

***Lyngbya majuscula* Blooms in an Enclosed Marine Environment**

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Abstract

Cyanobacterial blooms are a cause of concern because of their potential impacts on the marine environment. In Sentosa Cove, Singapore, *Lyngbya majuscula* blooms appeared regularly in the highly enclosed boat canals traversing the seafront residential development. This study investigated whether sediments resuspended by physical disturbance liberated nutrients that contribute to the blooms. Sediment resuspension events were mimicked in containers of sediment collected from the canals. *Lyngbya majuscula* that were incubated in containers with resuspended sediment attained greater biomass than those in filtered seawater only. Levels of iron, phosphates and nitrites in seawater with resuspended sediments were significantly higher than in those without. The results indicate that recurrent *L. majuscula* blooms in Sentosa Cove could be attributed to nutrient loading from sediment resuspension.

Keywords: *Lyngbya majuscula*; sediment resuspension; enclosed marine environment; Singapore

1. Introduction

Marine cyanobacteria are a rich source of natural products, with secondary metabolites from members of the genus *Lyngbya*, especially, having generated novel pharmaceuticals that are useful in furthering marine biotechnology and biomedical research (Tan and Goh, 2009; Tan, 2010; Tan *et al.*, 2010). The presence of *Lyngbya* blooms can, however, have wide-ranging effects on the marine environment, public health, as well as the economy. The blue-green filamentous marine cyanobacterium *Lyngbya majuscula* has been shown to cause tumours in turtles that ingest it (Arthur *et al.*, 2006), reduce recruitment and survivorship of scleractinian corals and gorgonians (Kuffner and Paul, 2004; Kuffner *et al.*, 2006), as well as overgrow and smother seagrass beds (Watkinson *et al.*, 2005). Similar to the effects of macroalgae flourishing in shallow soft bottom habitats, *L. majuscula* blooms can lower the nursery capacity of the habitat and affect the feeding behaviour of various fish species (Wennhage and Pihl, 2007; Gilby *et al.*, 2011). For humans, direct contact with toxin-producing strains of *L. majuscula* can trigger ailments such as dermatitis, eye irritations and respiratory problems, while accidental oral ingestion of the cyanobacterium or consumption of marine organisms such as fish or turtles which have eaten it can cause hallucinations, vomiting, diarrhoea and even death (Osborne *et al.*, 2001).

In recent years, incidences of *Lyngbya spp.* blooms have increased throughout the world, occurring on reefs in Florida, reef flats in Guam, and coastal bays in Australia (Thacker and Paul, 2001; Albert *et al.*, 2005; Paul *et al.*, 2005). The frequency of blooms and the severity of these impacts are causes for concern as cyanobacteria are predicted to thrive with global warming (Paul, 2008; Paerl and Paul, 2012).

Here, we report recurrent cyanobacterial blooms in a highly modified coastal environment in Singapore. Sentosa Cove, located on Sentosa Island, is a seafront residential development comprising a marina (ONE°15 Marina) nestled between two residential zones known as the Northern and Southern precincts. Construction of the Cove included the creation of a canal waterway that runs through each of the Northern and Southern precincts. The waterway is concrete with vertical brick wall sides and the bottom is lined with impermeable geotextile sheets. Water levels are maintained at a depth of 2.5 m by the use of sluice gates connected to the sea, resulting in reduced tidal influence, flow and water exchange, and accumulation of a sediment layer at the bottom. Residential bungalows with gently sloping manicured lawns lie on both sides of the waterway, each with a floating pontoon for the berthing of yachts. The development essentially created an enclosed marine system.

Thick floating mats of *L. majuscula* were initially observed in the Sentosa Cove waterway when the

construction of the first residential units commenced in 2004. Similar to that reported by Albert *et al.* (2005), bubbles trapped within the benthic *L. majuscula* matrix on hot sunny days enabled these clumps to float and aggregate at the water surface, possibly aiding in their dispersal. These blooms have occurred ever since with increasing frequency and become odorous, unsightly floating mats. Attempts by the management at amelioration have included regular removal of the mats. However, the impacts of persistent blooms are likely to be exacerbated in a highly enclosed marine environment.

That *Lyngbya majuscula* are dependent on phosphorus, nitrogen and iron for their primary mode of growth, and proliferate upon the introduction of these nutrients to *in situ* or *ex situ* systems (Elmetri and Bell, 2004; Ahern *et al.*, 2006a; Ahern *et al.*, 2008), suggested that nutrient loading was a principal cause of frequent *L. majuscula* blooms in this highly enclosed system. While nutrient influx from terrestrial sources was likely to occur due to the proximity of gardens and construction sites to the waterway, the possibility also existed for sediment resuspension – a ubiquitous process in aquatic environments that liberates nutrients – to influence cyanobacterial bloom development. The objective of this study was thus to establish the influence of sediment resuspension as a driver of the formation of *L. majuscula* blooms. We hypothesised that sediment resuspension events caused by the agitation

of the benthic sediment layer (e.g., via boat traffic or physical removal of the *L. majuscula* mats) in the waterway released nutrients into the water column and contributed to the frequent cyanobacterial blooms.

2. Materials and Methods

2.1. Study site

The study was conducted at the Northern Residential Precinct of the Sentosa Cove waterway ($1^{\circ}14'55''N$, $103^{\circ}50'40''E$) (Fig. 1) from July to December 2008. Light penetration was measured with a LI-193 Spherical Quantum Sensor, while temperature and salinity were measured using a YSI 85 multi-probe throughout the water column.

2.2. Effect of sediment resuspension on *Lyngbya majuscula* growth

Samples of bottom sediment, seawater and floating mats of *L. majuscula* from the study site were collected. All sediment, fauna and macroalgae that were trapped inside the cyanobacterial matrix were removed. An enclosed area illuminated by six 18W 600 mm full-spectrum fluorescent lamps (Arcadia, England) was constructed in the laboratory. Forty 700 ml glass containers, each covered with black duct tape such that light from the lamps only entered

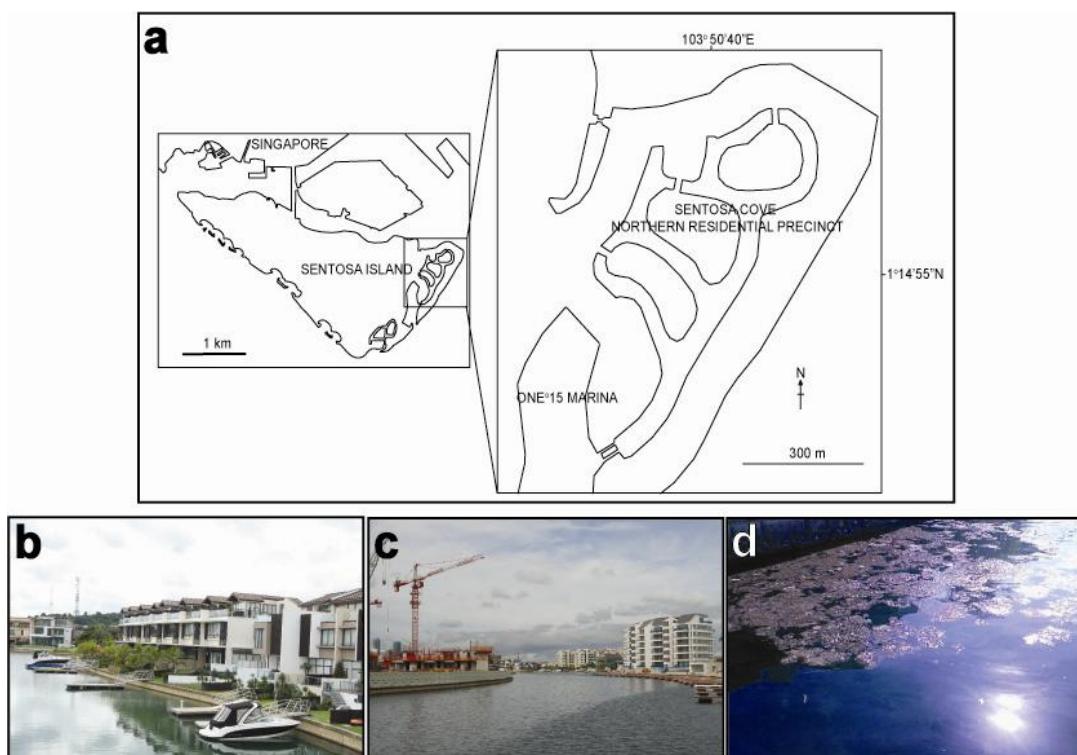


Figure 1. (a) Map of study site, (b) residences in the Northern Precinct, (c) site undergoing construction in 2008, (d) *Lyngbya majuscula* mats on the water surface

via the container mouths were placed in the area. Sixty-five grams of sediment were introduced to each of 20 containers ('treatments') and these were arranged alternately with 20 other containers without sediment ('controls'). Seawater from the study site was filtered with a 2 µm phytoplankton net and 500 ml was poured into each container. This served to agitate the sediment in the treatment containers to mimic sediment resuspension events. The containers were left to stand for one hour to allow the sediment to settle. A perforated plastic disc was then positioned horizontally across the middle of each container and 0.05 g (= 0.011 g dry weight) of cleaned *L. majuscula* was placed on it. The disc prevented direct contact between the cyanobacteria and the sediment at the bottom of the container. The experimental setup ran for 15 days and distilled water was added to top up the volume in the containers when necessary. Finally, the *L. majuscula* samples from each container were washed with filtered seawater, dried in an oven at 65°C for 48 hrs, and weighed.

2.3. Effect of sediment resuspension on nutrient levels

Seawater samples from the treatment and control containers were filtered again with a phytoplankton net and their nutrient content was analysed with Odyssey Hach powder pillows and an Odyssey DR/2500 Spectrophotometer. Nitrates were measured using the Cadmium reduction method with NitraVer® Nitrate

Reagent; nitrites using the Diazotization method with NitriVer®3 Nitrite Reagent; phosphates using the PhosVer 3 Absorbic acid method with PhosVer; and iron with the TPTZ method.

Using SPSS v17, the data were transformed where necessary, to satisfy the assumptions of normality and homogeneity of variances. They were then analysed using independent samples t-test.

3. Results

3.1. Environmental parameters at study site

The daytime temperature at the study site ranged from 27.9°C at the deepest part of the waterway to 31.5°C on the water surface. There was less temperature variation at night – from 31.1°C at the surface to 31.3°C at the bottom. Salinity of the water column increased with depth (29.4 ppt at the surface to 30.0 ppt at the bottom). Daytime light intensity in the waterway decreased with depth, dropping from 1023.00 µmol/sm² at the surface to 75.70 µmol/sm² at the bottom.

3.2. Effect of sediment resuspension on *Lyngbya majuscula* growth

Lyngbya majuscula samples incubated in the treatment containers had significantly greater biomass than those in the controls (0.0388 ± 0.0043 g

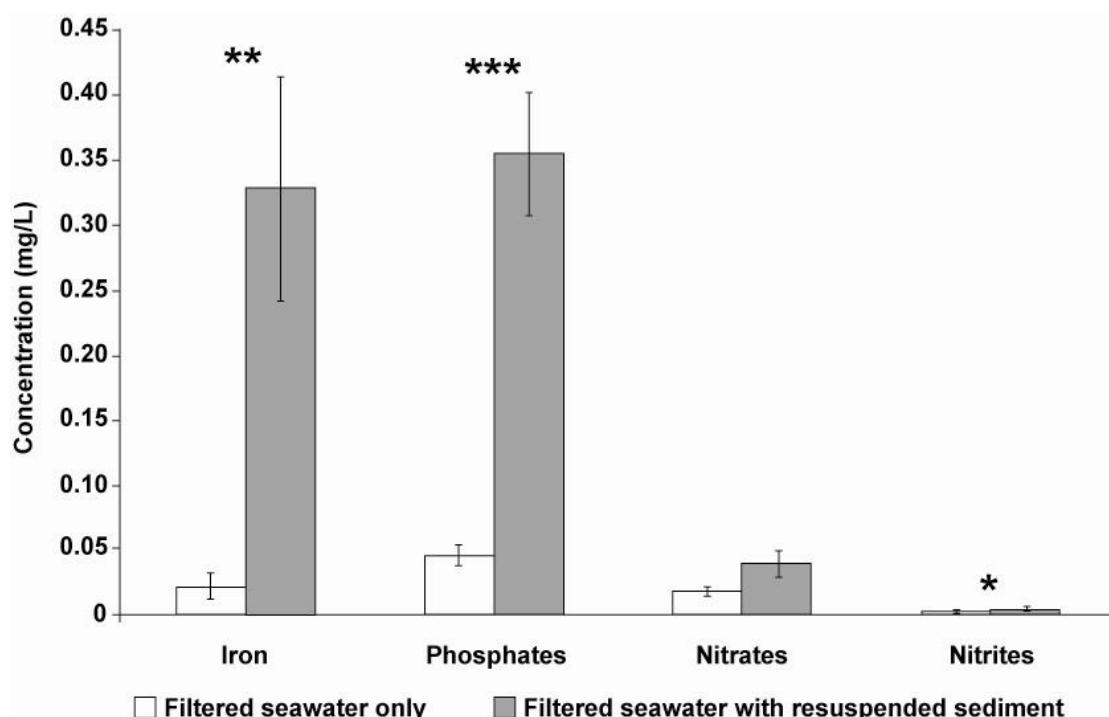


Figure 2. Nutrient content in control (filtered seawater only from the Sentosa Cove waterway) and treatment (filtered seawater with resuspended sediment) containers (* represents $p < 0.05$; ** represents $p < 0.005$; *** represents $p < 0.001$).

and 0.0210 ± 0.0032 g respectively) (log-transformed, $t = 3.844$, $df = 38$, $p < 0.001$).

3.3. Effect of sediment resuspension on nutrient levels

There were no significant differences in nitrate concentrations between the controls and treatments (log-transformed, $t = -1.868$, $df = 10$, $p = 0.182$). However, concentrations of the other three nutrients were significantly different (Fig. 2). Iron (fourth root-transformed, $t = -4.814$, $df = 10$, $p = 0.002$), phosphates (log-transformed, $t = -10.359$, $df = 10$, $p < 0.001$), and nitrites (square root-transformed, $t = -3.041$, $df = 10$, $p = 0.024$) were significantly higher in treatment containers than in the controls.

4. Discussion

In Singapore, *Lyngbya* spp. can be found in intertidal areas and lagoons, and are typically observed to develop into large benthic mats during the warmer season (Tan, 2011). Except for salinity readings which were comparable to those described from earlier studies conducted in the Singapore Straits (e.g., Chou and Hsu, 1987; Gin et al., 2000; Chou et al., 2004; Loh et al., 2006), the average light intensity, temperature, and levels of nitrites, nitrates and phosphates within the waterway of the Northern Residential Precinct of Sentosa Cove were all higher. As *Lyngbya majuscula* flourishes in shallow water bodies, under high light, high temperature, and in the presence of bioavailable nutrients (Albert et al., 2005; Watkinson et al., 2005), the ambient conditions in the Sentosa Cove waterway appeared especially conducive to the growth of *L. majuscula*.

The agitation of the sediments via the introduction of water into the glass containers mimicked the disturbance of the bottom of the waterway by activities such as the physical removal of *Lyngbya* mats, the passage of yachts and jet skis across the waterway, and the occasional opening of sluice gates. Nutrient levels, especially those of phosphates and iron spiked after the sediments were resuspended in the treatment containers, showing that the accumulated sediment layer in the waterway was a major source of dissolved nutrients which could be liberated in significant amounts if disturbed (Kalnejais et al., 2010). *Lyngbya majuscula* is able to fix nitrogen (Lundgren et al., 2003), so its growth is not limited in a low-nitrogen environment. Therefore, while nitrates and nitrites present in the Sentosa Cove waterway can encourage *L. majuscula* filament growth, they probably did not influence *L. majuscula* development to the extent as that of phosphates and iron, which promote nitrogen fixation, photosynthesis

and ultimately growth in *L. majuscula* (Elmetri and Bell, 2004; Ahern et al., 2006b; Ahern et al., 2007). The positive influence that resuspended sediment had on the growth of the cyanobacteria in Sentosa Cove was further corroborated by the significantly greater biomass of *L. majuscula* incubated in containers with sediment.

Additionally, the site experienced bouts of torrential rain over the course of the study. Surface water samples collected across the waterway after an episode of heavy rain revealed nutrient content much higher than those in the control containers. This was especially so for phosphates and nitrites, which were respectively 13 and 12 times more. Although this measurement was a one-off event, it suggested that rainfall events at Sentosa Cove can introduce substantial amounts of phosphates and iron into the waterway via terrestrial runoff and represent another source of stimuli for cyanobacterial growth. Our findings therefore indicate that the trajectory of *L. majuscula* bloom development in the Sentosa Cove waterway is spurred on by the influx of nutrients through channels such as sediment resuspension, and hint at the role of terrestrial runoff as a contributor as well. The effects of nutrient loading were also likely intensified by restricted flushing of the waterway.

The popularity of built up marine environments such as marinas, and increasingly, highly sheltered systems such as that in Sentosa Cove, point to the need for increased management of these artificial marine environments. It is thus imperative that anthropogenic disturbances (such as those in this study) be limited wherever possible. For example, runoff from the residents' gardens may be reduced by installing drainage systems to direct fertiliser-laden water away from the waterway. Increasing the water current within the waterway can enhance the dissipation of point sources of eutrophication and facilitate exchange – this may be achieved by installing pumps or making the influx and efflux of seawater more frequent. Frequent disturbance of the bottom sediment layer (and the associated triggering of downstream geo-chemical-physical effects) has even been suggested as a possible management solution by favouring the selection of new fauna unlike those selected for in eutrophic environments (Lenzi, 2010). The use of biological controls (e.g., seahares) may also be considered to reduce the intensity of *Lyngbya* blooms (Geange and Stier, 2010).

The environmental conditions in the waterway are by no means natural and are not entirely favourable to many marine species, but over time may have the potential to harbour certain lagoonal assemblages or even serve as nursery grounds for others. As with some

natural semi-enclosed marine systems (e.g., landlocked seas), a conceptual framework and an integrated approach may be necessary to ensure proper management of enclosed man-made marine ones (e.g., Kroeze *et al.*, 2008), or annual maintenance costs, such as those for Sentosa Cove (Tay, 2011), can be unnecessarily exorbitant.

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