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Role of salinity, nitrogen fixation and nutrient assimilation in prolonged bloom persistence of *Cyanothece* sp. in Lake St Lucia, South Africa

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ABSTRACT: Worldwide, cyanobacterial blooms are becoming more frequent, exacerbated by eutrophication and other anthropogenic actions and also associated with global climate change. In June 2009, a widespread bloom of the unicellular cyanobacterium *Cyanothece* sp. appeared in North Lake and False Bay of Lake St Lucia, a large (360 km^2) estuarine lake system in KwaZulu-Natal, South Africa, and persisted for 18 mo. It remains unclear how the bloom status was maintained for so long. This study investigated aspects of the nutrient (N and P) assimilation of *Cyanothece* sp. and how these may relate to maintaining a persistent bloom state during hypersaline conditions. The effects of salinity and nutrient limitation on the nutrient uptake dynamics of *Cyanothece* sp. were evaluated with $^{15}NO_3^-$ uptake, PO_4^{3-} uptake and $^{15}N_2$ fixation experiments. Nitrogen fixation was observed in this *Cyanothece* sp. isolate from St Lucia. Highest nutrient assimilation rates in all experiments were recorded at the lowest salinities, decreasing progressively up to a salinity of 120, with very little activity observed above this level. No $^{15}N_2$ fixation was measured above this salinity. Results indicate that *Cyanothece* sp. was well suited to take advantage of the conditions present during the onset of the bloom at salinities <100. However, once salinity increased above 120, nutrient uptake abilities would have been drastically reduced. Regardless, cells still survived under these extreme saline conditions, as most of their potential grazers and autotrophic competitors disappeared from the St Lucia Estuary.

KEY WORDS: $^{15}NO_3^-$ · $^{15}N_2$ · Cyanobacteria · *Cyanothece* · Lake St Lucia · Hypersalinity · Nutrient uptake · Nitrogen fixation

INTRODUCTION

Worldwide, cyanobacterial blooms are becoming more frequent and, together with eutrophication and other anthropogenic factors, have also been associated with climate change (Paerl & Huisman 2009). Eutrophication is a global issue, with many local consequences, resulting for instance in the world's largest trans-regional (km^2) macroalgal blooms during 2008 to 2012 in the Yellow Sea, China, where the clean-up cost ca. US$30.8 million (Liu et al. 2013). However, the mechanisms controlling the development and duration of algal, and particularly cyanobacterial, blooms are not always clear (Bianchi et al. 2000). Altered water characteristics (salinity in particular) influence ecosystem dynamics, which may lead to conditions conducive to cyanobacterial blooms (Paerl & Huisman 2009).

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Cyanobacteria grow optimally at relatively high temperatures, i.e. in excess of 25°C (Sellner 1997, Coles & Jones 2000). At these temperatures, the growth rates of most eukaryotic primary producers, such as diatoms, chlorophytes, cryptophytes and dinoflagellates decline (Jöhnk et al. 2008). Other factors that also contribute towards cyanobacterial dominance in water bodies include increased atmospheric CO_2 levels, accelerated anthropogenic nutrient loading, the accumulation of nutrients due to long water residence times and the effects of enhanced vertical stratification on the environment, such as increasing surface water temperatures (Paerl & Huisman 2009).

Nutrient dynamics are central to the establishment and maintenance of phytoplankton blooms. Cyanobacteria are capable of assimilating many forms of nitrogen, with a preference for the most reduced form i.e. ammonium (NH_4^+) (Herrero et al. 2001, Muro-Pastor et al. 2005). Many have been shown to be able to fix atmospheric nitrogen into NH_4^+ (Reddy et al. 1993, Bradley & Reddy 1997, Colón-López et al. 1997, Welsh et al. 2008, Sherman et al. 2010, Bandyopadhyay et al. 2011) and survive in conditions of extreme temperature, salinity and light exposure (Garcia-Pichel et al. 1998). Biological nitrogen fixation allows them access to a virtually unlimited pool of nitrogen that is not available to their non-diazotrophic competitors (Karl et al. 2002). This beneficial process of converting atmospheric N_2 to its reduced form is carried out by the nitrogenase enzyme complex (Montagna & Torres 2008, Zehr 2011). However, the nitrogen-reducing enzymes are highly sensitive to molecular oxygen (O_2), which irreversibly inactivates the enzyme (Schneegurt et al. 1994, Leigh 1995, Zehr 2011). To overcome the inhibitory effects of O_2 on N_2 fixation, many cyanobacteria have developed spatial and temporal strategies to separate oxygenic photosynthesis and nitrogen fixation (Fay 1992, Gallon 1992, Feng et al. 2010). For example, members of the focal species of this study (genus *Cyanothece*) have been shown to employ a temporal strategy to overcome the oxygenic inhibition of N_2 fixation by molecular oxygen: photosynthesis occurs during the day, while N_2 fixation is restricted to the night. It appears that these processes are governed by an underlying circadian rhythm, with the respective peaks in activity separated by 12 h (Reddy et al. 1993, Schneegurt et al. 1994, Colón-López et al. 1997, Schneegurt et al. 2000, Tucker et al. 2001, Stöckel et al. 2008, Toepel et al. 2008, Welsh et al. 2008, Červený & Nedbal 2009, Zehr 2011).

The St Lucia Estuary is part of the iSimangaliso Wetland Park, South Africa's first UNESCO World Heritage Site (Whitfield & Taylor 2009), and is the largest estuarine system in Africa. The 350 km^2 3 lake estuarine system, with an average depth of 0.9 m (Day et al. 1954), is particularly vulnerable to droughts and consequent hypersaline conditions. Wind-driven water movement is common in shallow water ecosystems (Schoen et al. 2014), yet despite the shallow nature of St Lucia, water has fairly long residence times. During mean water levels, dispersion of a tracer throughout the entire lake took about 3 to 4 mo, with little transfer between False Bay and North Lake (Schoen et al. 2014). Water residence time within the lake, specifically False Bay and North Lake, would have increased during the recent drought, with reduced water movement between the 3 basins (Schoen et al. 2014). In June 2009, a widespread bloom of an orange-pigmented planktonic organism appeared in North Lake and False Bay within Lake St Lucia and persisted for 18 mo. The organism was identified as a unicellular cyanobacterium of the genus *Cyanothece* (Muir & Perissinotto 2011). This was the first recorded bloom of this genus globally. Muir & Perissinotto (2011) suggested that the bloom was initiated by an unknown nutrient influx, which was subsequently augmented by a mass die-off of tilapia *Oreochromis mossambicus* as a result of an unusually cold winter. Elevated nutrient levels, coupled with the high salinity that reduced the grazing zooplankton species richness and abundance (Carrasco et al. 2010, Cyrus et al. 2010), resulted in its prolonged persistence within St Lucia (Muir & Perissinotto 2011). There must have been, however, a continuous nitrogen source (e.g. nitrogen fixation) in order to sustain bloom conditions.

Nitrogen fixation is an energetically demanding process (Gallon 1992, Colón-López et al. 1997, Vitousek et al. 2002), but has obvious ecological advantages in an environment where N may be limiting (Moisander et al. 2002, Marcarelli et al. 2006). Therefore, it was necessary to investigate whether this *Cyanothece* sp. isolate was able to fix N_2 and to what extent this occurred over the full salinity range observed during the bloom, since salinity also exerts a major influence on the metabolism of cyanobacteria (Moisander et al. 2002). We also investigated the impact of PO_4^{3-} availability on N_2 fixation, since this has also been shown to influence diazotrophic activity (Karl et al. 1997, Sañudo-Wilhelmy et al. 2001, Fu & Bell 2003). The ecophysiological experiments that were conducted shed further light on this *Cyanothece* sp. strain and how it could have per-

sisted for such a prolonged period of time in a hypersaline lake. Previous work by Muir & Perissinotto (2011) identified the bloom organism and provided preliminary information on the ecological drivers of the bloom. Further, du Plooy et al. (2014) showed that there were no interactive effects of environmental variables on the nitrogen uptake dynamics of this *Cyanothece* sp. Thus, the focus of the present study is on nutrient dynamics (uptake of N and P, and N_2 fixation) over a full salinity range, with the aim of expanding on the initial research to understand better the physiological aspects of *Cyanothece* sp. in relation to its bloom persistence.

MATERIALS AND METHODS

Culturing and preparation

Water samples were obtained during the height of the *Cyanothece* sp. bloom in June 2009 from Lister's Point in False Bay, St Lucia (Fig. 1). These were processed and cultures of *Cyanothece* sp. and its associated cobiont, *Flexibacter* sp., were prepared and maintained as described by Muir & Perissinotto (2011). Members of the genus *Flexibacter* are heterotrophic, utilising organic nitrogen sources (Lewin 1974, Wakabayashi et al. 1986, Raheb et al. 2007). Environmental conditions of the different cultures are summarized in Table 1. Stock cultures were prepared in 250 ml Erlenmeyer flasks (acid washed, autoclaved and fitted with cotton stoppers and foil) over a wide salinity range (0 to 300, at 60 salinity unit increments) with 200 ml ASN III media (Andersen et al. 2005) with N and P added (N- and P-replete conditions) according to Reddy et al. (1993). A step-wise acclimation from one salinity to the next was conducted at 30 salinity units every 48 h until the desired salinity was reached. From the stock cultures, working cultures with N- and P-limitations were made for the purpose of nutrient (both N and P) uptake and N_2 fixation experiments. These ASN III media alternatives included combinations of N and P concentrations (e.g. N-limited and P-limited; N-limited and P-replete; N-replete and P-limited). All the cultures were kept in a growth chamber with the temperature maintained at 30°C and light intensity at 150 µmol m^{-2} s^{1} with a day/night cycle of 12 h.

Nutrient concentrations (dissolved inorganic nitrogen [DIN]: NO_2^-, NO_3^-, NH_4^+ and dissolved inorganic phosphorus [DIP]: PO_4^{3-}) were determined from water samples collected during survey trips by the University of KwaZulu-Natal (UKZN) research team

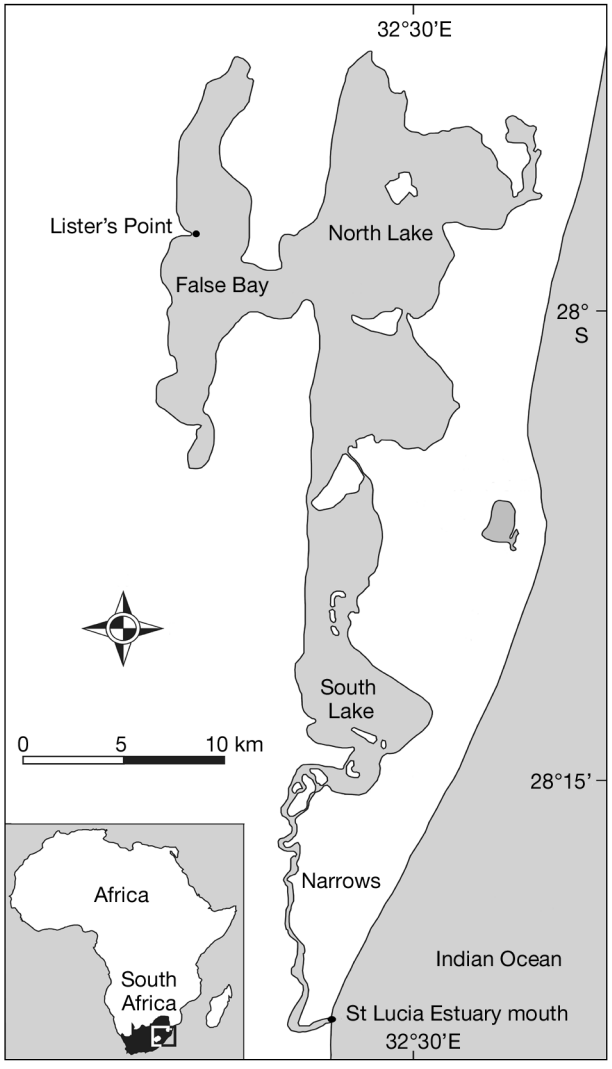

Fig. 1. Map of the St Lucia estuarine lake, showing the location (Lister's Point) where the *Cyanothece* sp. cells were collected in June 2009. Adapted from Miranda et al. (2010)

at the surface of the water column. Pore-water samples for the measurement of DIN and DIP were collected following the method described by Anandraj et al. (2008). All nutrient samples were placed in 500 ml acid pre-washed polyethylene bottles and analysed by the Durban branch of the Council for Scientific and Industrial Research utilising an Autoanalyzer III system using standard methods.

^{15}N (as $^{15}NO_3^-$) uptake

The uptake rates of N as $^{15}NO_3^-$ were calculated following the ^{15}N tracer method described by Dugdale & Goering (1967) and revised by Legendre &

Table 1. Environmental conditions during the bloom period (June 2009 to December 2010) of *Cyanothece* sp. in the St Lucia Estuary and the corresponding parameters that were controlled in the stock and experimental cultures. NA: not applicable

Parameter	Salinity	Temperature (°C)	Light intensity (µmol photons cm^{-2} s^{-1})	DIN (µM)	DIP (µM)	^{15}N (µM)	Atom % ^{15}N
Lister's Point	70–220[a]	15–55[a]	530.4[a]	Fig. 6	Fig. 6	No enrichment	Natural
Stock cultures	0, 60, 120, 180, 240, 300	30	150	8.8×10^3	3.3×10^3	No enrichment	Natural
Working cultures	0, 60, 120, 180, 240, 300	30	150	0, 8.8×10^3	2, 3.3×10^3	No enrichment	Natural
$^{15}NO_3^-$ uptake	0, 60, 120, 180, 240, 300	30	150	114	2, 16	11	~10
KH_2PO_4 uptake	0, 60, 120, 180, 240, 300	30	150	0, 114	16	No enrichment	Natural
$^{15}N_2$ fixation	0, 60, 120, 180, 240, 300	30	150	0	2, 16	0.5 ml 98 atom% $^{15}N_2$ gas	~13
N loss via N_2 fixation	0, 60, 120, 180, 240, 300	30	150	0	2, 16	0.5 ml 98 atom% $^{15}N_2$ gas	NA

[a]data from Muir & Perissinotto (2011)

Gosselin (1997). The uptake dynamics were investigated over the full range of salinity and under P-replete and P-limited conditions. The ASN III media had a final N concentration of 114 µmol l^{-1}, with the ^{15}N tracer ($^{15}NO_3^-$) added so that the final N concentration had a ^{15}N content of 10 atom% ^{15}N. Two P treatments were used, with PO_4^- concentrations adjusted to 16 µmol l^{-1} and 2 µmol l^{-1} for the P-replete and P-limited treatments, respectively. Each treatment consisted of 5 replicates of 50 ml Erlenmeyer flasks filled with 20 ml of the relevant tracer-labelled media and placed within a growth cabinet for pre-acclimation for 1 h. Thereafter, the experiments were initiated by adding 2×10^5 to 5×10^5 cells from the different salinity stocks to each flask. The incubation period was set at 6 h within the growth chamber under the same light and temperature conditions as described above. At the end of the incubation, solutions were processed with gentle vacuum filtration onto 25 mm pre-combusted Whatman GF/F filters with a nominal pore size of 0.7 µm. The filters were immediately oven dried at 60°C for 24 h, weighed and subsequently packaged for stable isotope analyses by IsoEnvironmental, Rhodes University, Grahamstown.

PO_4^{3-} (as KH_2PO_4) uptake

Uptake rates of P were measured following the method described by Maita et al. (1984). Similarly to the N uptake setup, the P uptake dynamics were investigated over a wide range of salinity under the N-replete and N-limited ASN III media conditions. The final P concentration was adjusted to 16 µmol l^{-1}. The experimental setup was exactly the same as described above for the N uptake experiment. However, after filtration the filters were dried and weighed only, while 20 ml filtrate from each sample, along with 5 replicates of the fresh media (initial P concentration) were manually analysed on a GBC UV/VIS 916 spectrophotometer for phosphate determination and the P uptake rates calculated following the method described by Murphy & Riley (1962).

$^{15}N_2$ fixation and NH_4^+ release

The N-limited stock cultures were used in the modified tracer assay of Montoya et al. (1996) to measure the N_2 fixation rates over the full salinity range and under P-replete and P-limited conditions. The assays were performed by filling 40 ml clear glass vials

(5 replicates per treatment) to capacity with the relevant media (ASN III N-limited), and sealing the vials with septum caps. Then, 0.5 ml of 98 atom% $^{15}N_2$ gas was injected into the vials with a gas tight syringe, the vials were vigorously shaken on a vortex shaker for 1 min to facilitate adequate gas diffusion and placed on a 100 rpm shaker tray overnight, within the growth chamber to allow media acclimation (Mohr et al. 2010). In the morning, 2×10^5 to 5×10^5 cells were injected into the vessels with a syringe to initiate the experiments within the growth chamber, with incubation lasting for 24 h. After the incubation, samples were immediately filtered onto 25 mm pre-combusted GF/F filters, oven dried at 60°C for 24 h and weighed and packaged for stable isotope analysis. The filtrate from each sample was collected into a 50 ml polyethylene vial for nutrient analysis. To determine the release/loss of N, as ammonium (NH_4^+), by *Cyanothece* sp. to the culture medium during N_2 fixation conditions, the NH_4^+ concentration was manually determined on a GBC UV/VIS 916 spectrophotometer from the filtrate samples using the phenolhypochlorite method as described by Solorzano (1969). Control treatments were similar, but were treated with N_2 gas injection instead of addition of labelled $^{15}N_2$ gas for the $^{15}N_2$ fixation assays, and additional incubations with only the media were incubated as controls for the N loss measurements.

Statistical analysis

Data from each experiment were analysed with 2-way ANOVA to account for salinity effects, nutrient effects (i.e. nutrient limited, nutrient replete), and salinity-nutrient interactions. Tukey's post-hoc analyses were carried out for all experiments for pairwise comparisons. Only the data from the N release experiment were normally distributed ($Z = 1.004$, $p = 0.226$), while the data from all other experiments were $\log_{10}$-transformed to satisfy the assumption of normality (N uptake: $Z = 0.698$, $p = 0.715$, N_2 fixation: $Z = 0.982$, $p = 0.289$, P uptake: $Z = 0.921$, $p = 0.364$). Equal variance between the residuals was observed in all experiments ($F = 0.000$, $p > 0.05$).

RESULTS

^{15}N (as $^{15}NO_3^-$) uptake

In general, highest uptake rates were measured at salinities below 120 (Fig. 2). Above this salinity, cells showed very slow uptake rates. Uptake of ^{15}N ($^{15}NO_3^-$) still occurred at salinities above 120 and even at 300, but only at µg atom N mg_{Cy}^{-1} h^{-1} rates, compared to the mg atom N mg_{Cy}^{-1} h^{-1} rates observed for salinities below 120. Interestingly, the highest uptake rate of 1.3 ± 0.7 (SE) mg atom N mg_{Cy}^{-1} h^{-1} was measured for the 60 salinity treatment, where PO_4^{3-} was at low/limiting concentrations (2 µM). This was also the only salinity treatment where a significant difference in uptake rates was measured between the 2 P treatments. There was no significant interaction between salinity and P treatments on uptake rates (ANOVA: $F = 1.357$, $p = 0.263$) and the P treatment did not significantly influence uptake rates (ANOVA: $F = 0.003$, $p = 0.960$). Salinity was the only factor to significantly influence ^{15}N uptake rates (ANOVA: $F = 12.013$, $p = 0.001$), as also shown in Fig. 2. A post-hoc Tukey HSD analysis indicated similar results to those observed in Fig. 2, where the uptake rates from the lower salinities only differed significantly to uptake rates from salinities above 120.

$^{15}N_2$ fixation

Nitrogen fixation rates recorded for the *Cyanothece* sp. strain isolated from the St Lucia Estuary were highest (2.0 ± 0.4 µmol N mg_{Cy}^{-1} h^{-1}) at the 0 salinity treatment (actual salinity ~4, no NaCl added), with PO_4^- added to the media. Rates decreased to 0.7 ± 0.4 (SE) µmol N mg_{Cy}^{-1} h^{-1}as the salinity increased to 60 (Fig. 3) and then drastically decreased to 0.02 ± 0.03 µmol N mg_{Cy}^{-1} h^{-1}at the 120 salinity treatments, with no N_2 fixation recorded above a salinity of 120. A considerable/significant drop in N_2 fixation activity was observed at a salinity of 120. ANOVA indicated

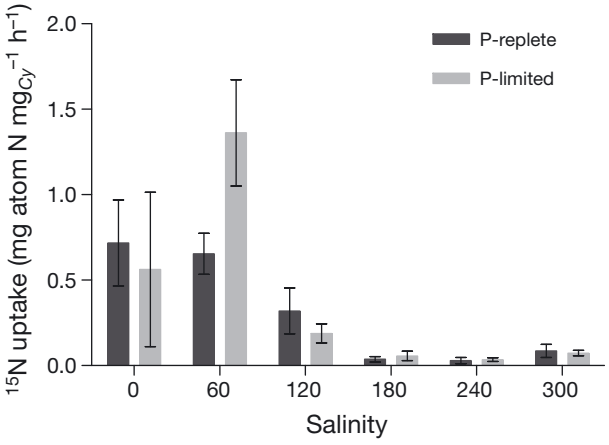

Fig. 2. Mean (±SE) ^{15}N (as $^{15}NO_3^-$) uptake by 1 mg *Cyanothece* sp. cells under different salinities and PO_4^{3-} treatments

that the P treatments (F = 1.540, p = 0.225) and the interaction between salinity and P treatments (F = 0.093, p = 0.963) did not significantly influence N_2 fixation rates. Salinity was the only variable to significantly influence N_2 fixation rates during this study (F = 23.527, p = 0.001). N_2 fixation rates at the 0 and 60 salinity treatments were not significantly different (p = 0.632) from one another, based on the Tukey HSD analysis. However, these 2 rates were different from all other treatments, particularly because no N_2 fixation was recorded at salinities higher than 120, and only very low fixation occurred at 120.

N release via N_2 fixation

While N_2 fixation was only recorded from 0 to 120 salinity treatments, NH_4^+ release was measured in all treatments, including those where no N_2 fixation was recorded (i.e. salinities 180 to 300, Fig. 4). The highest N (as NH_4^+) release/loss rate of 1.1 ± 0.9 (SE) µmol N mg_{Cy}^{-1} h^{-1} was measured for the 0 salinity and P-limited treatment. The lowest N release rate of 0.05 ± 0.07 µmol N mg_{Cy}^{-1} h^{-1} was recorded for the 120 salinity and P-replete treatment. No N release was measured in the 60 salinity and P-replete treatment. The interaction between salinity and P treatments (ANOVA: F = 1.932, p = 0.106) did not significantly influence the N_2 fixation rates by *Cyanothece* sp. However, salinity (ANOVA: F = 7.061, p = 0.001) and P treatment (ANOVA: F = 5.282, p = 0.026) significantly influenced the N release rates during this study.

PO_4^{3-} (as KH_2PO_4) uptake

For both N treatments, the P uptake rates generally showed a decrease in the uptake rate from low to high salinity (Fig. 5). The highest uptake rates were measured at the 0 salinity treatment in both the N-replete and N-limited incubations, with values of 5.3 ± 3.7 (SE) µmol P mg_{Cy}^{-1} h^{-1} and 0.3 ± 0.1 µmol P mg_{Cy}^{-1} h^{-1}, respectively. ANOVA results indicated that all factors significantly influenced P uptake rates (salinity: F = 20.829, p = 0.001; N treatment: F = 367.040, p = 0.001; Salinity × N treatment: F = 4.062, p = 0.005). This is clearly evident in Fig. 5, where the uptake rates for the 2 N treatments had to be separated onto separate axes, due to the order of magnitude discrepancy in values. Furthermore, uptake rates decreased exponentially in response to increases in salinity (Fig. 5).

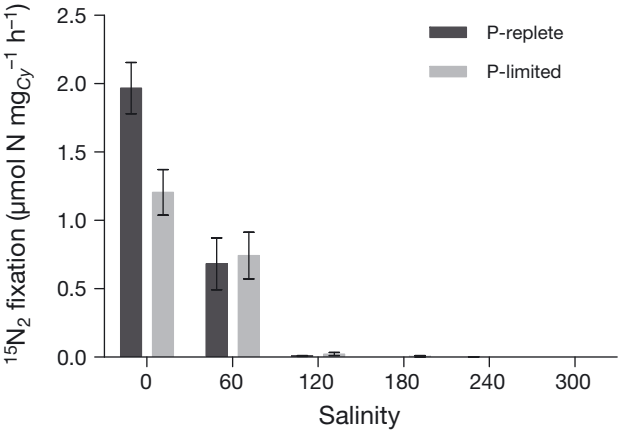

Fig. 3. Mean (±SE) $^{15}N_2$ fixation by 1 mg *Cyanothece* sp. cells under different salinities and PO_4^{3-} treatments

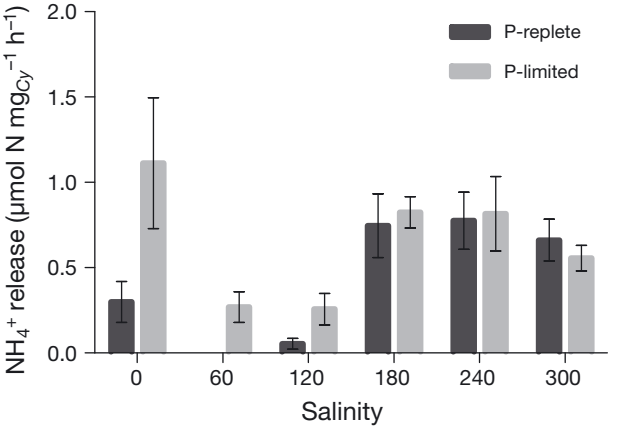

Fig. 4. Mean (±SE) release/loss of NH_4^+ by 1 mg *Cyanothece* sp. cells under different salinities and PO_4^{3-} treatments during the N_2 fixation assays

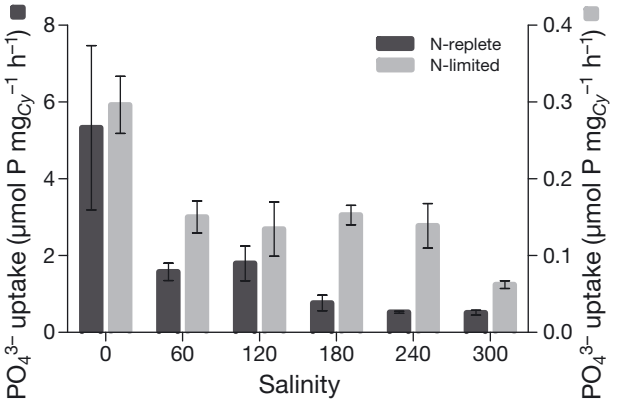

Fig. 5. Mean (±SE) uptake of PO_4^{3-} by 1 mg *Cyanothece* sp. cells under different treatment conditions

DISCUSSION

The occurrence of the 18 mo long cyanobacterial bloom from June 2009 to December 2010 in St Lucia (Muir & Perissinotto 2011) highlights the susceptibility of ecosystems to anthropogenic alterations, particularly freshwater abstraction and nutrient-overloading through upstream agricultural practices (Paerl & Huisman 2009). Experimental manipulations of this *Cyanothece* sp. illustrates how it is able to persist for a prolonged period of time under extreme hypersaline conditions (i.e. salinity well above 100). Ecologically, this is interesting in a system that has recently been shown to be drought-prone and exposed to regular development of hypersaline conditions.

In light of the persistence of the bloom, we report here a correction to the cell counts reported by Muir & Perissinotto (2011) as follows. In historical samples, cells similar to the organism of interest were first noted in May 2007 (80 to 100 cells ml^{-1}) and were present in very low numbers throughout the austral autumn and winter of 2007. A slight increase in numbers commenced in the spring and summer of 2008 (100 to 200 cells ml^{-1}), but between February 2009 and August 2009 the organism bloomed, reaching numbers of 5000 cells ml^{-1} by July 2009, when the bloom was first noted, and 10 000 cells ml^{-1} by January 2010. The cell counts throughout 2010 remained high (mean ± SD; 20 310 ± 5860 cells ml^{-1}) until November, when they began to fall at the onset of summer rains. Following the water level rise after very heavy rains in January 2011 (due in part to La Ninã effects that were apparent globally throughout the Southern Hemisphere), the bloom crashed and cells were then undetectable from hemocytometer counts.

Carrasco & Perissinotto (2012) recorded the development of a species-pauperate halotolerant community consisting of a simple food chain above a salinity threshold of 100 in the St Lucia Estuary. No eukaryotes were present once salinity increased above 140 (Muir & Perissinotto 2011, Carrasco & Perissinotto 2012), with only *Cyanothece* sp. surviving and persisting. This exemplifies how food webs may become truncated and how a system can change when hypersaline conditions persist for prolonged periods of time (Govender et al. 2011), and highlights the importance of freshwater inputs into a shallow water ecosystem such as the St Lucia Estuary. It appears that *Cyanothece* sp. is well-adapted to survive extreme hypersaline conditions within St Lucia. However, it is unclear what role the biomass of cyanobacteria played in the subsequent phase, after the demise of the bloom, since the bloom dispersed with the floods of December 2010 (Muir & Perissinotto 2011).

The dissolved nutrients (specifically N) were above limiting concentrations (20 µM < DIN < 500 µM) in North Lake prior to 2007 (Perissinotto et al. 2010), but more recently (2007 to 2011) nutrients were below limiting concentrations (Fig. 6) and therefore even small nutrient inputs to the bloom area may have played a major role in facilitating the bloom appearance. The nutrient concentrations observed during the bloom period indicate relatively low nutrient concentrations, especially of DIN. This highlights that an ability to fix atmospheric N_2 would have been crucial

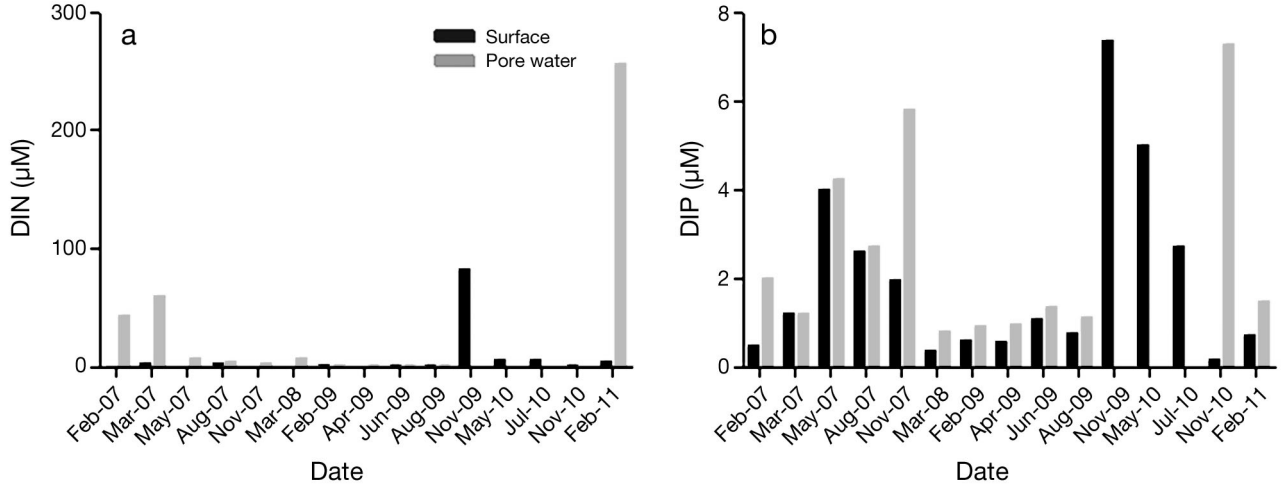

Fig. 6. (a) Dissolved inorganic nitrogen (DIN) and (b) dissolved inorganic phosphorus (DIP) concentrations at Lister's Point during the period February 2007 to February 2011

in meeting N demands by *Cyanothece* sp., and possibly driving bottom-up processes to facilitate the survival of non-nitrogen-fixing phytoplankton.

In general, *Cyanothece* sp. showed high nutrient assimilation rates (Figs. 2 to 5) in response to the addition of specific nutrients. Nitrogen uptake rates are higher than those reported for cyanobacteria in general (e.g. Carpenter & Dunham 1985, Gu & Alexander 1993, Bradley et al. 2010, Kim et al. 2011) but still within documented uptake rates (Chevalier et al. 2000). Most significantly, this study established that this *Cyanothece* sp. isolate is capable of N_2 fixation (Fig. 3), and that uptake activity (including N_2 fixation) can be demonstrated at salinities as high as 120, although there is a significant drop in metabolic activity (i.e. N uptake, N_2 fixation and P uptake) at a salinity of 120 (Figs. 2 to 5). This is also one of the highest salinities at which N_2 fixation has been documented, with Severin et al. (2012) also documenting cyanobacterial N_2 fixation at salinities of 165. It is possible that the nitrogenase activity may have been limited due to structural changes in the enzyme, as a response to increasing intracellular ionic or osmotic stress (Herbst 1998). It must be considered that the released NH_4^+ may play an inhibitory role on the N_2 fixation capabilities of *Cyanothece* sp. (Holl & Montoya 2005). Nitrogen-containing compounds have been documented to inhibit N_2 fixation; however, in this study the *Cyanothece* sp. cells were placed into fresh media containing no dissolved N. Alternatively, inhibition of N_2 fixation may be related to reduced photosynthetic fixation of carbon needed to support nitrogenase activity (Herbst 1998).

Similar trends were observed in the non-heterocystous filamentous cyanobacterium *Oscillatoria* in the hypersaline Mono Lake sediments by Herbst (1998). Here, extreme hypersaline conditions inhibited N_2 fixation activities. During the bloom period in St Lucia, the salinity was well over 100 (Muir & Perissinotto 2011), which suggests that the *Cyanothece* sp. cells were under salinity stress and that their uptake abilities were drastically reduced (Moisander et al. 2002, Marcarelli et al. 2006). However, as this study shows, the nutrient uptake rates must have been high at salinities below 60, indicating that cells may have been able to take advantage of the conditions present at the onset of the bloom, to acquire the nutrient resources needed for growth (Yannarell & Paerl 2007).

Apart from the nutrient acquisition abilities demonstrated by *Cyanothece* sp. under a wide salinity range, the subsequent release/loss of NH_4^+ from *Cyanothece* sp. cells under N_2 fixation conditions is also of significance (Fig. 4). Previous work by Agawin et al. (2007) and Ritchie (2013) has demonstrated that the loss of N into the environment by *Cyanothece* and *Synechococcus*, respectively, is possible. This may occur by passive leak-out ('pump/leak' systems) (Agawin et al. 2007, Mulholland 2007, Ritchie 2013), release of exopolysaccharides (EPS) (Trabelsi et al. 2009), viral cell lysis (Hewson et al. 2004, Hewson & Fuhrman 2006) and cell death (Berman-Frank et al. 2004). It was of interest to investigate whether the isolate from St Lucia also released N into the environment, as this may be a possible N source for other autotrophs that are unable to fix their own N, thereby driving bottom-up ecosystem processes in the estuary (e.g. facilitation between nitrogen-fixing cyanobacteria and non-nitrogen-fixing phytoplankton species sensu Agawin et al. 2007). The results obtained indicate 2 different scenarios that occurred during the N_2 fixation assays (Fig. 4). Firstly, there was NH_4^+ present within the media of the experimental treatments, while none was measured in the controls (media incubations with no inoculation) after the fixation period was terminated, and the rate of the measured release was higher under conditions where P was at limiting concentrations. Secondly, there was a clear increase in the release of N at salinities above 120, with overall rates much higher at these compared to the lower salinities. This is particularly interesting, since there was no N_2 fixation observed above a salinity of 120 (Fig. 3), indicating other possibilities such as the release of previously fixed N as glycoproteins through EPS secretion, cell lysis or viral cell lysis. At salinities above 120, a combination of processes may have been at play, such as the secretion of glycoproteins together with the release of N from small internal ammonium pools through sufficient lysis of the population. Muir & Perissinotto (2011) observed this *Cyanothece* sp. producing large amounts of mucilaginous slime, which may have contributed to the release of N into the media as glycoproteins (Trabelsi et al. 2009). This correlates well with results of the N and P uptake experiments, which show that little net uptake was measured at salinities above 120.

Cyanothece sp. cells show great resistance to changing environmental variables and are capable of surviving conditions that other competitors and grazers cannot withstand. This study shows that *Cyanothece* sp. cells maximise their nutrient acquisitions when conditions are favourable and then utilise those nutrient reserves to survive the harsh conditions. We suggest that at low salinities, in the normal estuarine range, organisms such as *Cyanothece* are a rare com-

ponent of the phytoplankton. They are extremely halotolerant (De Philippis et al. 1993, Garcia-Pichel et al. 1998, Carrasco & Perissinotto 2012), however, and as salinities in Lake St Lucia rose (>35) they remained able to take up nutrients and metabolise efficiently, with the added advantage that they were also able to fix atmospheric nitrogen. While P is seldom limiting in Lake St Lucia, low N levels can block microalgal production and thus diazotrophy confers a considerable advantage (Fig. 6). There may have been an initial nutrient pulse, augmented by a fish kill in the system (Fig. 6). With the elimination of eukaryotic competitors and normal components of the plankton due to increasing salinities (Carrasco & Perissinotto 2012), *Cyanothece* sp. was able to continue blooming up to a threshold in salinity >60 and <120, by which point all eukaryote competitors had been eliminated (Carrasco & Perissinotto 2012). Above a salinity of 120, the *Cyanothece* sp. population resorted to a survival mode: able to maintain minimal cellular structure with minimal uptake and metabolic activity, but unaffected by predation. Although inactive, cells remain viable at very high salinities. Muir & Perissinotto (2011) demonstrated that the cells maintained a limited cellular architecture with reduced cytoplasm and thylakoids, but that even without a reduction in salinity, they were able to reconstitute cell structures very rapidly, provided that enough nutrients were supplied. Du Plooy et al. (2014) also observed cell viability through cellular chl *a* fluorescence in natural and sub-cultured cultures that had been left untouched for 2 yr. Preliminary PAM fluorescence observations on these cultures also indicate viability through photosynthetic activity. However, slow ^{15}N uptake rates were recorded above salinities of 120 during the experiments (Fig. 2), and it is plausible that the cells (collectively) released nutrients through mucilage production, with part of the colony benefiting from the nutrients made available and prolonging its persistence. The population maintained high cell numbers for a long time even during extreme environmental conditions (Muir & Perissinotto 2011). However, the heavy rainfall and subsequent floods that occurred in St Lucia during December 2010 sealed the demise of the bloom, as salinities suddenly decreased (resulting both in dilution of the bloom and probably in bursting of cells under hypo-osmotic conditions) and as a diverse trophic community returned, including competitors and grazers of *Cyanothece*.

The recent connection of the Mfolozi River to the St Lucia Estuary (Whitfield et al. 2013), combined with heavy rainfall during the early months of 2013, has resulted in a shift towards oligohaline conditions (Nel 2014). This substantial reduction in salinity has resulted in the return of a diverse trophic community with many eukaryotic competitors and grazers taking full advantage of the current conditions. As the system now moves into a wet phase (Fauchereau et al. 2003, Lumsden et al. 2009), it is unlikely that *Cyanothece* sp. will reappear, since the return of a diverse trophic community will result in competition with eukaryotic autotrophs, and *Cyanothece* sp. may become subject to top-down control by grazing by zooplankton. However, despite the beach spillway that now connects the Mfolozi River to Lake St Lucia, the estuary mouth remains largely closed to the ocean and, if drought was to reoccur, conditions conducive to a *Cyanothece* bloom might arise again.

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