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Structural and functional responses of microbial mats to reductions in nutrient and salinity stressors in a Bahamian hypersaline lagoon

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ABSTRACT: Microbial mats in Bahamian hypersaline lagoons are affected by the combination of salinity fluctuations and external nutrient inputs, both of which are seasonally variable. The purpose of this study was to examine the singular and combined effects of salinity and nutrient (N+P) stress on primary production, extracellular enzyme activity, and the composition of the photoautotroph community in this episodically varying extreme environment. Anoxygenic phototrophic bacteria were able to increase their relative abundance when nutrients were supplied under hypersaline conditions (300 g l^{-1}). When salinities were lowered (38 g l^{-1}) and nutrients added, extracellular enzyme activity (aminopeptidase, α-glucosidase, and β-glucosidase), rates of oxygenic photosynthesis, and phototroph biomass increased in the oxic surface layers of the mat. Once salinity stress had been lowered, oxygenic photosynthesis allowed the proliferation of *Cyanobacteria*, heterotrophic activity, and a corresponding reduction in the abundance of anoxygenic phototrophic bacteria. On reduction of nutrient stress, mat phototrophs responded by increasing biomass (using either anoxygenic or oxygenic photosynthesis, or both). In this hypersaline system, seasonal as well as short-term (days) variations in environmental conditions may promote structural changes in the mat community which alter the rates of major processes such as oxygenic photosynthesis and heterotrophy, and illustrate the cyclic behavior of microbial dormancy and proliferation in this extreme environment. Cycles in nutrient input and salinity are primary forcing factors for the maintenance of a dynamic and diverse (both structurally and functionally) benthic microbial community in a small hypersaline lagoon, Salt Pond, on San Salvador Island, Bahamas.

KEY WORDS: *Cyanobacteria* · Photopigments · HPLC · Enzymes · Productivity

INTRODUCTION

Hypersaline systems, such as lagoons, evaporative lakes, and salterns are commonly inhabited by benthic microbial mats composed of phototrophic *Cyanobacteria*, diatoms, and diverse chemoheterotrophic and chemoautotrophic bacteria (Cohen & Rosenberg 1989, Stal 1995, Oren 2000, Abed et al. 2008, Green et al. 2008). Calm, protected conditions and clear, shallow waters provide ideal environments for the prolific growth of structurally and functionally well-developed microbial mats (Black 1933, Neumann et al. 1970, Pinckney et al. 1995). These systems are frequently nitrogen depleted, and primary productivity relies on inputs of nitrogen through the activity of nitrogen-fixing diazotrophs (*Cyanobacteria* and diverse members of *Alpha-*, *Beta-*, and *Gammaproteobacteria*) as well as allochthonous sources in rainfall and runoff (Stal 1995, Camacho & de Wit 2003, Omoregie et al. 2004, Yannarell et al. 2006).

In Salt Pond, a small hypersaline lagoon on San Salvador Island, Bahamas, the microbial mats, which are dominated by *Cyanobacteria*, are well-developed in terms of abundance, biomass, and primary production

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(Paerl et al. 1993, Pinckney et al. 1995, Yannarell et al. 2006, Yannarell & Paerl 2007). Maximum rates of production and nitrogen fixation occur in a <10 mm thick surface layer during the daytime and nighttime, respectively (Paerl et al. 1996). Purple anoxygenic photosynthetic bacteria (*Chromatium* sp.) and chemolithotrophic bacteria (*Beggiatoa* sp.) migrate from deeper layers to the surface during periods of anoxia in the sediments (Paerl et al. 2003). In the dry months, evaporation leads to hypersaline conditions in which *Cyanobacteria* are the primary nitrogen-fixers. During wet periods, a more diverse community of bacterial diazotrophs develops within the mat (Yannarell et al. 2006). On annual time scales, the primary environmental control on microbial activity in this lagoon appears to be salinity, which may range from as low as 60 to 90 g l^{-1} in the wet season (September–January) to a maximum of 340 g l^{-1} at the end of the dry season in July and August (see Fig. 1) (Yannarell et al. 2006). Lower salinity generally promotes higher metabolic activity, and experimental reduction in salinity results in elevated rates of primary production and nitrogen fixation in less than 2 d (Paerl et al. 2003).

The relatively small size (5.0 ha) and shallow waters (<1 m) of Salt Pond allow rapid, major changes in salinity. Freshwater inputs from runoff and direct deposition during rainfall events affect submerged mats as well as those subaerially exposed in the littoral zone. Salinity can range from freshwater (rainfall on salt-encrusted subaerial mats) to reductions of pondwater salinity by as much as 60% following major rains (Yannarell et al. 2007). Furthermore, rain may import allochthonous nutrients and organic matter that stimulate microbial activity (Yannarell et al. 2007). The focus of our research was to quantify the effects of these rapid salinity alterations on microbial mat structure and function using a series of manipulative bioassays.

Previous studies have demonstrated that microbial mats in Salt Pond are affected by the combination of salinity fluctuations and external inputs of the growth-limiting nutrients nitrogen (N) and phosphorus (P), both of which are variable over time (Pinckney et al. 1995, Pinckney & Paerl 1997, Paerl et al. 2000, 2003, Yannarell & Paerl 2007, Paerl & Yanarell 2010). However, the primary focus of previous studies was the determination of functional responses of the community (e.g. primary productivity, nitrogen fixation) rather than detailed examinations of structural changes of the phototrophic community in response to nutrient/salinity manipulations. Microbial responses to rapid and simultaneous changes in both of these factors offer insight into the processes that define the structural and functional characteristics of this diverse and tightly-coupled community. The purpose of the present study was to examine the individual and synergistic effects of salinity and nutrients (N+P) on both the structural (photoautotroph relative abundances) and functional (primary production, enzyme activity) responses of the microbial mat community in Salt Pond to assess the potential impacts of rapid changes in nutrient and salinity conditions similar to those encountered during episodic rainfall events.

MATERIALS AND METHODS

The island of San Salvador, Bahamas (24° 05′ N, 74° 30′ W), has a subtropical, semiarid climate, with a mean annual rainfall of 101 cm. In a typical year, just under half of the rainfall occurs during tropical storms in the wet season from mid-September through late November (Shaklee 1996, Paerl et al. 2003). Salt Pond (24° 01′ N, 74° 27′ W) is a shallow (<1.0 m) hypersaline lagoon (5.0 ha) located on the central eastern side of San Salvador. The water volume of Salt Pond varies with the wet and dry seasons, and salinity ranges from 67 to 340 g l^{-1} (median = 168 g l^{-1}) (Fig. 1). Carbonate sands, including portions of the arid littoral zone, are covered by microbial mats consisting of benthic diatoms and non-heterocystous *Cyanobacteria* of the orders Oscillatoriales, Chroococcales, and Pleurocapsales (Pinckney & Paerl 1997, Paerl et al. 2003, Yannarell et al. 2006). Deep cores reveal alternating periods of prolific mat growth, sedimentation events, and a layer of gypsum ($CaSO_4 \cdot 2H_2O$) at ca. 1 m in the sediment. The pH of the hypersaline water in Salt Pond is a constant 7.95 to 8.00. Six-year (2000–2006) median values for nutrients (NO_2^-+NO_3^-, NH_4^+, PO_4^{3-}, Si) in the waters of the lagoon were 0.8, 14.5, 0.3, and 33 µmol l^{-1}, respectively, and mean N:P and N:Si molar ratios were 71 and 0.9, respectively (Paerl et al. unpubl.).

On 26 June 2004, cores of subaerially-exposed microbial mat (4.0 × 4.0 × 1.0 cm) were collected from moist sediments in the littoral zone midway between the waterline and shoreline during a period of extreme hypersalinity (300 to 330 g l^{-1}) and temperature (>40°C) in Salt Pond (Fig. 1). Six cores of mat material were randomly placed upright in 12-well polystyrene tissue culture plates (Falcon). Experimental manipulations consisted of 12 replicate mat core incubations in Salt Pond water (300 ± 10 g l^{-1}, mean ± SD), seawater (38 ± 5 g l^{-1}), and freshwater (2.0 ± 2 g l^{-1}). Nitrate and phosphate (500 µmol l^{-1} NO_3^- as KNO_3 and 500 µmol l^{-1} PO_4^{3-} as KH_2PO_4, final concentrations) were added to one-half of the samples (Table 1). Culture plates were submerged to a water depth of 10 cm in plastic containers (50 × 50 × 25 cm) filled with the incubation water for the different salinity and nutrient treatments. This design resulted in

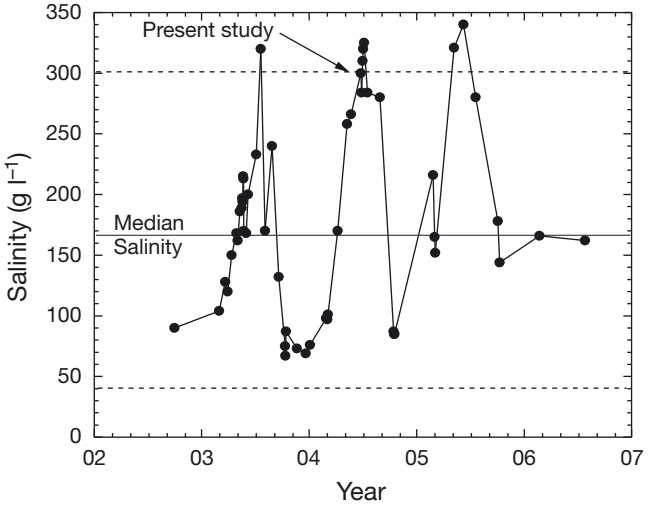

Fig. 1. Salinity (g l^{-1}) in Salt Pond, San Salvador Island, Bahamas, from 2002 to 2006. Salinity at the time of the bioassays is indicated by an arrow, and the horizontal dashed lines show the salinities for the bioassays. Solid horizontal line indicates the median salinity over the time period

12 replicates (in 2 culture plates) for each of 6 treatment levels in a fully-crossed statistical design. Incubation containers were placed outside under ambient irradiance in small tanks continually flushed with flowing seawater (0.05 l s^{-1}) for temperature control (30 to 36°C). Salinity was checked daily using a refractometer (±0.2%) and adjusted (by adding freshwater) to maintain the desired concentrations. After 10 d, the incubations were terminated and assayed for primary productivity, photopigment abundance, and enzyme activity. This manipulative experiment was designed to simulate salinity and nutrient exposures under ambient conditions as well as following a rain event (Table 1). Thus mats were not pre-acclimated to nutrients or salinities prior to the manipulations because the overall goal was to determine the short-term (10 d) mat responses to the extreme ranges of salinity and nutrients likely experienced in Salt Pond. Episodic rain events are common and reflected by rapid changes in pondwater salinities (Fig. 1).

Gross primary production was measured with oxygen microelectrodes (Unisense; 20 µm tip diameter) using the light/dark shift method (Revsbech & Jørgensen 1986). Productivity measurements consisted of illuminating the sample with a fiber-optic light (ca. 1500 µmol photons m^{-2} s^{-1}) and measuring the initial slope of oxygen decrease at 100 µm depth intervals within 1 to 2 s after darkening the sediment surface (Revsbech & Jørgensen 1986, Pinckney & Zingmark 1993). All measurements were undertaken after oxygen concentrations had achieved a steady state as determined by microelectrode profiles in the sample. Contact between the tip of the microelectrode and the mat surface was observed with a small magnifying lens (25×). Productivity was measured at successive intervals of 100 µm until there was no detectable response within 5 s of the light/dark shift. Data were acquired and processed using Sloper software (Unisense). The measured rate at each depth interval was then integrated over all depth intervals to give a depth-integrated areal estimate of gross primary production (GPP). Three vertical profiles of production were obtained at random locations within each sample. Depth-integrated GPP for all 3 profiles was averaged to provide an estimate of total community production for each sample. GPP was determined for 6 of the 12 samples in each of the 6 treatments (108 production profiles, 36 GPP estimates, 6 treatments, 6 replicates). Productivity measurements were obtained under subaerial (i.e. not submerged) exposure conditions to minimize the potential effects of salinity-related oxygen diffusion constraints (Sherwood et al. 1991, Garcia-Pichel et al. 1999) and therefore represent maximum potential rates of GPP for comparisons among the different treatments.

Subsamples (1.0 × 1.0 × 0.3 cm) from each of the productivity samples were collected for photopigment analysis, stored in 2 ml microfuge tubes, and immediately frozen. High-performance liquid chromatography (HPLC) was used to determine chemosystematic photosynthetic pigments for mat photoautotrophs. Mat samples were lyophilized for 24 h at –50°C, placed in 90% acetone (1.00 ml), sonicated, and extracted at –20°C for 18 to 20 h. Filtered extracts (200 µl) were injected into a Shimadzu HPLC apparatus equipped with a monomeric (Rainin Microsorb-MV, 0.46 × 10 cm,

Table 1. Bioassay design summary and corresponding abbreviations used in the text. There were 12 replicates for each treatment for a total of 72 individual core samples (each 4.0 cm^2) used in the experiment

Designation	Description	Salinity (g l^{-1})	Addition (µmol l^{-1})
FW Ctrl	Freshwater, control	2	Ambient nutrients
FW N+P	Freshwater, nutrient addition	2	500 NO_3^-+ 500 PO_4^{3-}
SW Ctrl	Seawater, control	38	Ambient nutrients
SW N+P	Seawater, nutrient addition	38	500 NO_3^- + 500 PO_4^{3-}
SP Ctrl	Salt Pond water, control	300	Ambient nutrients
SP N+P	Salt Pond water, nutrient addition	300	500 NO_3^- + 500 PO_4^{3-}

3 µm) and a polymeric (Vydac 201TP54, 0.46 × 25 cm, 5 µm) reverse-phase C18 column in series. A nonlinear binary gradient consisting of the solvents 80% methanol:20% 0.50 M ammonium acetate and 80% methanol:20% acetone was used for pigment separations (Pinckney et al. 1996). Absorption spectra and chromatograms (440 ± 4 nm) were acquired using a Shimadzu SPD-M10av photodiode array detector. Pigment peaks were identified by comparison of retention times and absorption spectra with pure standards (DHI). The synthetic carotenoid β-apo-8′-carotenal (Sigma) was used as an internal standard.

Ectoenzymatic activity of the microbial community was assayed as an indicator of the response of the heterotrophic community to the experimental manipulations. 4-methylumbelliferone-conjugated substrate analogs (MUF) were used to measure enzyme activity associated with the breakdown of carbohydrates (i.e. β-glucosidase and galactosidase), and mono-phosphoesters (i.e. alkaline phosphatase; Sigma) (Hoppe 1983). An aminomethyl-coumarin-conjugated substrate analog (AMC) was used to measure leucine-aminopeptidase activity (Sigma). Due to limited quantities of mat material, assays were conducted in duplicate for each of the incubation treatments at the termination of the experiment, with a single heat-killed control. Three sub-cores were taken from each core and divided into quarters, and the upper 3 mm of mat material was transferred to a test tube. Water (3 ml) from respective salinity treatments was added to the sample with 100 µM of MUF/AMC-substrate. Samples were incubated at *in situ* temperatures for 10 min. Prior to measuring the fluorescence signal (excitation 365 nm, emission 460 nm) the sample was briefly vortexed to mix the liquid without disrupting the structure of the core. Then, a 1.5 ml sub-aliquot was taken, measured and returned to the test-tube. Samples were incubated for an additional 10 min and a second measurement was taken. A subset of the cores was dried and weighed. The fluorescence signal was calibrated using either MUF or AMC standards prepared using water from the respective salinity/nutrient treatments and normalized to time and sample dry weight.

RESULTS

Primary productivity

Gross primary production (GPP) measured after 10 d incubation ranged from 0 to 2.39 ± 0.44 µmol O_2 cm^{-2} h^{-1} (mean ± SD) (Fig. 2). A single-factor ANOVA indicated a significant treatment effect ($F_{5,30}$ = 62.63, p < 0.001) and a Dunnett's T3 post hoc comparison of means indicated 3 homogeneous groups (p ≤ 0.05). The

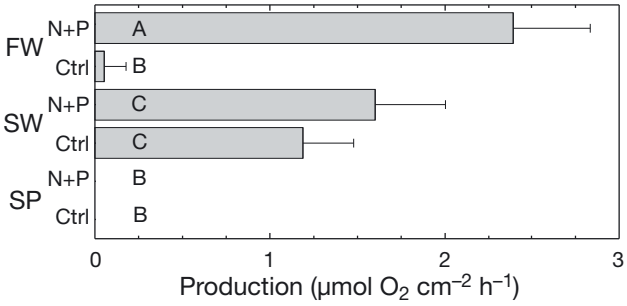

Fig. 2. Gross primary production for the 6 experimental treatments (see Table 1). Bars represent mean + SD for 6 replicates. Letters A, B, and C indicate the results of *a posteriori* multiple comparisons of means. Bars which share the same letters were not significantly different (p > 0.05). No oxygenic photosynthesis was detected in the initial (time 0) or the SP samples

FW N+P (freshwater, nutrient addition) treatment had the highest GPP, followed by SW Ctrl (seawater, control) and SW N+P (seawater, nutrient addition). GPP in all other treatments was undetectable. Vertical profiles of steady-state dissolved O_2 concentrations in the mats were consistent with the productivity results (Fig. 3). The upper 15 mm of mats exposed to the SW Ctrl, SW N+P, and FW N+P treatments showed supersaturated concentrations of O_2 while the other 3 treatments were essentially hypoxic/anoxic.

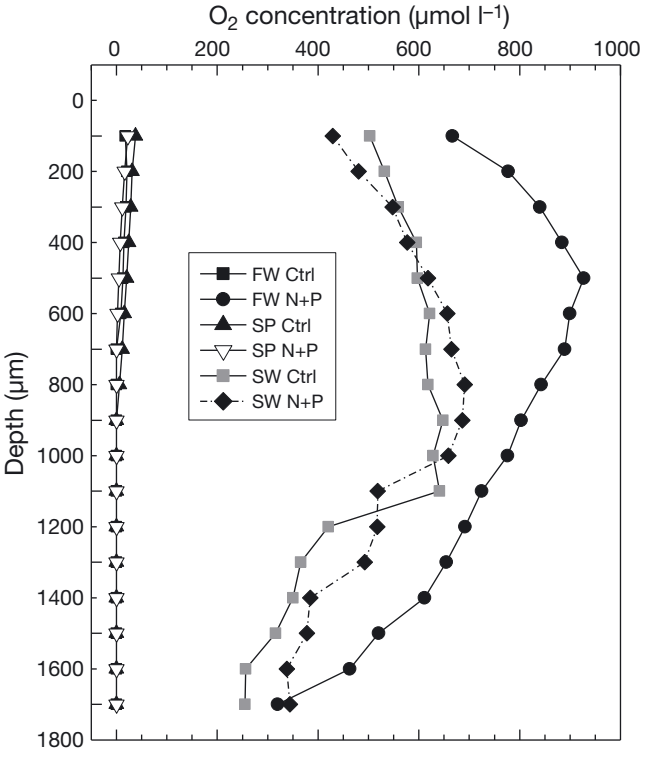

Fig. 3. Representative profiles of the vertical distribution of steady-state dissolved oxygen concentrations for each of the experimental treatments (see Table 1)

Ectoenzyme activity

Enzyme activity measurements on duplicate samples for α-glucosidase activity ranged from 2.93 to 8.31 nmol g^{-1} h^{-1} and was highest in the SW N+P treatment (Fig. 4). The activity of β-glucosidase had a range of 2.43 to 12.62 nmol g^{-1} h^{-1} with higher rates in the SW (seawater) incubations. Similarly, aminopeptidase activity was highest in the SW treatments and ranged from 2.06 to 129.2 nmol g^{-1} h^{-1}. Alkaline phosphatase activity was highly variable (6.81 to >500 nmol g^{-1} h^{-1}), with the highest rates in the SP (Salt Pond) treatments, followed by the SW control, SW N+P, and FW (freshwater) treatments. With the exception of alkaline phosphatase, enzyme activities were generally higher in the treatments that exhibited oxygen supersaturation in the upper mat layers. The highest alkaline phosphatase activity occurred in the anoxic samples.

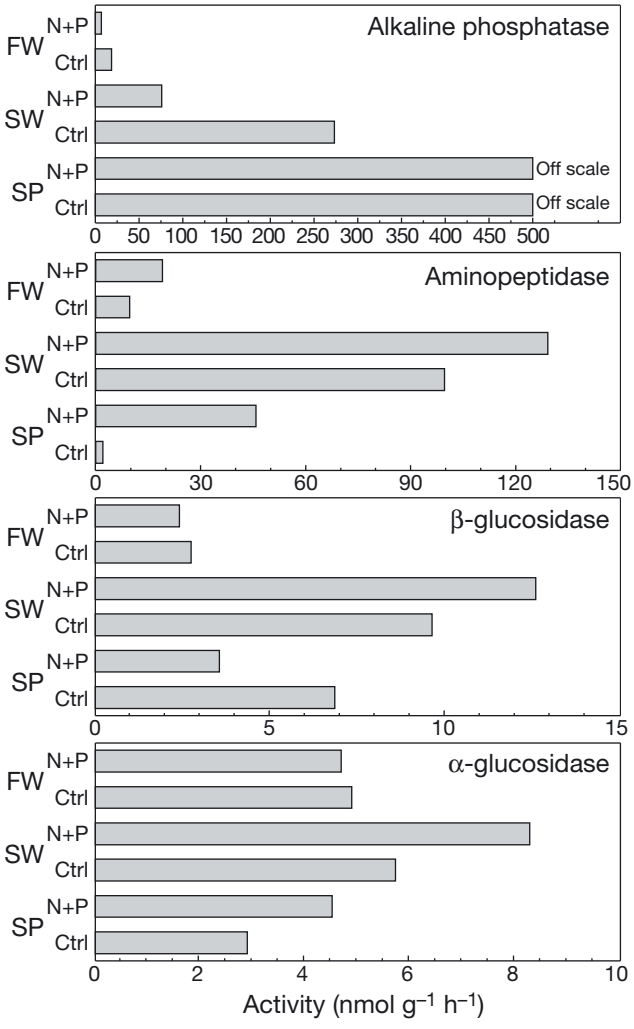

Fig. 4. Ectoenzyme activities for each of the experimental treatments. Bars represent the average of duplicate measurements (see Table 1)

Photopigment abundances

The most commonly detected photopigments were chlorophyll *a* (chl *a*), bacteriochlorophyll *a* (bchl *a*), echinenone myxoxanthophyll, zeaxanthin, α-carotene, β-carotene and the photoprotective pigment scytonemin. The photopigment composition indicated that the phototroph community was composed almost exclusively of *Cyanobacteria* and anoxygenic phototrophic bacteria (Fig. 5). For the bioassays, photopigment abundances were analyzed using a single-factor multivariate analysis of variance (MANOVA) with treatment as the factor (6 levels) and pigment abundances as the variates (10 pigments). The MANOVA indicated a significant treatment effect (Pillai's Trace, $F_{50,110}$ = 5.63, $p < 0.001$). The univariate ANOVAs showed sig-

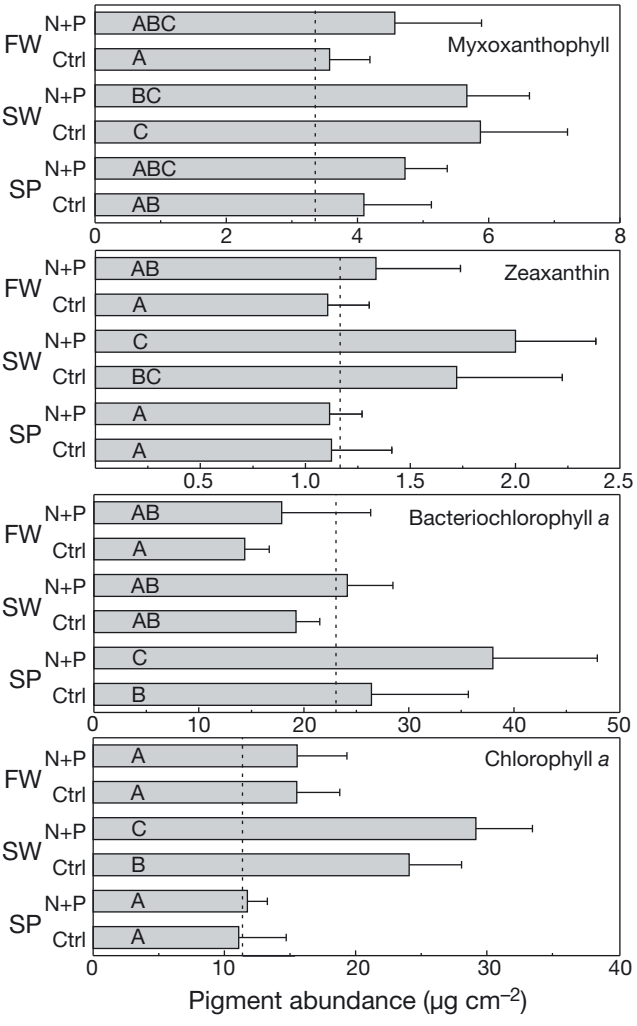

Fig. 5. Photopigment abundances for each of the experimental treatments (see Table 1). Bars represent mean + SD for 6 replicates. Letters A, B, and C indicate the results of *a posteriori* multiple comparisons of means. Bars which share the same letters were not significantly different ($p > 0.05$). Vertical dashed lines indicate initial pigment concentrations (time 0)

nificant treatment effects for all pigments ($p \leq 0.05$, power ≥ 0.71) except echinenone ($p = 0.945$, power = 0.096). Mean abundances for each treatment level were compared using the Bonferroni multiple comparisons test ($p \leq 0.05$) (Fig. 5). For chl *a*, abundances were higher in the SW treatment. The indicator pigment for anoxygenic phototrophic bacteria, bchl *a*, was highest in the SP water and lowest in the FW treatment. The cyanobacterial pigments zeaxanthin and myxoxanthophyll were highest in the SW incubations. The only significant nutrient addition effects were for chl *a* in SW and bchl *a* in SP.

Discriminant analysis was performed following the MANOVA to build a predictive model of group membership based on the observed photopigment abundances for each treatment level in the bioassay (Fig. 6). The first 2 discriminant functions explained 74.7 and 11.2% of the variance, respectively (cumulative total = 85.9%) (Wilks' $\lambda = 0.002$, $p < 0.001$). Using these functions, 93.9% of the samples were correctly classified according to the incubation treatment level. The group centroids, derived from the canonical discriminant functions, suggest that the photopigment composition of mats incubated at lowered salinities differed from those in SP water. Furthermore, photopigment abundances in the N+P additions differed from those in the corresponding control (Ctrl) treatments. The notable exception is an absence of a nutrient addition effect for the SP incubated samples. The differences between most of the treatments were best explained by the first discriminant function, while the second function explained the difference between the N+P and Ctrl treatments for the FW incubated mats. The primary difference between the 2 functions was related to a change in sign (i.e. +/–) for the coefficients for myxoxanthophyll and chl *a*, suggesting that mat phototroph community differences were primarily attributable to changes in cyanobacterial biomass.

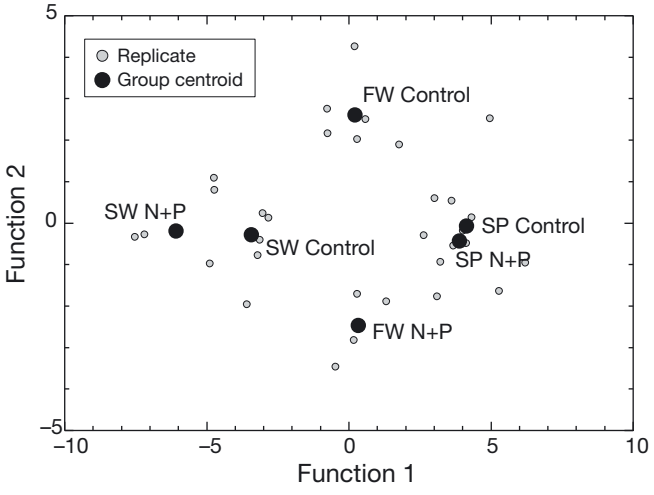

Fig. 6. Canonical discriminant function plot for pigment data classified by experimental treatment (see Table 1). Centroids are labeled with respective treatment levels, and functions for individual data points are indicated by the small circles

Summary of mat responses

The microbial mat enzyme activity, productivity, and photopigment responses to the N+P additions in the bioassays relative to their respective controls (i.e. no nutrients added) were summarized for each of the salinity treatments (Table 2). The highest increase in chl *a* occurred in the SW incubation followed by the SP and FW treatments. Bchl *a* increased in all treatments, with the largest increase in the SP water. β-carotene increased in the SP and SW treatments and declined in FW. For echinenone, abundances declined in all treatments while myxoxanthophyll declined in the SW treatment and scytonemin was detected in the SP water only. Zeaxanthin increased in SW and FW but remained relatively constant in the SP water. GPP was not detectable in the SP treatment, showed a 35% increase in SW, and was highest (4500%) in the FW incubation. For enzymes, α-glucosidase activity increased in SW and FW while β-glucosidase activity decreased in SP and FW. Alkaline phosphatase activity was highest (>500 nmol g^{-1} h^{-1}) in FW but lower relative to controls in SP and SW. Aminopeptidase activity was highest in FW and an order of magnitude lower in SP and SW.

Table 2. Summary of microbial mat responses to nutrient (N+P) additions. Values are percent higher or lower than respective measurements for controls incubated at the 3 salinity levels (Salt Pond = 300 g l^{-1}, seawater = 40 g l^{-1}, freshwater = 2 g l^{-1}). These results highlight the response to nutrient additions within each of the 3 salinity levels

	Salt Pond	Seawater	Freshwater
Chlorophyll *a*	5.9	21.1	0.2
Bacteriochlorophyll *a*	43.8	25.4	24.5
β-carotene	4.0	15.6	36.4
Echinenone	–20.1	–20.2	–5.8
Myxoxanthophyll	15.2	–3.5	27.8
Scytonemin	21.5	[a]	[b]
Zeaxanthin	–0.7	16.3	20.8
Gross primary production	0.0	34.8	4498
α-glucosidase	55.2	44.5	–4.0
β-glucosidase	–47.9	30.8	–12.0
βminopeptidase	2124	29.6	96.6
Alkaline phosphatase	[c]	–72.2	–63.1

[a]Scytonemin was not detected in the N+P treatments
[b]Scytonemin was not detected in either control or N+P treatments
[c]Activity was off-scale and >500 nmol g^{-1} h^{-1}

DISCUSSION

Seasonal rainfall and evaporation at Salt Pond result in an annual salinity range of 60 to 350 g l^{-1}. Hypersalinity, coupled with a high exposure to ultraviolet light and summer water temperatures exceeding 40°C, create extreme environmental conditions within the lagoon. The historical N:P ratios (0 to 1600) suggest a strongly P-limited system, and nutrient concentrations are highly variable over the range of salinities within the pond. However, nutrient concentrations are generally highest when salinities are >100 g l^{-1} (Paerl et al. unpubl.). At salinities <100 g l^{-1}, nutrient concentrations are much lower, possibly a result of more rapid utilization and most likely reflects higher activity and growth rates (Abed et al. 2007, Oren 2009). Wieland & Kühl (2006) reported that a salinity of 100 g l^{-1} seems to be a transition point for microbial mats at which a further increase results in higher respiration and lower rates of oxygenic photosynthesis. In Salt Pond, rates of nitrogenase activity and CO_2 fixation decrease with increasing salinity (Pinckney et al. 1995, Pinckney & Paerl 1997, Paerl et al. 2003). The observed relationship between salinity and nutrient concentrations in Salt Pond may reflect physiological changes in mat activity.

In the present study, manipulative bioassays were used to quantify the structural and functional responses of the microbial mat community to short-term changes in salinity and nutrient concentrations reflecting the extreme range experienced in Salt Pond over an annual cycle. The manipulations simulated exposure of mats in the littoral zone to rainfall (freshwater) and responses were measured after allowing sufficient time (10 d) for acclimation to the altered salinity and nutrient conditions. Previous studies have shown measurable changes within 2 d (Pinckney & Paerl 1997, Paerl & Yanarell 2010). The nutrient additions for the bioassays were ca. 5 to 10× higher than previously measured levels in the lagoon. These high concentrations were used to minimize the possibility of nutrient limitation during the bioassay. Samples incubated in hypersaline SP water did not exhibit any detectable oxygenic photosynthesis and vertical profiles showed anoxic conditions in surface layers. Alkaline phosphatase activity exceeded 500 nmol g^{-1} h^{-1} in both the control and N+P amended samples and suggests severe P-limitation under hypersalinity and anoxia. However, the addition of nutrients resulted in an increase in the abundance of the photopigments of purple anoxygenic phototrophic bacteria and illustrates the ability of these microbes to grow under anoxic, hypersaline conditions when nutrient levels are elevated. Paerl & Yanarell (2010) have suggested that the high microbial diversity provides a rapid response to environmental variability because specific members of the community can assume key production and nutrient cycling functions depending on their salinity optima.

An increase in pigment abundance may reflect an increase in the abundance of respective phototroph groups and/or photoacclimation responses of cells. Because irradiance levels were similar for all treatments, photopigment changes due to light acclimation were unlikely. Furthermore, all experimental treatments were compared to appropriate controls for statistical tests.

The incubations at seawater salinity (38 g l^{-1}) showed high rates of photosynthesis relative to the controls, and increases in the abundances of chl *a* and the cyanobacterial pigment zeaxanthin. This response indicates an increase in oxygenic photosynthesis and growth by *Cyanobacteria* when salinity stress is reduced. The elevated activities of the extracellular enzymes aminopeptidase, α-glucosidase, and β-glucosidase suggest a similar heterotrophic response to lower salinity and oxygenation of the surface layers of the mat. Previous studies in Salt Pond have demonstrated the rapid uptake of dissolved organic carbon and organic nitrogen compounds by mat microbes following reductions in salinity (Paerl & Yanarell 2010). The addition of inorganic nutrients (N and P) at the lowered salinity resulted in higher glucosidase activities and higher chl *a* abundances but lower alkaline phosphatase activities relative to the corresponding control treatment. This response indicates that once salinity stress is reduced, nutrient concentrations become an important determinant of mat activity. The lowered alkaline phosphatase activity in the nutrient treatment provides some evidence that P may be the primary limiting nutrient under these conditions. Although the cyanobacterial pigment zeaxanthin did not exhibit a significant increase in abundance in the nutrient treatment, the general trend mirrored the increase in chl *a* and suggests cyanobacterial growth. The reduction in salinity from 300 to 38 g l^{-1} stimulated mat oxygenic photosynthesis for both the N+P and Ctrl treatments. The enhanced rates of GPP without a large increase in cyanobacterial biomass provides indirect evidence that *Cyanobacteria* can quickly switch from anoxygenic to oxygenic photosynthesis depending on the ambient environmental conditions (Jørgensen et al. 1986, de Wit et al. 1988, Pringault & Garcia-Pichel 2000).

At a salinity of 2 g l^{-1}, there was a reduction in the abundance of bchl *a* along with a low alkaline phosphatase activity, and only the N+P treatment showed a significant increase in GPP ($p < 0.05$). The observed vertical distribution of dissolved oxygen is consistent with the productivity measurements and shows that the SP and FW control treatments were anoxic. Previ-

ous studies have shown the potential importance of anoxygenic photosynthesis by *Cyanobacteria* (using H_2S as the electron donor), especially when exposed to high concentrations of sulfide (Padan 1979, 1989, Cohen et al. 1986, de Wit et al. 1988, Pinckney & Paerl 1997), which may be attributed to a reversible inhibition of photosystem II (PSII) by H_2S when concentrations exceed 3 µM (Jørgensen et al. 1986, Pringault & Garcia Pichel 2000, Wieland & Kühl 2000). Furthermore, salt stress results in an increase in the amount of P700 and PSI (photosystem I) reaction centers for some *Cyanobacteria*, possibly enhancing anoxygenic photosynthesis (Sudhir & Murthy 2004). Although the quantum yield for cyanobacterial anoxygenic photosynthesis is low, there is sufficient carbon fixation for survival (Oren et al. 1977). For mats in Salt Pond, the rates of CO_2 fixation in mats exposed to hypersalinity were nearly one-half the rates at normal salinities while oxygenic photosynthesis was not detectable (Pinckney & Paerl 1997, Paerl et al. 2003). The addition of seawater likely reduced sulfide concentrations in the upper mat layers directly by dilution and indirectly by enhancing cyanobacterial oxygenic photosynthesis and subsequent oxygenation of mat surface layers.

The discriminant analysis suggests that the collective changes in photopigment abundances were related to both nutrient status and incubation salinity, except for mats incubated in the hypersaline Salt Pond water. Under hypersaline (300 g l^{-1}) conditions, anoxygenic phototrophic bacteria were the only group to show a small but significant response to nutrient additions. In these microbial mats, both salinity and nutrient status seem to determine, interactively, the structure and function of the photoautotroph community. Nutrient additions alter community structure (photopigment composition), but the nature of the response depends on salinity. Yannarell et al. (2006), using *nifH* and 16S rDNA sequence data, demonstrated that the composition of the cyanobacterial community in Salt Pond was less sensitive to desiccation stress and hence dominated the nitrogen-fixing community during dry months. However, when salinities were lowered, the community diversity of diazotrophs increased.

Collectively, these results suggest that, under hypersaline conditions, *Cyanobacteria* fix carbon using anoxygenic photosynthesis while anoxygenic phototrophic bacteria increase their relative abundance when nutrients (N and P) are supplied in excess (Fig. 7). Once salinity and/or sulfide stress is lowered, oxygenic photosynthesis allows the proliferation of *Cyanobacteria* and a corresponding reduction in the abundance of anoxygenic phototrophic bacteria. When nutrient stress is reduced, mats respond by increasing biomass (using either anoxygenic or oxygenic photosynthesis, or both). High and low salinity conditions control the diversity and location, both horizontally and vertically, of key microbes responsible for carbon and nitrogen cycling within the mat community (Paerl & Yanarell 2010). In this hypersaline system, seasonal variations in environmental conditions result in structural changes in the mat community which alter the rates of major processes, such as oxygenic photosynthesis and heterotrophy, and illustrates the cyclic behavior of microbial dormancy and proliferation in this extreme environment.

The ability of the Salt Pond benthic microbial community to rapidly (within days) undergo alterations in community structure (relative abundances of photoau-

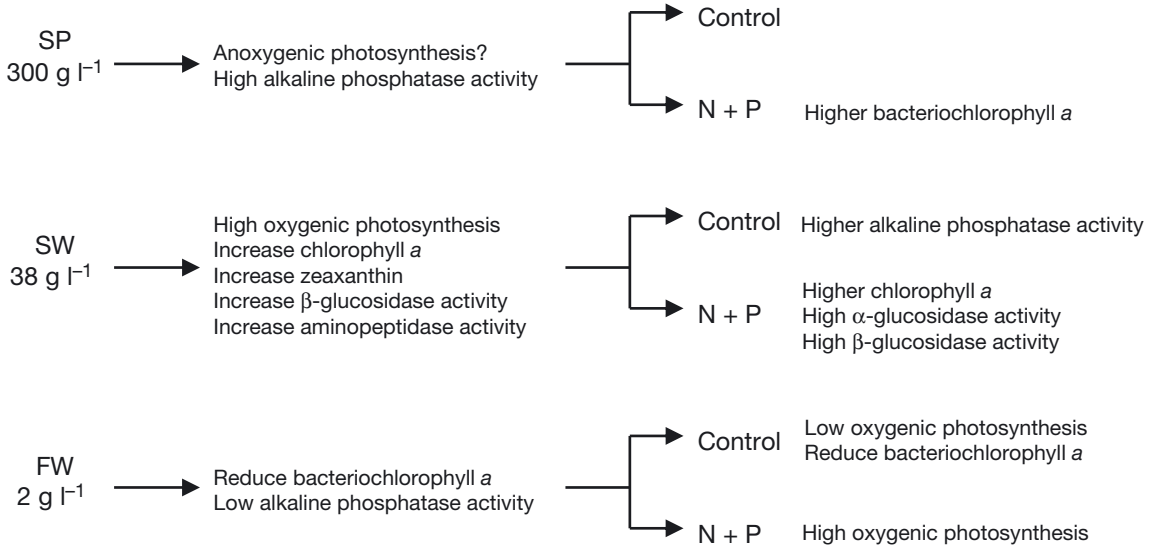

Fig. 7. Summary of microbial mat responses to changes in salinity and nutrient additions. FW = freshwater, SW = seawater, SP = Salt Pond, N+P = nutrient addition

totrophs) and function (rates of photosynthesis and heterotrophy) in response to major salinity and nutrient changes illustrates the close coupling of biotic and abiotic processes in this extreme environment. Furthermore, the community response to the alleviation of salinity and nutrient stress individually differs from the simultaneous removal of both stressors. Both salinity and nutrients regulate community structure and function in this system. Cycles in nutrient input and salinity are primary forcing factors for the maintenance of a dynamic and diverse benthic microbial community in this hypersaline lagoon.

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