularly with regard to nitrogen species. However, N:P ratios are highly variable, from ~4 (well below Redfield and therefore nitrogen limited) to over 30 (which is phosphorus limited and close to very high ratios of ~50 recorded in areas of the Mediterranean known for high phosphorus limitation (Laroche and Breitbarth 2005).

Typical N:P ratios recorded earlier in the Bahamas are 10.2-14.0 (Lapointe, Littler et al. 1992), well within nitrogen limitation under Redfield assumptions. The elevated N concentrations in Highborne suggest that cyanobacterial nitrogen fixation does not offer the stromatolites a competitive advantage in an otherwise N-deficient environment. Conversely, the high rate of diazotrophy within the stromatolites (Steppe, Pinckney et al. 2001) by cyanobacteria that are the pioneers of Highborne mat communities (Reid, Visscher et al. 2000) is a possible indicator that the excess nitrogen in the system may not be available to the microbes within the mats, possibly due to diffusion limitation, or other unknown factors. However, rates of N-fixation measured in the mats did exhibit a diel cycle (Steppe, Pinckney et al. 2001), and nitrogen that is fixed at night may not be available to the mats during daytime primary production.

Given the extremely high variance, there were no significant differences between average nutrient concentrations on the west (marina) vs. east (study site) side of the Cay,

which suggests that nutrient supply alone does not explain stromatolite absence in the western location. There was, however, a possible seasonal difference in nutrient dynamics, as July 2006 average NO_3 concentrations in the study site differed significantly from the December 2005 NO_3 average (ANOVA, $F_{2,62}=36.55$, $p<0.0001$). This could be due to variability in organic matter supply, nitrogen cycling, or differences in speciation due to differential rates of nitrification, denitrification, or uptake kinetics (Harrison, Harris et al. 1996) relative to nutrient inputs in the system. Future r measurements are needed for a definitive conclusion.

All nutrient concentrations over time for December 2005 are shown in Figure 7,and illustrates the high temporal variability in concentrations among nitrogen species. There was a trend of increasing variability during the sampling period that seems to coincide with tide – plotted at the bottom of the figure. While a regression of the coefficient of variation (CV) of each triplicate sample of both nitrogen species against tide was significant (NH_4 r-square=0.6158 p=0.0034; NO_3 r-square=0.2469, p=0.0483), regression analysis of nutrient concentration vs. tidal height revealed no significant relationship. These analyses do illustrate that tide has a significant effect on nutrient variability, possibly due to wave-driven advection of nutrients from the sediment or tidal supply from an external source.

December 2005 nutrients plotted against the distance in hours from high tide (HT) (figure 8) show a significant relationship for nitrogen species (NH_4 r-square=0.31 p=0.05, NO_3 r-square=0.34 p=0.03). There is a bimodal rise in nutrient variability when tidal influence is highest (between high and low tide) and an overall decreasing trend exists in nutrient concentration from –6 hours from HT to +6 hours, indicating that nutrient

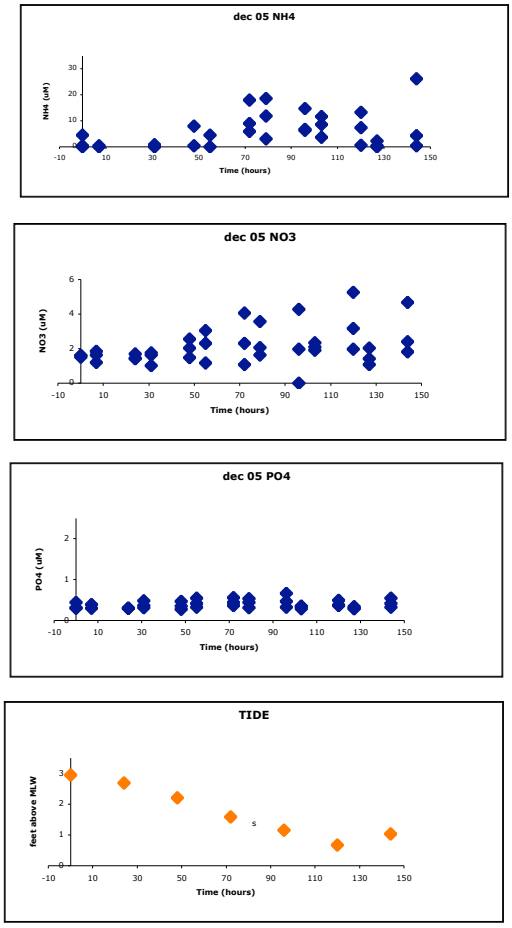

Figure 7: Plots of nutrient concentrations taken in triplicate 6-12 December twice daily at 1030 and 1730 (n=3), and a plot of tidal height in feet above mean low water (MLW). A correlation seems to exist for this study period between nutrient variability and tidal height.

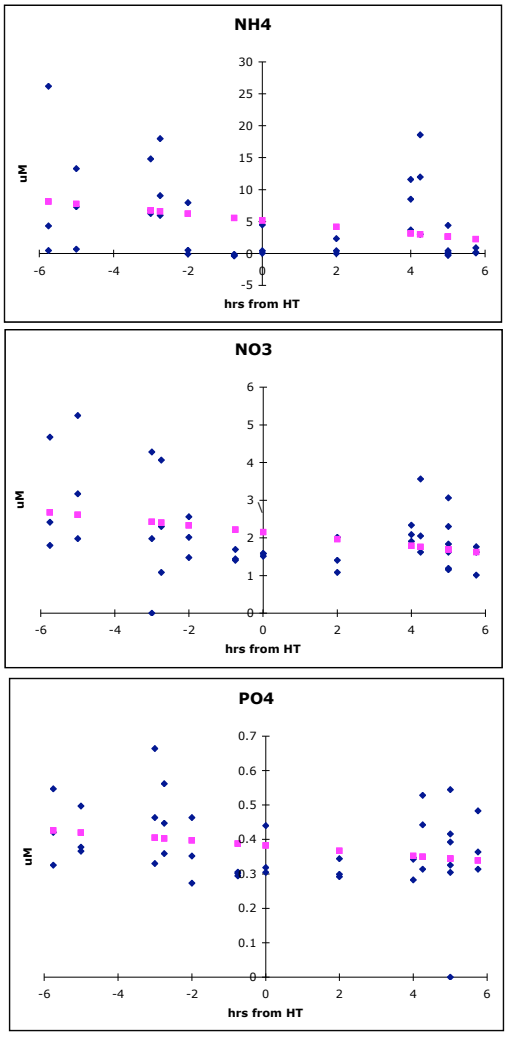

Figure 8: December 2005 nutrient concentrations (blue points) plotted against hours from high tide on the x axis, where 0 is high tide, and ±6 hrs is low tide. Pink data points are expected y values according to the regression model. Nitrogen species are significantly related to hours from high tide, but phosphate is not (NH_4 r-square=0.31 p=0.05, NO_3 r-square =0.34 p=0.03, PO_4 r-square =0.26 p=0.1). There is a bimodal trend of variability when tide is strongest, and a decreasing trend in concentration from –6 to 6, meaning nutrient variability and concentration are greater when the tide is advancing than when it is receding.

concentrations are higher when the tide is advancing than when it is receding. If the retreating tide facilitated the pumping of nutrients from groundwater or a terrestrial source, the opposite relationship between tide and variability would exist. The trend in these data suggests several possibilities including that: 1) nutrients could be supplied from an offshore source, or that 2) tidal pumping is driving nutrient release via increasing pressure on the seabed. Figure 9 is a plot of all nutrient concentrations over time for July 2006, and highlights the trend in nutrient variability, but for different solutes than December 2005. Both the week-long and day-long sample plots show a change in nutrient variability for NH_4 and PO_4, but not for NO_3. The only solute with a significant relationship with tide is the week-long CV of PO_4 (r-square=0.3545, p=0.0076), however during the 28 July sampling period, no relationship existed between tide and solutes. This variability in July 2006 PO_4 also occurred in the sediment data, though further analysis is needed to ascertain whether the two sources of variability are related. Inorganic nutrients showed variability in December 2005 vs. July 2006 samples, though these data are insufficient to explain that variability or predict which species may be variable or elevated at any given time.

Benthic diatoms colonize the surface of Highborne mats and silicate is expected as an important nutrient in the system. Yet silicate concentrations were below detection limits among all samples (data not shown), suggesting severe limitation. A previous study measured the ratio of zeaxanthin:fucoxanthin as a proxy for the relative abundance of cyanobacteria vs. diatoms in mats – called the "ZF ratio" (Pinckney and Reid 1997). In mats on a nearby island ratios of 4.26 ±1.21 (mean ± 1 standard deviation) to 21.42 ± 13.50 were seen, leading to the hypothesis that diatoms may be important producers in

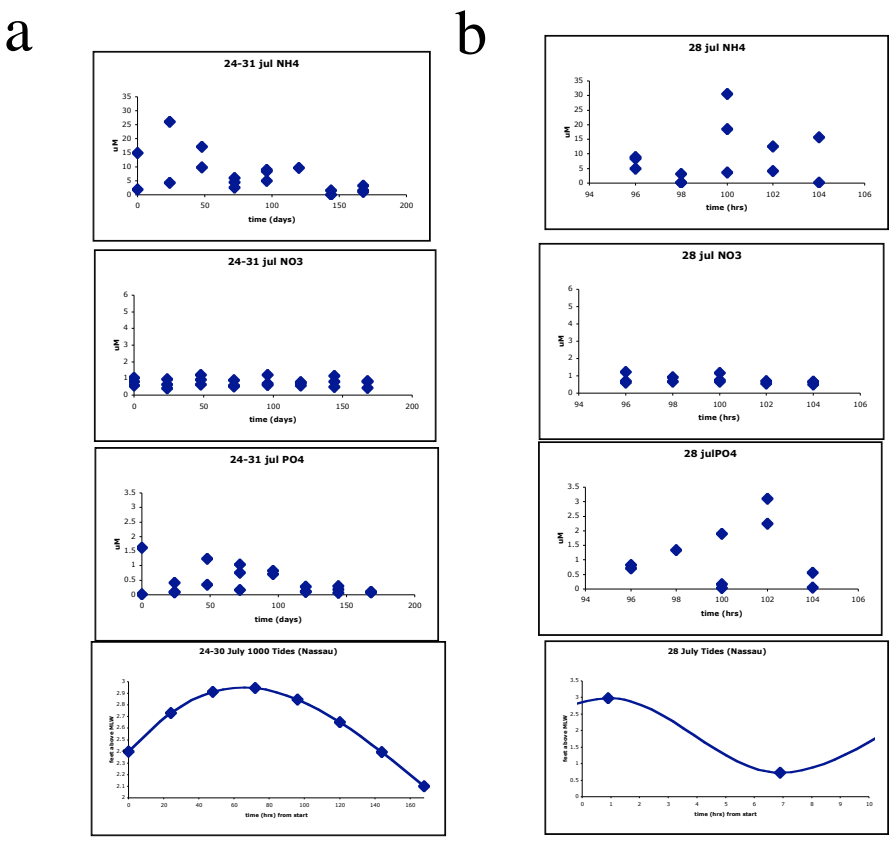

Figure 9: Plots of triplicate nutrient samples taken a) 24-31 July 2006 at 1030 daily from one location in the study site, along with tide height in feet above MLW, b) taken 28 July 2006 of triplicate samples taken at 2-hour intervals for a total of 8 hours from 1030-1830. In descending order, the plots are: NH_4, NO_3, PO_4, and tide height. Contrary to December 2005 data, these data suggest that no relationship exists between nutrient concentration or variability, and tidal height.

the mats of this and surrounding areas. The data herein suggest that diatoms are constrained by silicate supply, the limitation of which may prevent them from outcompeting the stromatolites on which they are found.

Conclusion

The waters around Highborne Cay are not oligotrophic, but can contain high concentrations of inorganic nitrogen relative to phosphorus in the system. It is therefore unlikely that nutrient supply (both in magnitude or N:P ratio) would benefit N-fixing cyanobacteria by limiting water column supply to competitors and therefore favoring N-fixing cyanobacteria within the mats and on which the mats' consortia are based. Furthermore, nutrient concentrations in the study site do not differ significantly from those on the west side of the cay – an area in which no stromatolites occur. It is unlikely that the nutrient regime in Highborne Cay is the factor responsible for making this site a suitable environment for stromatolite persistence by being harsh enough to facilitate mat survival. Though a relationship between tide and nutrient concentrations seems to exist, the relationship was variable between study periods, suggesting other possible mechanisms operate either seasonally or temporally.

Nutrient concentrations in the sediments measured during the same time periods were generally higher than the water column data – particularly with respect to nitrogen, which had higher averages and peaks than were found in the water column. These may be due to advective flow, which can supply electron acceptors and facilitate the removal of decomposition products, enhancing the microbial decomposition activities in the upper sediment layer (Rasheed, Badran et al. 2002).

CHAPTER 3: Microcosm Flux Chamber Experiments

Introduction

Stromatolites are laminated structures that contain a microbial population (Riding 1999; Riding 2000), and most examples of these microbial mats exist in niche environments such as highly saline ponds and embayments, or tidal flats (Paerl, Joye et al. 1993) that are sheltered from high tidal flows or wave energy (Monty 1976; Cohen, Castenholz et al. 1984). However the stromatolites of Highborne Cay exist in a normal marine environment (Reid, Visscher et al. 2000) characterized by high wave energy, significant sediment transport (Eckman, Andres et al. 2008) and relatively low amounts of benthic flora and fauna (Andres and Reid 2006).

Stromatolite formation in Highborne Cay is microbially-mediated – a process of trapping and binding sand grains (ooids) that are suspended in the water column, facilitated by the production of copious and sticky exopolysacchride (EPS) on the mat surface (Decho, Visscher et al. 2005) and subsequent lithification. Lithification is facilitated by production of aragonite crystals, which weld together ooids and provide structure. Living stromatolites in Highborne Cay are classified according to the composition of the microbial assemblage resident in its outermost "living" layer (Reid, Visscher et al. 2000), which is primarily composed of differing combinations of: photoautotrophs (cyanobacteria), sulfate reducers (SRBs), sulfide oxidizers (SOBs), and aerobic heterotrophs (Visscher and Stolz 2005). Mats are categorized as one of four main types according to the relative abundance of these communities.

The first mat type is a CYN, or "cyanobacteria" mat, so named because it has a relatively simple microbial community, primarily filamentous cyanobacterium *Schizothrix gebeleinii* (see (Golubic 1991) and references within), which helps reinforce the mat's structure. The cyanobacteria are diazatrophs, supplying the mat with a portion of its basic nitrogen requirements (Steppe, Pinckney et al. 2001), and it is upon this nutrient supply that the mat is thought to rely (Reid, Visscher et al. 2000). CYN mats generally contain a homogeneous autotrophic population that is less developed than the other mat types, with photosynthesis, respiration and fermentation as the main pathways (table 3, line 1).

The second type is called DIA, or "diatom" mat, which includes benthic branching diatoms that (along with macroalgae of the genera *Batophora* and *Chondria* (Andres and Reid 2006)) colonize the exterior of the mat, likely due to the hard substrate the mats provide. Other than the obvious colonization by diatoms, DIA mats often closely resemble CYN mats (P. Reid pers. Comm.). Therefore the main reactions within the mat should be similar, though the inclusion of autotrophic diatoms on the mat surface would likely differ, possibly increasing production and respiration (Table 3, line 2).

The third type of mat is an EPS mat, named for its relatively thick biofilm of exopolysacchride (EPS) that covers the mat surface and fills the spaces between the grains. A thicker, more developed layer of *Schizothrix* underlies the EPS, and embedded in the EPS matrix are aragonite needles. These needles are precipitated by cyanobacteria, and are closely related to the activity of SRBs remineralizing the EPS and SOBs (table 3, line 3) recycling sulfide (Decho, Visscher et al. 2005). This assemblage may decrease the net daytime oxygen production, increase nighttime oxygen

MAT	DAYTIME REACTIONS	NIGHTTIME REACTIONS
CYANO	1. Carbon fixation (photosynthesis): $CO_2+H_2O \rightarrow CH_2O+O_2$	1. Fermentation (H_2 production) $5CH_2O+H_2O \rightarrow HCO_3+H+4CH_3O$ 2. N-fixation, glycogen degradation $N_2+8H^+ \rightarrow 2NH_3+H_2$
DIATOM	1. Carbon fixation (photosynthesis): $CO_2+H_2O \rightarrow CH_2O+O_2$	1. Fermentation (H_2 production) $5CH_2O+H_2O \rightarrow HCO_3+H+4CH_3O$ 2. N-fixation, glycogen degradation $N_2+8H^+ \rightarrow 2NH_3+H_2$
EPS	1. Carbon fixation (photosynthesis): $CO_2+H_2O \rightarrow CH_2O+O_2$ 2. Sulfide Oxidation $H_2S+2O_2 \rightarrow SO_4+2H$ 3. Carbon fixation $2CO_2+H_2S+H_2O \rightarrow 2CH_2O+SO_4+2H$ 4. Carbon oxidation (sulfate respiration): $2CH_2O+SO_4 \rightarrow 2HCO_3+2H_2S$ 5. Carbonate respiration: $4H_2+CO_2 \rightarrow H_4+2H_2O$ and $2CH_2O \rightarrow CH_4+CO_2$	1. Fermentation (H_2 prod): $5CH_2O+H_2O \rightarrow HCO_3+H+4CH_3O$ 2. N-fixation, glycogen degradation: $N_2+8H^+ \rightarrow 2NH_3+H_2$ 3. Denitrification: $5CH_2O+4NO_3 \rightarrow 5HCO_3+H^++2N+H_2O$
MATURE	1. Carbon fixation (photosynthesis): $CO_2+H_2O \rightarrow CH_2O+O_2$ 2. Sulfide Oxidation $H_2S+2O_2 \rightarrow SO_4+2H$ 3. Carbon fixation $2CO_2+H_2S+H_2O \rightarrow 2CH_2O+SO_4+2H$ 4. Carbon oxidation (sulfate respiration): $2CH_2O=SO_4 \rightarrow 2HCO_3+2H_2S$ 5. Carbonate respiration: $4H_2+CO_2 \rightarrow H_4+2H_2O$ and $2CH_2O \rightarrow CH_4+CO_2$	1. Fermentation (H_2 prod): $5CH_2O+H_2O \rightarrow HCO_3+H+4CH_3O$ 2. N-fixation, glycogen degradation: $N_2+8H^+ \rightarrow 2NH_3+H_2$ 3. Denitrification: $5CH_2O+4NO_3 \rightarrow 5HCO_3+H^++2N+H_2O$

Table 3: Mat types and the reactions that are associated with them in the light and the dark.

consumption, and introduce denitrification. Furthermore, though EPS mats may contain more photoautotrophs, it is postulated that the overlying EPS layer on mats of this type may act as a barrier and help seal them against the environment (Decho, Visscher et al. 2005), thereby stifling nutrient loss to the water column and dampening any signal these mats may produce.

The last type of mat is a mature mat (MAT), because at this stage, the four microbial populations (cyanos, SOBs, SRBs and aerobic heterotrophs) all flourish, and the consortia between them are the most developed. MAT mats are characterized by an abundant coccoid species of cyanobacterium called *Solentia*, that bores into ooids, allowing the grains to be more effectively fused by the precipitation of aragonite crystals, which is a byproduct of heterotrophic metabolism (DesMarais 1997). At this stage, a geochemical mass balance within the living layer of the mat suggests stromatolites may require little more than light to function (Visscher and Stolz 2005).

Each mat type forms a different physical structure based on internal metabolic processes (table 3) and/or the interaction of metabolism with the external environment, with differing physical characteristics and attendant growth rates of the mat structure (Decho, Visscher et al. 2005). Transition between these structures causes lamination, which provides structure to the mat and is what differentiates stromatolites from other microbe-containing macrofabrics such as dendrolites and thrombolites (Reid, Visscher et al. 2000; Riding 2000; Andres and Reid 2006). Lamination is most likely created by a transition between the microbial populations that constitute the most metabolically active portion of the mats (Reid, Visscher et al. 2000; Visscher, Reid et al. 2000; Andres and Reid 2006). As noted above, stromatolite lamination may be caused by internal processes

alone (Ginsburg 1991), or by a combination of internal and external physical forces (Gebelein 1976). However, the factors that cause transition between mat types, and subsequently mat lithification and lamination, are poorly understood.

Because each mat type may have different external resource needs as a function of varying microbial communities within the mat and the consortial relationships among those communities, nutrient dynamics between Highborne Cay stromatolites and their external environment may play an important role in mat transition, lithification, growth and morphology. The external nutrient environment may confer a competitive advantage to certain types, and perhaps drive a shift in relative populations of mat constituents and lithification. It is therefore important to determine the extent to which nutrient exchange between stromatolites and the water column differs with mat type.

Highborne mats are exposed to a nutrient-rich, turbulent water column and episodic – sometimes long-term – burial in sediments containing inorganic nitrogen concentrations an order of magnitude higher than exist in the water column (see chapter 1 and 2). As mats transition between CYN, DIA, EPS and MAT mat types, the diversity of the populations within the mat and the corresponding metabolic reactions within the mat also change. It is reasonable to expect the net intake and output of oxygen and nutrients from the mats to differ as well.

In order to investigate the potential exchange between mats and their external environment, this study's goal was to quantify mat productivity (O_2 production and consumption), as well as uptake and release of inorganic nutrients to the water column relative to that production, to answer the following questions:

Q1: Do mats exchange solute between the living layer and the water column?

Q2: Is there is a difference in primary productivity and nutrient fluxes among mat types?

Q3: Are mats limited by one or more nutrients (suggesting that nutrients govern mat transitions)?

Methods

General Overview:

Question 1:

To examine whether stromatolites exchange solutes with the overlying water (Q1 above) mat samples were placed in stirred microcosm chambers and nutrient and oxygen fluxes were measured over an incubation period that either included light/dark treatments via dark shade, or incubation times that allowed the mats to progress through a natural light/dark cycle so that measurements of productivity could be compared to nighttime respiration. Oxygen and the inorganic nutrients NH_4, NO_3, and PO_4 were measured to determine whether a signal could be detected, suggesting solute flux is occurring between the mats and their environment. Significant fluxes would suggest mats exchange nutrients and oxygen with their environment as a function of metabolism within the living mat layer.

Question 2:

To examine whether different mat types have different nutrient cycling and productivity (Q2 above), incubation experiments consisted of samples of one particular

mat type, with replicates. These experiments determine whether there was a significant difference of fluxes among types relative to any between-run variability. Oxygen was measured as an estimate of net primary productivity and to determine whether rates of oxygen flux are affected by mat type. The inorganic nutrients NH_4, NO_3, and PO_4 were measured. Alkalinity was also measured to calculate total CO_2 production as a proxy for productivity, however alkalinity data were compromised and therefore are not reported here. Mats were subsampled for pigment analysis, specifically chlorophyll, fucoxanthin and zeaxanthin, as proxies for the relative amounts of diatoms vs. cyanobacteria in the mats. These relationships allowed investigation of the relationship between fluxes and biomass, and also investigation of the amount of diatoms in DIA mats relative to other mat types, helping to determine the differences – if any – between mat types.

Question 3:

In order to answer whether Highborne mats are limited by one or more nutrients (Q3 above), additions of PO_4 and Si – potentially limiting in Highborne Cay (chapter 1) – were performed [table 4: experiment 6, MAT P addition; experiment 9, DIA P and Si addition] by adding 30 moles of KH_2PO_4 and 15 moles of Na_2SiF_6 to each ~1L chamber. These treatments were to investigate whether PO_4 or Si addition would stimulate production – P via direct uptake by cyanobacteria during primary productivity, and Si by supporting the diatoms that colonize on the mat surface. Oxygen fluxes were measured to determine whether the addition of potentially limiting nutrients affected a significant response in productivity or respiration, which would suggest nutrient limitation. In experiments where limiting nutrients were added, controls consisted of mats with ambient seawater and no nutrient addition.

RUN	DATE	MAT TYPE	SITE	LAT	LONG
1	21-Jul-06	CYN	8	N24 43.027	W78 49.135
2	22-Jul-06	DIA	12	N24 44.138	W76 48.717
4	24-Jul-06	EPS	2	24,42'45.5"N	76,49'10"W
5	25-Jul-06	EPS	2	24,42'45.5"N	76,49'10"W
6	26-Jul-06	MAT	4	24,42'51"N	76,49'9.5"W
8	29-Jul-06	control blank			
9	30-Jul-06	DIA	6	N24 42.911	W76 49.159

Table 4: All experimental runs, including site names and coordinates.

Microcosm Chambers

Microcosm flux chambers were constructed almost entirely of clear acrylic to allow sunlight to pass through to the sample (with the exception of the base and the small impeller, both of which were constructed of gray pvc) (figure 10). The chamber consists of a cylinder 11.4 cm in diameter and 11 cm high affixed to a base. The lids are clear acrylic with a butyl o-ring machined onto the underside, and sealed to the chamber cylinder by securing them to the base with four threaded PVC bolts and stainless steel nuts and washers.

Stirring paddles are 5.1 cm wide by 2.54 cm tall, and their movement is facilitated by a water-driven pvc impeller mounted on the exterior of the chamber lids and enclosed in a clear acrylic housing. The impellers are powered by water flowing from a single head tank (figure 11) mounted >1.5m above the chambers to allow for sufficient head pressure to drive the stirring mechanisms. A jet pump fills the tank, and 3/8" tubing runs from the tank to the impeller housing of each chamber, and stirring speed was regulated with in-line pinch valves (Cole Parmer). Chambers were tested for leakage to ensure no mixing of impeller and chamber water occurred by filling chambers with DI and driving the impellers with ~11psu Chesapeake Bay water prior to deploying the equipment in the field. After ~8h, water salinity and inorganic nutrients were examined and found to be 0 (results not shown). Chamber lids are equipped with two luer lock sampling ports (Cole Parmer) – one each for sample extraction and simultaneous replacement of overlying water. Port valves remained closed except while sampling to ensure the chambers were gas tight.

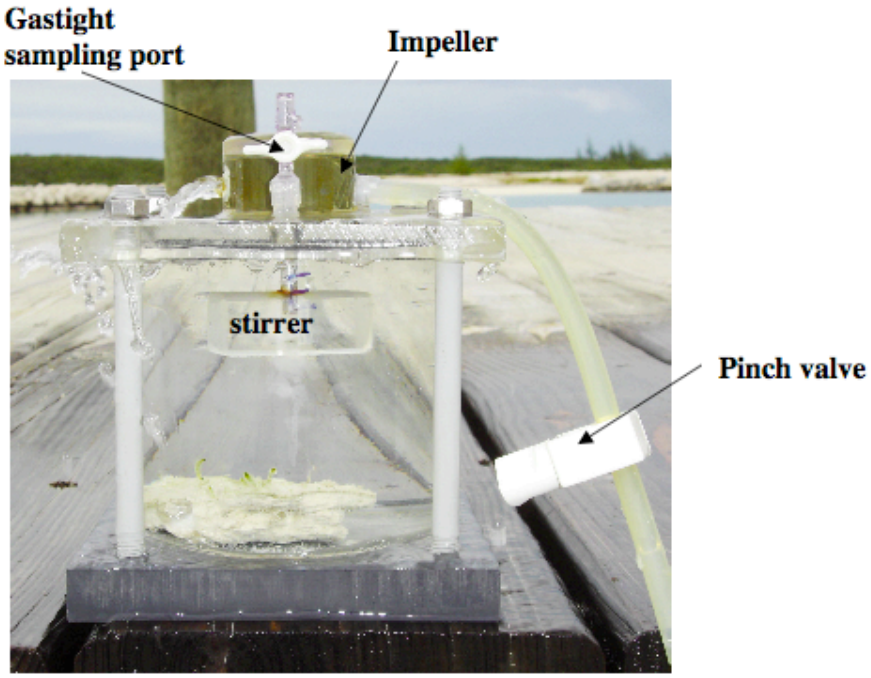

Figure 10: Photo of a stirred microcosm flux chamber used in the December 2005 and July 2006 studies.

Figure 11: Photos of the flux chamber, circulating bath, and head tank for stirring chambers as deployed December 2005.

A previous microcosm flux study 21-26 July 2005 included chambers constructed of flexible bags. This design was tested to incorporate prior studies suggestion that productivity in coral is highly dependent on water flow over the coral heads (Carpenter, Hackney et al. 1991), and that corals can die if kept in still water (Adey 1983). Models of stromatolite growth and morphology differ greatly depending on whether the mats' environment is diffusion dominated (Merks, Hoekstra et al. 2003), suggesting that stromatolites may also be sensitive to flow. If mats respond like coral, traditional stirred chambers may underestimate productivity (Sanford 1997). However, flexible bag chambers proved unreliable because the wave energy of the study site was too high. The site's sediments were highly permeable and wave energy pumped water through the sediment, scouring sediment out from under the chamber housing and creating a gap between the bottom of the chamber and the sediment.

Sites and Sampling

21-31 July 2006, samples of CYN, DIA, EPS, and MAT stromatolites were collected from various locations along the Highborne Cay study site (table 4). Sampling was performed by cutting portions of mat from larger sections with a knife, relocating samples to a circulating water bath on the west side of the cay, and allowing them to equilibrate for ~16 hours. Equilibration time was needed to ensure that any living mat material that may have been damaged during the sampling had ample time for the reduction of solutes, and that any solutes or other material that may be stored beneath the living layer of the mat had sufficient time to diffuse out before sampling and would not confound the flux signal.

Samples were placed into each of 12 microcosm chambers, which were filled with filtered water with no headspace remaining, and sealed. Overlying water for chambers was collected from the study site <1h prior to each run and passed through a 3 μm prefilter and 0.2 μm capsule filter in order to exclude the bacterial population in the water column and limit their potential impact on nutrient signals. Chambers were then sealed, and submerged in a circulating water bath. During preliminary trials (December 2005), two Tidbit temperature recorders were deployed (Onset Corporation) – one in the water bath alongside the samples, and one in the nearby water column off the dock – to ensure recirculation in the bath was sufficient to keep it at ambient *in situ* water temperatures. There was an average difference of 0.67°C (over 8 runs) and maximum difference between chambers and water column of 0.87°C during any of the experimental runs.

Samples collected for all experimental runs were incubated in the microcosms for either ~8 hours with two separate light/dark treatments (dark simulated by covering the chambers with a foil-covered box containing a HOBO light meter), or allowed to progress through a natural light dark cycle during a longer ~17-hour incubation. 5-ml samples were taken for oxygen, alkalinity, and the inorganic nutrient species NH_4, NO_3, PO_4, and Si. Sample water was collected using a 5-ml syringe affixed to a luer lock valve on the port (Cole Parmer), passed through a 0.45 μm Acrodisc syringe filter, placed into a sterile 5-ml vial (Evergreen Scientific), and frozen at –20°C until analysis.

Following the incubations, samples were photographed for quantification of surface area by ImageJ image analysis software (Wayne Rasband, http://rsb.info.nih.gov/ij/). Samples were then weighed for later calculation of mat volume in order to correct for water displacement in the chamber when converting

nutrient values from molar to moles. Finally, mats were subsampled for post hoc pigment and C:P analysis. Pigment analysis provides a proxy for biomass in order to normalize fluxes as well as investigate any differences in magnitude of biomass by mat type, while C:P analysis has been shown to be a useful indicator of P-limitation (Elser, Watts et al. 2006). Subsampling was performed using a plastic corer of diameter 1 cm driven approximately 2 cm into the mat surface to incorporate the entire depth of the living layer (~8mm (Visscher, Reid et al. 2000)). Sample was weighed, and frozen at –20°C until analysis.

Chemical Analysis

Samples were analyzed for alkalinity, NH_4, NO_3, PO_4, and Si on a Westco SmartChem Discrete Analyzer (Westco Scientific Instruments, Inc.) – alkalinity according to a methyl orange method (EPA 310.2), NH_4 according to a modification of the phenyl-hypochlorite method (Koroleff 1976), NO_3 using the cadmium reduction method, PO_4 according to a modification of EPA standard 365.2 and Eton et al. 1995, and silicate according to Strickland and Parsons 1972. pH was measured using an electrode (VWR scientific model 8000).

Samples were taken for oxygen using a 500μL gastight syringe (Hamilton Company) and analyzed using either a Winkler titration, or a Clark-style mini electrode (Diamond General Development Corp.). During runs for which the electrode was used, it was calibrated at each sample period via a four-point standard curve using Winkler titrations of ambient seawater bubbled with N_2 gas.

In order to ensure the flux signals detected during these experiments were truly a function of processes within the stromatolite and not processes within the water column,

a separate experimental run of blanks was conducted by filling all chambers with filtered seawater alone for an 8-hour incubation of light and dark treatments.

The C:P ratio of stromatolite biomass was calculated from mat subsamples, and DOC concentrations of the overlying water were also collected, however the data for both these analyses were compromised and therefore their results are not included here.

Pigments

Mat samples were analyzed for Chlorophyll a and the accessory carotenoids Fucoxanthin and Zeaxanthin. Fucoxanthin is found in diatoms, while Zeaxanthin is largely found in cyanobacteria (SCOR-UNESCO 1966; Wright, Jeffrey et al. 1991). The ratio of the two carotenoids is the ZF ratio, a useful proxy for the relative amounts of diatoms to cyanobacteria within the mat (Pinckney and Reid 1997). Chlorophyll a was measured to normalize the flux data against the biomass of the primary producers and to investigate whether the relative amounts of biomass differ across mat types. Fucoxanthin is important to investigating whether the abundance of diatoms relative to cyanobacteria differs by mat type, and it is also a key to understanding whether signals of nutrient flux and production in the mats are related to diatom abundance on the surface.

Mat subsamples were thawed and pigment was extracted within 1h by the addition of 100% acetone with sonication for 10 minutes, and centrifuged at ~3,400 rpm for 6 minutes. The supernatant was filtered through a sterile, DMSP-treated cotton plug filter, and placed into a glass vial. This process was repeated until supernatant was clear (a minimum of 3 repetitions), then acetone was evaporated with N_2 and dry sample was frozen at -70°C until analysis.

Frozen samples were brought to a known volume with acetone, and immediately analyzed according to (Airs, JE et al. 2001) on an Agilent 1100 series HPLC (Agilent Technologies). Samples were analyzed for Chlorophyll a, Fucoxanthin and Zeaxanthin. Chlorophyll was quantified by calibration with a known standard (Sigma). Fucoxanthin and Zeaxanthin were identified by combined spectra (SCOR-UNESCO 1966) and molecular weight data via LC-MS (Airs, JE et al. 2001)).

Pheophytin is a known degradation product of Chlorophyll(Whitney and Darley 1979), and high amounts of this pigment were found in the samples. Pheophytin was identified using LC-MS, and a standard curve was constructed by acidification of a Chlorophyll a standard via the addition of concentrated glacial acetic acid (SCOR-UNESCO 1966), a method that converts all chlorophyll to pheophytin.

Statistical analysis

For all investigations, oxygen and nutrient fluxes were calculated from the time series measurements of oxygen and nutrient concentrations within each chamber. Solute concentration data were first normalized to mat surface area, with fluxes were calculated by a regression of concentration versus time. For oxygen fluxes, regressions were typically significant, with r-square > 0.7 and $p < 0.05$. While the p values of nutrient flux data were commonly not significant at an alpha=0.05, clear trends in the regression analysis were evident. Low p values in these data may be due to an absence of net flux, and not necessarily high variability. For this reason, regression slopes with an r-square $>$ 0.30 were accepted as valid fluxes, while r-square values < 0.30 were visually inspected, and those with visible trends but slopes too shallow to yield a high r-square were also

included. Fluxes were tested for assumptions of normality using the Shapiro-Wilk test and normal probability plot (SAS 9.1).

To investigate whether mats exchanged solutes with their environment, oxygen and nutrient fluxes were analyzed using a t-test against a mean of 0 and standard deviation of 1 in order to determine if flux data was significantly different than 0 (table 5, line 1). Oxygen and nutrient fluxes were then analyzed using a one-way ANOVA with flux as the dependent variable, and light/dark treatments as independent variables to determine whether mats displayed a clear diel pattern of oxygen uptake and production indicative of primary production and respiration. To investigate whether a difference exists between the fluxes of different mat types, a one-way ANOVA was used with nutrient and oxygen flux as the dependent variable, and mat type and experimental run as independent variables for both light and dark treatments.

To determine whether mat productivity was nutrient limited, a one-way ANOVA was performed on light treatments of the nutrient addition experiments with oxygen flux as a dependent variable, and nutrient-addition treatment as the independent variable (table 5, line 2. This analysis would determine whether the addition of potentially limiting inorganic nutrients phosphate, silicate or the combination of the two altered oxygen production or consumption, and whether nutrient flux differed by mat type, which would suggest the possibility that different mat types have different nutrient requirements and possibly experience differential nutrient limitation.

In order to test covariates that may explain potential differences in oxygen fluxes between mat types, all oxygen fluxes were analyzed via linear regression against environmental light levels, chlorophyll concentration in the mat, and water bath

QUESTION	METHODS	STATISTICS	RESULT
Do mats exchange solute between the living layer and the water column?	Unaltered (control) treatments of mats in light and dark	T-test of all fluxes against a mean of 0 and standard deviation of 1	All solutes, mat types, and treatments differed from 0, suggesting mats exchange solutes with the environment
Is there is a difference in primary productivity and nutrient fluxes among mat types?	Control treatments of mats in light and dark by mat type	ANOVA; solute as dependant variable and mat type as independent variable	Most significant difference was between two runs of DIA mats (dark: $F_{2,10}$=74.4, p<0.0001, light: $F_{2, 10}$=17.1, p=0.014). Day-to-day variability is greater than difference by mat type
Are mats limited by one or more nutrients (suggesting that nutrients govern mat transitions)?	Nutrient addition vs. control treatments in light and dark for all mat types	ANOVA; oxygen as dependent variable and nutrient addition as dependent variable.	P-addition increased productivity in DIA mats ($F_{2,3}$=4.63, p=0.098) and decreased it in MAT mats (ANOVA $F_{2, 5}$=59.23, p=<0.0001).

Table 5: An overview table of research questions, as well as methods and statistics employed to answer them, and results.

temperature (table 5, line 3). As the final analysis of oxygen data, a regression was performed on daytime vs. nighttime oxygen fluxes in order to investigate whether the magnitude of the two are related.

Results

Question 1

Concerning the first question of whether Highborne mats are exchanging solutes with their environment, results show clear trends of nutrient and oxygen uptake and production by Highborne mats. Oxygen time series measurements yielded trends with high r-square values (e.g. figure 12). The rates of all oxygen measurements sorted by treatment (e.g. figure 13) show daytime oxygen production via net photosynthesis, and nighttime consumption via respiration.

NH_4 fluxes (figure 14) were highly variable, with none significantly different than 0 – however NO_3 fluxes showed less variability and were significant (figure 15). Many of the daytime NO_3 fluxes showed a net uptake, and most dark treatments displayed a net release likely due to a combination of decomposition and N-fixation, which is known to have a strong diel (nighttime) pattern in Highborne mats (Steppe, Pinckney et al. 2001). Seawater control experiments confirm that flux signals are indeed from mat samples and not from the water column because seawater blank results yielded much smaller variability than the magnitude of flux signals. Control treatments rates were: Oxygen=14.30±5.2 nmol cm^{-2} h^{-1}, NH_4=-0.25 ±0.16 nmol cm^{-2} h^{-1}, NO_3 = -0.08 ± 0.37 nmol cm^{-2} h^{-1}, PO_4 = 0.18 ±0.128 nmol cm^{-2} h^{-1}.

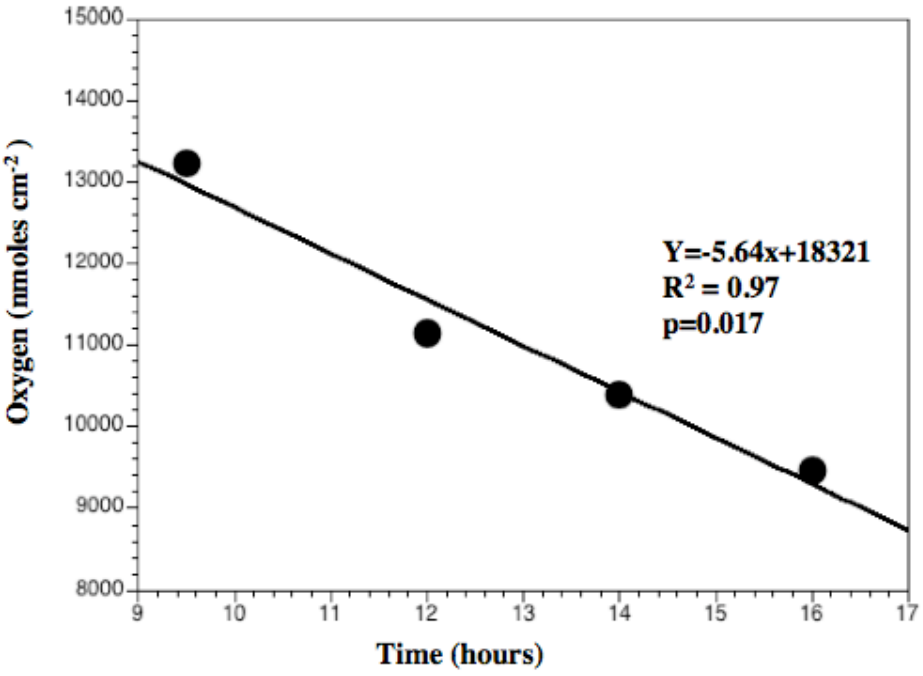

Figure 12: Example of a time series measure for fluxes. This time series was taken from oxygen measurements of DIA mats during nighttime respiration.

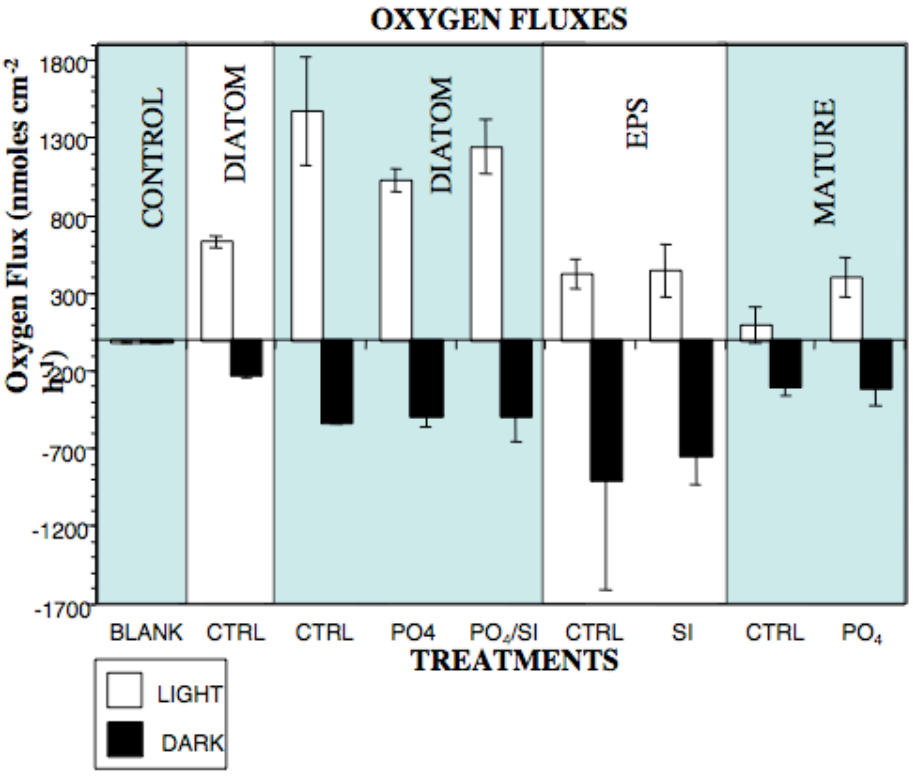

Figure 13: Oxygen fluxes for all mats and treatments July 2006. Oxygen fluxes for all mats and treatments July 2006. Y axis is oxygen flux. X axis are treatments where blank = filtered water blank, ctrl = controls with no nutrient addition, and po4 and si = additions of phosphate and silicate. The shaded areas delineate experimental runs by mat type. The magnitude of daytime oxygen production does not correspond with nighttime respiration, and day-to-day variability is higher than variability my mat type.

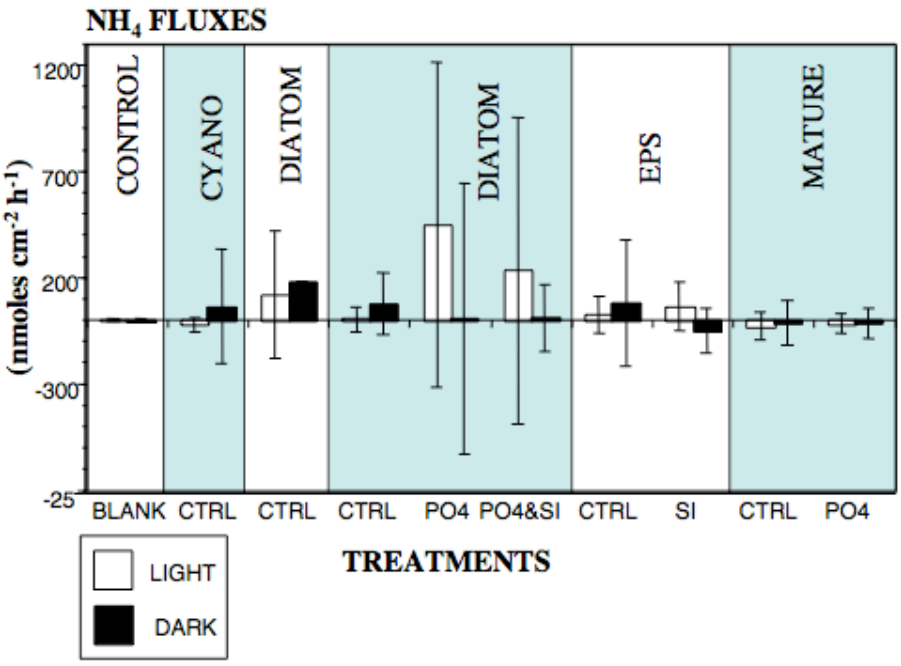

Figure 14: An overview of NH_4 fluxes all experimental runs. Y-axis is solute flux. X-axis are treatments where blank = filtered water blank, ctrl = controls with no nutrient addition, and po4 and si = additions of phosphate and silicate. The shaded areas delineate experimental runs by mat type.

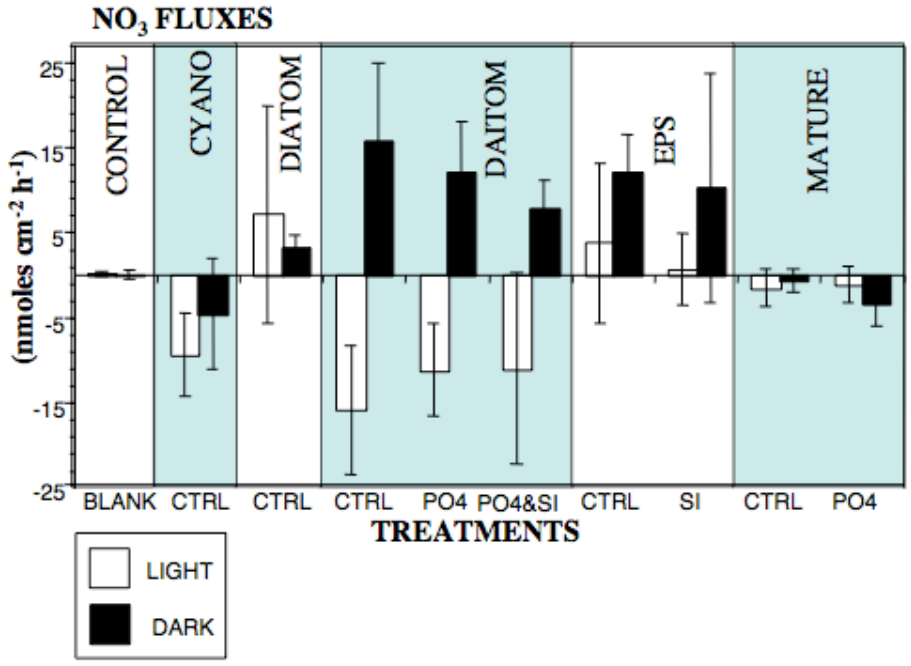

Figure 15: An overview of NO_3 fluxes obtained from flux measurements over all experimental runs. The Y-axis is solute flux. The x axis are treatments, where blank = filtered water blank, ctrl = controls with no nutrient addition, and po4 and si = additions of phosphate and silicate. The shaded areas delineate experimental runs by mat type. Many light treatments showed a net uptake of NO3, and most dark treatments showed a net release, also possibly due to nighttime N-fixation. Mature mats had the lowest flux, supporting the hypothesis that these mats are more self-sufficient and sealed to the environment.

Question 2

Concerning the question of whether there is a difference in productivity by mat type, the data suggest that the daily difference in productivity and nutrient flux is greater than variability due to mat type. The plot of oxygen flux of all runs (figure 13) shows variability, however plots of separate samples of DIA mats on separate days within the figure illustrate that diel variability is higher than any difference by mat type since the same type of mat had significantly different oxygen signals on two separate days (ANOVA dark: $F_{2,10}$=74.4, p<0.0001, light: $F_{2,10}$=17.1, p=0.014,). Table 5 provides a matrix table of all ANOVA results of oxygen fluxes for all combinations of different mat types in both light and dark. Though there are other significant differences between types, none of them has a stronger difference than DIA vs DIA. To attempt to explain this diel variability, oxygen fluxes were regressed against the possible covariates chlorophyll, light, and temperature, and there were no significant relationships between them.

There were, however, more differences in oxygen fluxes between types in dark treatments (table 6), with the most significant difference between CYN and EPS mats ($F_{2,7}$=5.80, p=0.047, as well as CYN and DIA mats ($F_{2,14}$=21.85, p<0.001). The same analysis for nutrients found no corresponding differences in nutrient fluxes by mat type.

An ANOVA was performed on the ZF ratios to determine the relative abundance of diatoms to cyanobacteria in the mats (Figure 16a). There was no significant difference between mat types, which is unexpected since DIA mats are thought to have more diatoms present on the mat. There is a difference in ZF ratios by nutrient-addition treatment with higher ratios in P-addition treatments, however only one sample was

	DIA	DIA	EPS	MAT
DIA		**$F_{2,5}$=14.04 *p*=0.02**	$F_{2,4}$=0.316 *p*=0.61	$F_{2,6}$=13.4 *p*=0.15
DIA	$F_{2,5}$=51.7 *p*<0.001		$F_{2,4}$=21.5 *p*=0.020	$F_{2,6}$=2.47 *p*=0.18
EPS	**$F_{2.4}$=72.0 *p*=0.003**	**$F_{2.4}$=23.6 *p*=0.012**		**$F_{2,5}$=36.7 *p*=0.003**
MAT	**$F_{2.7}$=7.47 *p*=0.03**	$F_{2.7}$=0.15 *p*=0.71	$F_{2,6}$=5.10 *p*=0.07	

Table 6: ANOVA of oxygen fluxes of all mat types. Shaded squares are nighttime respiration fluxes. Bold numbers are significant.

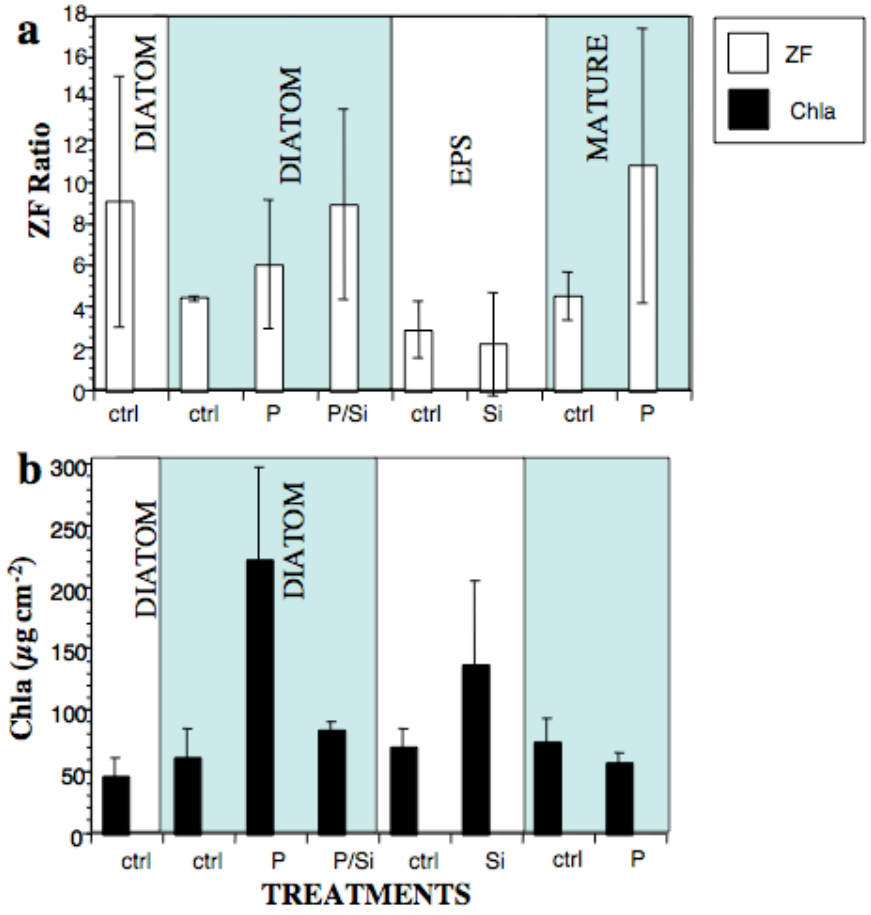

Figure 16: A figure of ZF ratios and Chlorophyll a (Proxy for Biomass) across all treatments. An overview of ZF ratios (in white), which are a proxy of the ratio of Zeaxanthin:Fucoxanthin as a proxy for the ratio of cyanobacteria to diatoms in the mats. A higher ZF ratio indicates more cyanobacteria relative to diatoms, which is expected if diatoms are Si-limited, however a further increase in ZF ratio seems to be apparent in P&Si additions, which is opposite of what would be expected since Diatoms require Si, and remains unexplained.

taken, and in order to conclude that pigments changed relative to one another during the course of the incubations, T_0 and T_f samples needed to be collected.

Question 3

Regarding the question of whether mats are limited by one or more nutrients, the data are unclear. The results of microcosm P-addition treatments do not suggest a response of increased productivity, and therefore do not conclusively support the hypothesis that P might be a limiting nutrient for the mats. Figure 17 is a plot of both p-addition experiments for DIA mats and MAT mats. There were differences in daytime productivity between control and p-addition treatments for both mats (MAT: ANOVA $F_{2,5}$=59.23, p=<0.0001, DIA: ANOVA $F_{2,3}$=4.63, p=0.098), however MAT productivity increased with p addition while DIA productivity decreased, which is unexpected if the mats are limited by phosphate, and cannot be attributed to difference by mat type.

Despite a lack of response by productivity in the mats during p-addition treatments, the mats took up PO_4 in high quantities and at high rates. Figures 18a and 18b are plots of PO_4 fluxes of control treatments on the y-axis against expected PO_4 fluxes calculated from Redfield with oxygen on the x axis. The expected slope is 1, while the slope of figure 18a is ~100x steeper, meaning that ~100x more PO_4 is fluxing into the mats compared to the PO_4 requirement. A partial explanation is that oxygen signals are a net signal of photosynthesis and respiration, while internal cycling within the mat may be very high, transferring more nutrients to biomass. (Visscher and Stolz 2005). It is, however, unlikely that internal cycling would be ~100x higher than net oxygen production, therefore the rapid uptake of PO_4 is likely due to abiotic adsorption. The mechanism behind this is possibly coupled daytime photosynthesis and H_2S oxidation in

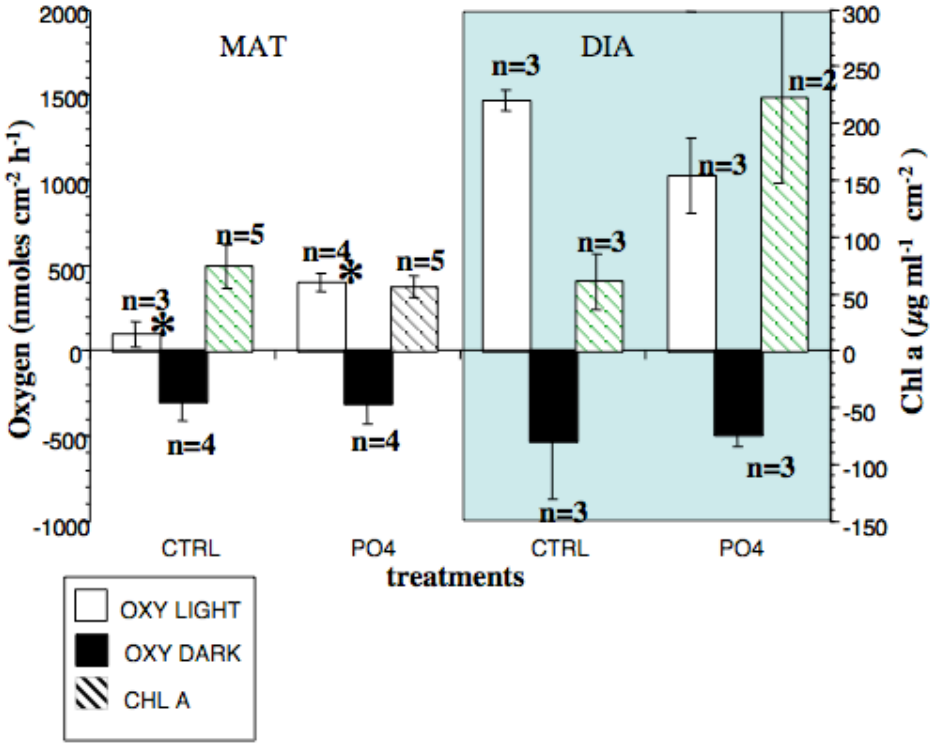

Figure 17: Oxygen response to PO_4 addition by mat type. Flux for Mature and Diatom mats on two different days under light and dark conditions. Control = no amendments to water column. P = phosphate addition. Mature mats had increased productivity with added phosphate, while Diatom mats had a decrease in productivity. However day-do-day variability between runs makes it impossible to say whether this difference can be attributed to mat type.

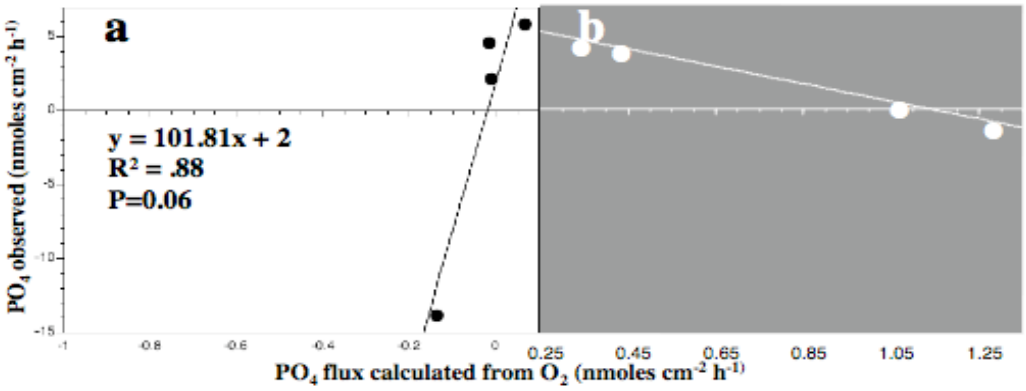

Figure 18: PO_4 flux expected from Oxygen (nmoles cm^{-2} h^{-1}) vs. expected phosphate release as calculated from oxygen flux data, assuming Redfield. Each data point is the average flux of one treatment of one experimental run. Values are plotted against actual PO_4 flux values on the y axis. A slope of 1 indicates an expected flux of PO_4 at Redfield. Overall, slopes suggest that abiotic adsorption and desorption coupled with biotic $CaCO_3$ precipitation and dissolution control PO_4 fluxes (and therefore supply) of mats in this system.

the mats (Visscher and Stolz 2005), which precipitate $CaCO_3$ and dissolve it respectively, but with a net daytime precipitation. A greater precipitation of $CaCO_3$ has an attendant adsorption of PO_4 into that matrix (Tunesi, Poggi et al. 1999; Millero, Huang et al. 2001).

Furthermore, the mats may be diffusion limited (Merks, Hoekstra et al. 2003). Figure 19 is a plot of PO_4 flux against its initial concentration in the water column. As water column concentrations rise, so does the rate at which P is taken up in the mat – whether by adsorption or biotic uptake. This further points to the possible importance of flow, as higher flow would facilitate advection into and out of the mat and alleviate any potential limitation due to diffusion.

To examine whether nitrate fluxes are related to oxygen flux and, if so, to determine what percentage of the mats' nitrogen supply is met by NO_3, plots were made of nitrogen demand in light and dark as calculated from oxygen fluxes using the Redfield ratio, plotted against NO_3 fluxes (figures 20a and 20b). There is a significant relationship between nitrogen and oxygen, with NO_3 accounting for ~10% of the daytime N requirement, and ~14% of expected nighttime nitrogen production/release. Both the greater percentage that NO_3 accounts for nighttime supply, and the positive y-intercept of the daytime plot, support that N-fixation is occurring within the mats. This analysis was done on NO3 rather than on NH4 because NH4 fluxes were inconsistent likely due to internal cycling and therefore that solute could not be used.

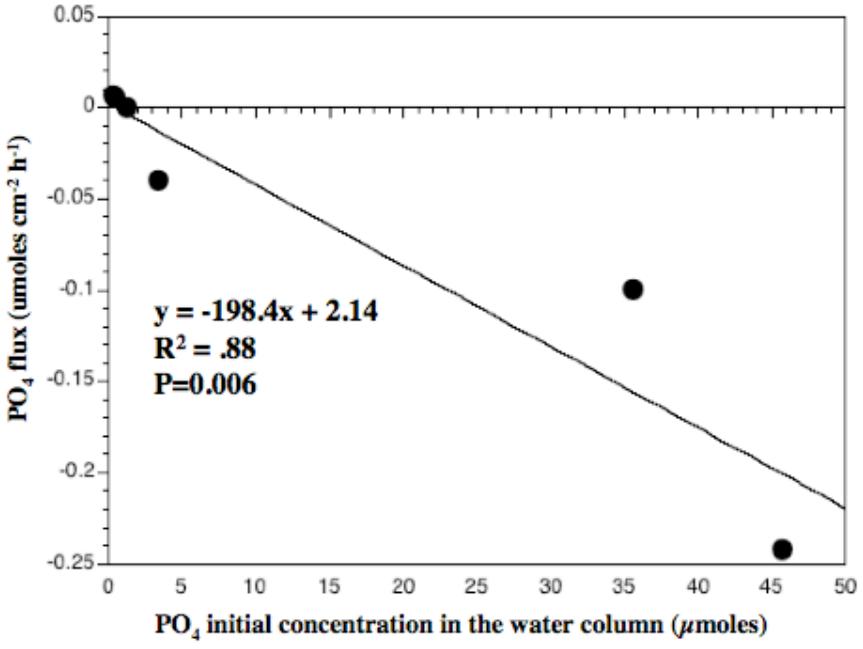

Figure 19: A plot of the initial water column concentration of phosphate against the average flux per treatment for both control and P-addition treatments.

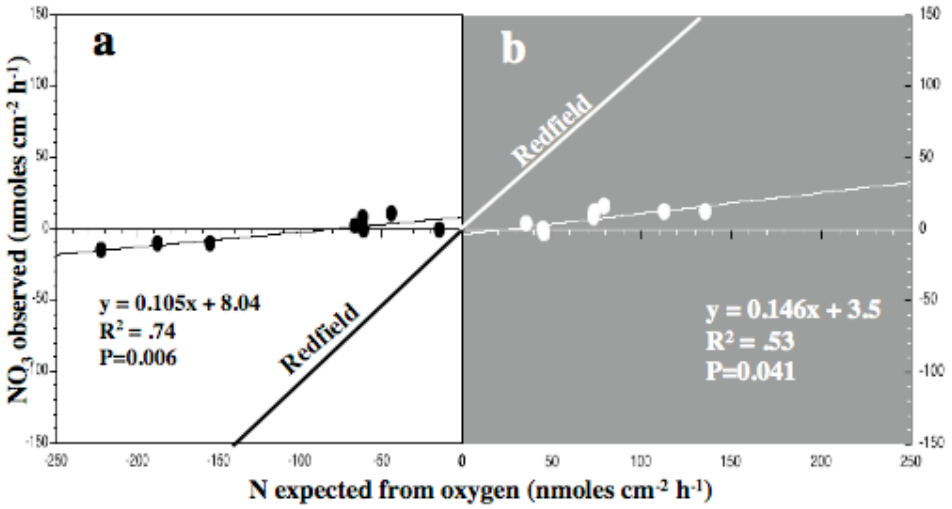

Figure 20: Expected Nitrogen uptake during the day (a), and release at night (b) as calculated from oxygen flux data, assuming Redfield stoichiometry. Each data point is the average flux of one treatment of one experimental run. Values are plotted against actual NO_3 flux values on the y axis.

According to the slopes above, daytime NO_3 uptake accounts for ~10% of the mats' net N requirement, while nighttime release is ~14% of expected nitrogen release from the mats for CO_2 production.

Discussion

Oxygen measurements suggest that Highborne mats have high rates of productivity. Microbial mats are known to have high rates of photosynthesis and aerobic respiration (Canfield and Des Marais 1993; DesMarais 1997), however rates of primary production recorded by this study (figure 13) are even higher than the production of similar intertidal mats on a nearby island (Pinckney and Reid 1997), which were: maximum production of 327 ± 55 nmol O_2 $cm^{-2}h^{-1}$, and average production of 188 ± 73 nmol O_2 $cm^{-2}h^{-1}$). This difference in production can either be attributed to this study's use of stirred chambers compared to the previous study's use of straw cores with little or no flow, or that Highborne mats are much more productive than others in the area.

This study was unable to find a difference in productivity (as oxygen production), nutrient flux, and chlorophyll between mat types, therefore suggesting that the various microbial communities that compose the different mat types may not promote different nutrient uptake or release rates, or rates of productivity. There are lower NO_3 fluxes (figure 16b) for MAT mats, which supports the hypothesis that the more developed consortia within this mat would result in tighter geochemical coupling and less apparent fluxes (Visscher and Stolz 2005). However, there is also day-to-day variability in both NO_3 and oxygen (the fluxes of which this study finds are tightly coupled), and this variability is greater than the difference by mat type, therefore it is impossible to say that NO_3 fluxes definitively answer the question of whether there are differences in flux by mat type.

It is therefore unlikely that the nutrient regime in Highborne Cay plays a role in the transition between mat types because any difference in nutrient and oxygen fluxes by

mat type is smaller than day-to-day variability in those fluxes. If there is a difference, this study's experimental structure was not able to detect it.

It is also important to note that, while some suggest that microbial mats are closed systems (see (Guerrero, Piqueras et al. 2002) and references therein), figures 11 and 12b both suggest that Highborne mats are actively taking up nutrients from overlying water during daytime production and releasing at night. The answer to this question is an important step toward discovering whether the environment's nutrient regime plays a role in stromatolite persistence because it means the geochemical cycling within a mat cannot be viewed as a closed system.

The diel cycle of nitrogen fixation in Highborne mats (Steppe, Pinckney et al. 2001) on which their consortia (Visscher and Stolz 2005), and those of similar mats (Steppe, Olson et al. 1996), are thought to be based, is supported by figures 14b, 15a, and 15b which also suggests that these mats fix nitrogen at night when it is least needed for production, then lose it to the water column, and still require nitrogen uptake from the environment in the daytime when photosynthesis requires. However not included in this analysis is NH_4, the rates of which were higher in magnitude but sporadic with no clear daytime uptake or release. The fact that Highborne Cay has relatively high concentrations of nitrogen in the water column (chapter 2), with even higher concentrations in the sediments (chapter 1), further supports the fact that mats may not be producing much-needed nitrogen via fixation as was once thought. If they are limited, it is likely by a different nutrient.

Mats in Highborne Cay are likely limited by P since any available phosphate is bound to the carbonate matrix, as evidenced by the high rates of uptake (figure 18a) and

the fact that nighttime fluxes of PO_4 (figure 18b) display an opposite trend than what would be expected during oxygen consumption and remineralization. While remineralization is likely occurring, so is microscale anoxia within the mat. This anoxia would likely act as a sink for even more phosphate into the carbonate matrix due to higher rates of $CaPO_4$ chemosorption when more Ca^+ ions are present, concentrations of which can be 2-5x higher in Highborne mats than in the surrounding seawater (Visscher and Stolz 2005). Phosphate has also been shown to limit productivity in similar carbonate systems (LaPointe 1987), and water column nutrient concentrations of Highborne (chapter 2) suggest phosphate may be limiting in Highborne Cay.

Phosphate uptake into the mats, particularly during phosphate-addition treatments, was much higher than is likely biotically. The average PO_4 flux of DIA p-addition treatments was -242 nmoles cm^{-2} h^{-1}, and of MAT p-addition treatments was ~100 nmoles cm^{-2} h^{-1} while no extra nitrogen was taken up to compensate. (In control treatments, PO_4 uptake ranged from 5 to -13 nmoles cm^{-2} h^{-1}.) This suggests phosphate uptake is controlled abiotically. Comparable sediments have an adsorption coefficient $K^*=20$ (where K^* is phosphate adsorbed/equilibrium concentration) for oxic calcium carbonate (Cole 1953). In addition, all uptake of PO_4 may be diffusion limited (figure 19).

Phosphate can be adsorbed into the study site's carbonate sediments by complexing with ferric oxyhydrates (Millero, Huang et al. 2001), and also by adsorption to $CaCO_3$ which is likely heightened due to the aragonite precipitation inherent within the mats' lithification (Reid, Visscher et al. 2000; Andres, Sumner et al. 2006). Aragonite has more active adsorptive sites than calcite (Millero, Huang et al. 2001) as well as a higher

K value of adsorption (Krom and Berner 1980). Due to this mechanism, carbonate can exert control over PO_4 chemistry (Ames 1959; Kanel and Morse 1978).

Given the carbonate makeup, increased aragonite precipitation, and high rates of phosphate uptake of Highborne mats, it is likely that any excess phosphate in the water column is quickly bound to the carbonate in the mats and the sediments. This is supported by the fact that water column concentrations of PO_4 typically average ~0.5 μM (detection limit of ~0.01 μM; chapter 2). This is the same concentration at which the kinetics of adsorption decrease significantly (Tunesi, Poggi et al. 1999), and it is therefore likely that the water column concentration and supply of PO_4 to the mats is controlled abiotically by the kinetics of adsorption.

While phosphate may be the main nutrient limiting the mic robial communities within the mats, the diatoms colonizing the surface of the mats may be limited by silicate, which is below detection limits (chapter 2). Despite this, Si-addition treatments failed to promote productivity (figure 13), and no increase in Si uptake was evident in the Si-addition or combined Si/P treatments. This may be due to the high stirring rates within the chambers which tended to scour the mat surface and may have kept diatoms from building biomass. Another possible explanation could be co-limitation with a micronutrient found within the mats such as Fe or Mn.

Diatoms also may be limited by a combination of phosphate and silicate, though outcompeted for phosphate by adsorption. However, given these likely disadvantages, benthic branching diatoms may be a more important geochemical constituent than was previously believed. A plot of biomass (chl a) vs. ZF ratio (figure 21) reveals that mats with the highest biomass (delineated in the figure by shading), also have a low ZF ratio,

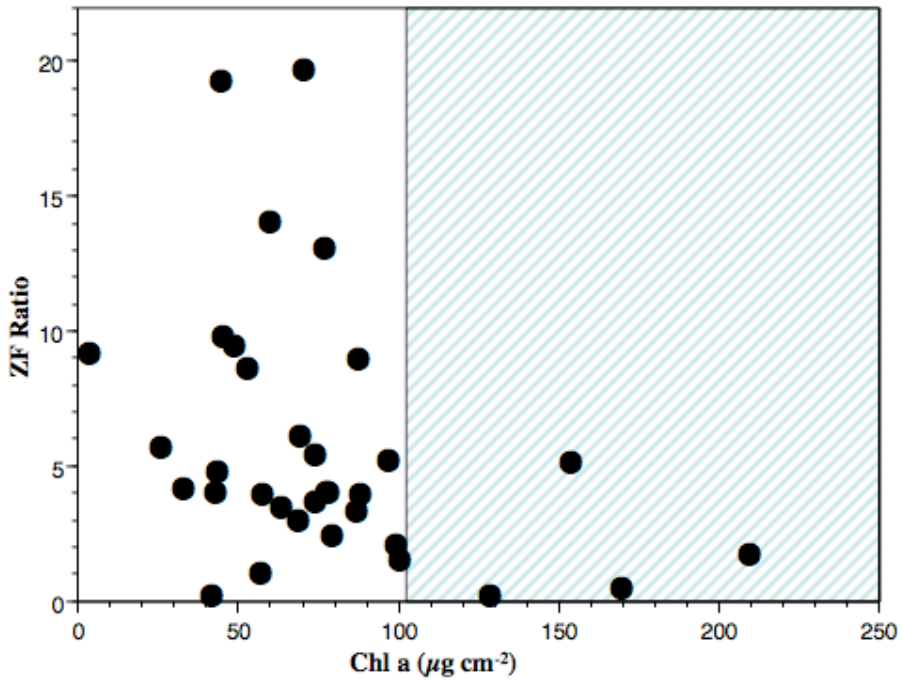

Figure 21: Chl a concentrations plotted against ZF ratios. Beyond ~100 μg cm^{-2} Chl a, mats have lower ZF ratios, suggesting that high biomass in mats is driven by diatoms rather than cyanobacteria, both of which contain Chl a. However, oxygen and nutrient flux data suggest that however high diatom biomass may be, it is not driving productivity.

indicating more diatoms relative to cyanobacteria. This suggests that diatoms may play a role in driving bulk biomass in the mats, which is an important finding since the role of diatoms in stromatolites and the extent to which they affect growth and morphology is largely unknown (Riding, Awramik et al. 1991; Browne, Golubic et al. 2000).

DIA mats that had higher productivity, paradoxically, had lower chlorophyll concentrations and therefore lower biomass, and p-addition treatments for these mats yielded higher productivity (figure 17). MAT mats, however, showed the opposite: p-addition treatments had lower productivity with higher concentrations of chlorophyll. Though the variation in productivity between the two mats cannot be compared (as mentioned earlier), the relationship between the response to P addition and chlorophyll raises further questions about what fraction of biomass is productive in the mats, and which types may be generally more efficient producers or recyclers. Furthermore, a relationship exists between the ZF ratio and the rate of respiration (figure 22), wherein mats with lower ZF ratios (and therefore a higher diatom fraction) also had a higher rate of respiration. Given this, and the fact that the nitrogen being fixed by the mat's cyanobacteria is an anaerobic process, one explanation may be that diatoms respiring above the cyanobacteria may help shift the anoxic boundary layer and create a greater zone of anoxia within the mat, thereby promoting nitrogen fixation.

Considering this data, it is unlikely that nutrients play a role in the transition between mat types in Highborne Cay stromatolites, since variability in nutrient fluxes between types are smaller than diel variability between experimental runs. This is supported by the fact that nutrient concentrations at the study site are the same as those on the western (marina) side of the cay (chapter 2) where no stromatolites are found,

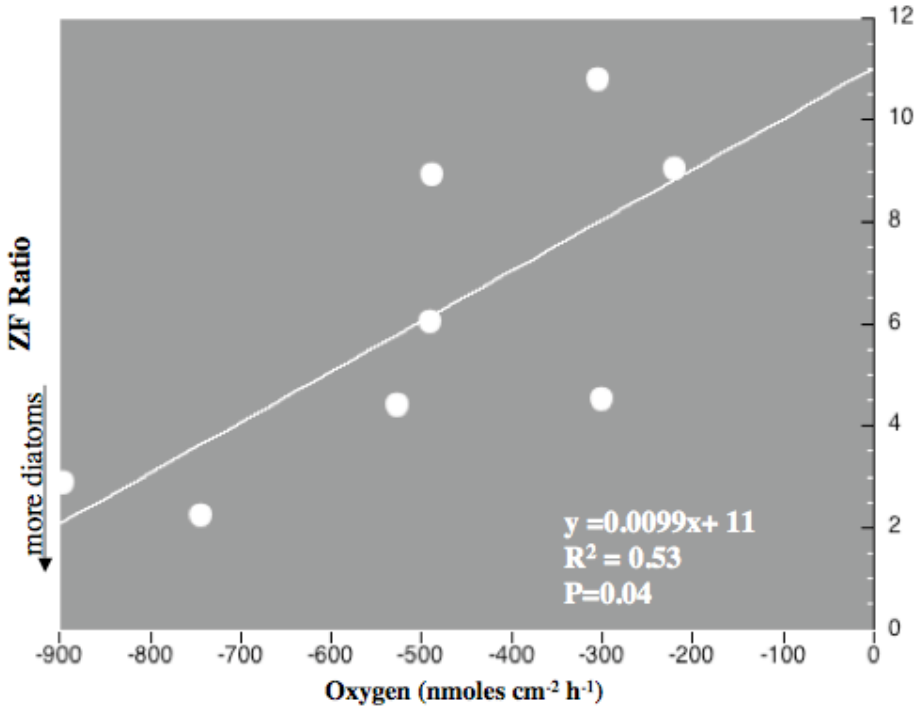

Figure 22: Relation of ZF Ratio to Oxygen and NO_3 Flux Across All Mats and Treatments. The lower the ZF ratio, the greater the oxygen uptake through respiration. Greater diatom abundance increases respiration, suggesting diatoms are an important constituent in Highborne mats.

though these environmental data are limited and much only be taken as a possible indicator. More extensive environmental monitoring is needed to make a judgement conclusively. However this conclusion is further supported by the fact that PO_4 additions do not seem to promote productivity despite that abundant EPS created by the mats and low ambient PO_4 in the water column both suggest otherwise. Therefore, environmental variability in nutrients – and most importantly in P – likely would not affect the geochemical cycling of the mat and not force a transition between types.

It is most likely that physical forcing such as magnitude of turbulent flow and burial promote the transition between mat types. The physical regime may be responsible for the transition by augmenting the recruitment of diatoms to the mats, a factor which may to have significant implications in mat constitution and chemical cycling given this study's data on the possibly importance of diatoms in the mats.

Conclusion

Research into modern marine stromatolites suggests that they are a well-developed microbial consortia that carry out significant geochemical recycling. Though this consortia has been thought to explain why stromatolites exist in Highborne Cay assuming the study site is oligotrophic, typical for tropical areas, this study's experiments suggest that nutrient supply does not play a likely role in stromatolite persistence at this site. The nutrient regime in highborne offers abundant nitrogen, and though concentrations of phosphate could be limiting, addition of this nutrient to the mats spurred no change in productivity.

The findings also suggest Highborne mats exchange solutes with an environment that can be high in nutrients. This may reduce the significance of N-fixation on which the mats were thought to rely, and changes the way studies must look at the internal recycling within the mats, because given the high turbulent flow in which these mats exist, recycling within the mat may be slower than the rate at which solutes are lost to the water column. The fact that there is no difference in solute fluxes by mat type further supports this, as fluxes from the mat may be driven by external factors such as water column solute concentrations and flow rates, rather than internal ones such as community composition.

In light of these findings, stromatolite formation in Highborne Cay is likely a function of an internal response to external, physical mechanisms that limit the growth of competitors such as diatoms and macroalgae.

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