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*Research Article*

# Effects of Salinity and Suspended Solids on Tropical Phytoplankton Mesocosm Communities

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**Abstract**

Phytoplankton play a fundamental role in marine food webs but are affected by both natural and anthropogenic fluctuations in environmental conditions. Here, to simulate a dynamic coastal environment, we used mesocosms to examine how different salinity levels and suspended solids concentrations (SSCs) impact a natural phytoplankton assemblage collected from a tropical estuary in Singapore. Significant differences in the phytoplankton composition between the baseline and treatments with medium and high SSC were found, but not among the three salinities tested. Differences can be attributed to nutrient limitation (particularly silicate) and the use of kaolinite for the suspended sediment. Silicate limitation is likely to have caused the observed switch in dominant genus from *Skeletonema* sp. to *Chaetoceros* sp. and the occurrence of weakly silicified genera such as *Cylindrotheca*. Kaolinite affected phytoplankton abundance through effects such as shading, flocculation, and nutrient adsorption. These results demonstrated how the combination of various physicochemical effects of suspended solids can influence tropical phytoplankton communities. Furthermore, as suspended solids such as kaolin can be found in the natural environment, this study showed that their potential effects should be evaluated beyond just their concentration.

**Keywords**

anthropogenic activities, kaolinite, nutrients, Singapore, species composition, tropical estuary

As primary producers, phytoplankton form the base of most marine food webs, providing approximately 50% of carbon directly and indirectly for almost every aquatic organism (Field et al., 1998). Light is an essential resource for phytoplankton but, due to the dynamic nature of the ocean environment, its intensity varies in space and time. For example, rapid coastline development and associated activities such as dredging and land reclamation frequently result in elevated suspended solid concentrations (SSCs) and turbid conditions (Field et al., 1998). Even though phytoplankton possess the capacity to acclimatize to varying light conditions through mechanisms such as altering their photosynthetic pigment content and ratio (Dubinsky & Stambler, 2009; Nymark et al., 2009) and adjusting the number and size of their photosynthetic units (Falkowski & Raven, 2007), the shading effect created by turbidity can still impact phytoplankton primary productivity (Grobbelaar, 1985), especially within short time scales.

Changes in coastal water salinity occur due to natural causes such as heavy rainfall and terrestrial runoff and/or anthropogenic sources such as at outfalls of power plants. Changes in salinity can affect aspects of the phytoplankton community, such as species composition, due to interspecific differences in salinity optima (D'ors et al., 2016; Lionard et al., 2005; Qasim et al., 1972). Rapid and large salinity changes subject algal cells to osmotic stress (Lionard et al., 2005), disturbing cellular

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homeostasis. Suboptimal salinities can also lead to the suppression of photosynthesis, carbon fixation and cell division (D'ors et al., 2016), as well as affecting nitrogen metabolism (Dohler & Biermann, 1985), all of which are tied to cell growth rate. As salinity affects growth rate, it plays a role in phytoplankton competition that can lead to changes in community composition (Muylaert et al., 2000). These effects are generally more pronounced in coastal than in oceanic ecosystems, where the larger body of water buffers against salinity fluctuations (Smayda, 1958).

Studies investigating the interacting effects of SSCs and changes in salinity on phytoplankton are generally field-based surveys conducted in estuaries. Estuaries have a natural salinity gradient due to the freshwater input from upriver and saltwater intrusion at the river mouth and usually have concurrent high sediment levels. However, as a consequence of data being collected longitudinally (e.g., Lueangthuwapranit et al., 2011; Muylaert et al., 2009; Zhang et al., 2014), the phytoplankton community sampled analyzed along estuaries are often not independent. The effects of salinity and SSC are also potentially confounded by other factors such water flow (Lueangthuwapranit et al., 2011). Furthermore, the great majority of these studies have been carried out in temperate countries (Cloern et al., 1989; Kromkamp & Peene, 1995; Lionard et al., 2005; Muylaert et al., 2009; Zhang et al., 2014) with very few performed in the tropics (Lueangthuwapranit et al., 2011; Palleyi et al., 2008; Rochelle-Newall et al., 2011). To better understand the interaction effects of salinity and SSC on coastal phytoplankton communities, and minimizing influence from other factors, it is important to conduct manipulative experiments under controlled conditions.

The aim of our study was to determine the effects of SSC and salinity on a phytoplankton community collected from an estuary in tropical Singapore. This was achieved through testing three SSCs and three different salinity levels in combination using mesocosms. We hypothesized that the treatments with the highest concentration of SSC and with a change in salinity level from ambient would have the lowest phytoplankton density and species richness.

## Methods

### *Experimental Design*

The experiment was conducted outdoors, at ambient temperature and light intensities. The phytoplankton community was collected from Pandan River (1°18′9.29″N, 103°45′15.33″E) an estuarine water body with mean salinity of ~30.0 ‰ at the collection site. A total of three salinities (Pandan River salinity and ±3‰) and three sediment concentrations (5 mg $l^{-1}$, 20 mg $l^{-1}$, and 80 mg $l^{-1}$), were tested in a fully crossed design ($n = 3$), bringing the total number of experimental units (mesocosms) to 27 (Figure 1). Kaolin powder (KM20, Kaolin (Malaysia) Sdn Bhd, Appendix, Table A1) was used to simulate the physical effects of SSCs within the experimental units.

Each experimental unit was filled with 20 L of ultraviolet radiated, filtered (0.2 µm) natural seawater from Pandan River. Other than introduced kaolin, the chemical components (e.g., TN, TP) were assumed to remain unchanged. The desired salinity (+3‰, no change and −3‰) treatments were achieved through the addition of Dionised water and aquarium salt ($H_2$Ocean pro+) to the designated units.The treatments of ±3 psu were chosen with the aim of simulating a scenario of a heavy downpour buffered by an adjacent ocean body, a common occurrence in the coastal environments of Singapore. The experimental units were homogenized via manual stirring with a paddle and allowed one day to stabilize, after which the salinity was checked again. Natural phytoplankton populations were collected from Pandan River at 0.5 m depth during high tide (12:00 hr), using a 5 L niskin water sampler (General Oceanics) in triplicates. Immediately, subsamples were taken for the analysis of SSC, after which the remaining water was filtered through a 35 µm mesh to remove larger zooplankton. Further subsampling was then carried out for water quality analysis of other parameters (total nitrogen, total phosphorus, nitrates, nitrites, ammoniacal nitrogen, silicate, and *chlorophyll a*) and phytoplankton composition and abundance. Water quality samples were processed and analyzed according to the respective methods of the American Public Health Association (APHA, 2012; Appendix, Table A2). After subsampling, 100 ml of the remaining water was then inoculated into each experimental unit. The first replicate collected from Pandan River was used to inoculate the first replicate for each of the respective treatment combinations, and the second was used for second replicate and so forth. In total, 900 ml was taken from each of the 5 L replicates. From the time when samples were taken from Pandan River for inoculation, the whole experiment was established within an hour. The experimental units were distributed randomly using a random number table, so as to minimize the effect of location, and sheltered with clear plastic sheeting to prevent rain affecting salinities. Five loggers measuring photosynthetic active radiation (PAR; Odyssey, Dataflow Systems Ltd) were distributed among the 27 units to capture light distribution across the whole setup and to obtain PAR measurements from the different SSC treatments: two were placed in the low suspended solid treatment and one in the medium and two in the high SSC treatment. Temperature loggers (HOBO, Onset Computer Corporation) were placed

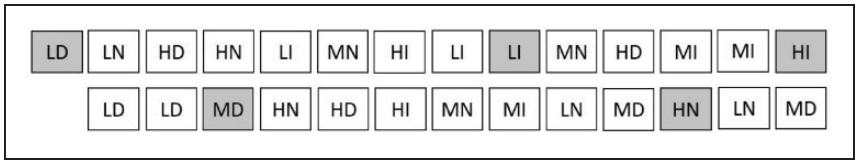


**Figure 1.** Randomized Layout of Experimental Units and Treatments. PAR Loggers Were Immersed Within Selected Experimental Units (Shaded Gray). SSC Treatments →L = Low; M = Medium; H = High; Salinity Treatment→ D = Decrease; N = No Change; I = Increase. Note That the Units Were Placed in a Single Line on Site.

within each unit. The experiment ran for a total of seven days, during which experimental units were gently stirred hourly between 09:00 and 17:00 hr with separate paddles to minimize cross contamination. The duration of seven days was chosen to identify the short-term changes that are characteristic of a dynamic nearshore environment. The salinity, temperature, and dissolved oxygen were checked at the beginning of each day using a portable YSI multimeter (YSI, Professional Plus). The experiment was terminated on the seventh day, and water samples were taken for analysis of nutrients (total nitrogen, total phosphorus, and silicate), *chlorophyll a*, and phytoplankton composition and abundance. Data from the PAR and temperature loggers were then extracted. Samples for phytoplankton composition and abundance were preserved with acidic lugol. All water samples were taken back to the laboratory (DHI Singapore Pte Ltd) in cooler boxes to minimize the influence of temperature changes. Water quality samples were analyzed as described earlier. Phytoplankton samples were enumerated and identified to the lowest possible taxon using the Utermöhl method (Utermöhl, 1931, 1958) with an inverted microscope (Olympus, CKX41) at 200× magnification and assessed for phytoplankton composition.

### *Statistical Analysis*

Statistical analyses were carried out in R (R Core Team; Pinheiro et al., 2017). All data were checked for normality and variance. If the data were found to be normally distributed, with no significant difference in variance, an analysis of variance (ANOVA) was performed. Pairwise multiple comparisons were made using Tukey's honest significant differences (Tukey's HSD). If the data did not fulfill either of the assumptions, a linear mixed effects model (lme) (Pinheiro et al., 2017) or Kruskal–Wallis rank sum test was applied. For LME, all models were checked for heteroscedasticity and, if present, variance structures were input and the model with the lowest Akaike information criterion (AIC) was chosen. Pairwise multiple comparisons were then carried out using the "glht" function from the "multcomp" package (Hothorn et al., 2008).

Relationships between phytoplankton and environmental data were analyzed in Primer (7 with PERMANOVA+, Plymouth, UK) using canonical analysis of principle coordinates (CAP). For the CAP, the abundance of individuals within each genus was used as the input variable for each replicate. Due to the large range in plankton counts and the presence of a few highly abundant genera (e.g., *Chaetoceros* sp.), the data set was square root transformed to reduce the influence/bias of these relatively abundant genera when carrying out Bray–Curtis dissimilarities (M. J. Anderson & Willis, 2003). Following this, a distance matrix was created from Bray–Curtis similarity values. The CAP was then carried out on this distance matrix. The CAP plot was then overlaid with the phytoplankton genera and the water quality parameters (correlation >0.4) separately.

## Results

Before the start of the experiment, samples from the Pandan River (PR; hereafter referred to as the baseline) were analyzed to assess water conditions. The mean salinity ($\pm SE$) was $30.06 \pm 0.03$‰, SSC concentration was $14.31 \pm 0.66$ mg $l^{-1}$ and nutrients averaged $0.026 \pm 0.002$ for μM for total nitrogen, $0.0006 \pm 0.00005$ μM for nitrate, nitrite was below the detection limit (<0.0002 μM or <0.01 mg $l^{-1}$) in all samples, $0.0069 \pm 0.0003$ μM for ammoniacal nitrogen, $0.00179 \pm 0.00008$ μM for total phosphorus, and $0.00025 \pm 0.00001$ μM for phosphate. *Chlorophyll a* had a mean concentration of $3.60 \pm 0.30$ μg $L^{-1}$ and silicate $0.0130 \pm 0.0004$ μM (Table 1).

Baseline (i.e., initial) phytoplankton samples had a total of 27 genera which were grouped into four categories (Diatoms, Dinoflagellates, Cyanobacteria, and Others; Table 2). The most abundant group of organisms was Diatoms followed by Dinoflagellates. The mean cell abundance was 231 cells $ml^{-1}$, of which, the dominant genus was *Skeletonema* sp. with a mean abundance of 181 cells $ml^{-1}$, followed by *Pseudo-nitzschia* sp. at 14 cells $ml^{-1}$. The genera with the lowest mean abundance were from *Coscinodiscus* sp., *Karenia* sp., and *Licmophora* sp. at 0.1 cells $ml^{-1}$ each. The mean

**Table 1.** Baseline (Pandan River, PR) Water Quality Parameters ($n = 3$).

| Parameter | Average (μM) | SD | SE |
|---|---|---|---|
| Suspended sediment concentration (SSC) | 14.31 mg $l^{-1}$ | 1.15 | 0.66 |
| Total nitrogen (TN) | 0.026 | 0.003 | 0.002 |
| Nitrate ($NO_3^-$) | 0.0006 | 0.00008 | 0.00005 |
| Nitrite ($NO_2^-$) | n.d. | n.d. | n.d. |
| Ammoniacal nitrogen ($NH_3^-N$) | 0.0069 | 0.0006 | 0.0003 |
| Total phosphorus (TP) | 0.00179 | 0.00015 | 0.00008 |
| Phosphate ($PO_4^{3-}$) | 0.00025 | 0.00001 | 0.00001 |
| *Chlorophyll a* | 3.60 μg $L^{-1}$ | 0.51 | 0.30 |
| Silicate ($SiO_3^-$) | 0.0130 | 0.0006 | 0.0004 |

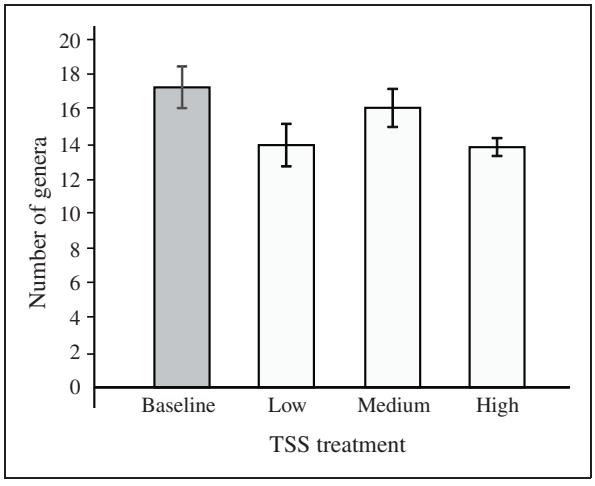


**Figure 2.** Mean (± *SE*) Genera Richness Recorded at the Baseline (Before) and in the Three Different SSC Treatments at the End of the Experiment (Seven Days Later). TSS = Total Suspended Solids.

number of genera collected in baseline samples was 17.3 (Figure 2).

The experiment ran for seven days, April 7–14, 2015. On the second day, four experimental units from different treatments were removed due to disturbance (building maintenance). At the end of the experiment, water temperature across the days had a mean (± *SE*) of 27.6 ±0.05°C, with a range of less than 2°C. Dissolved oxygen had a mean concentration of 4.17 ±0.44 mg $l^{-1}$ and daytime PAR levels ranged from a mean of 968 to 1,490 μmol photon $m^{-2}$ $s^{-1}$. The total number of observed phytoplankton genera had increased from 27 (baseline data) to 35. The dominant group remained diatoms (Table 2). Among the SSC treatments, the medium SSC concentration had the highest mean (± *SE*) number of genera (16.1 ±1.1), whereas the high SSC treatment had the lowest mean number of genera (13.9 ±0.5). There was no significant difference in the genera richness across all treatments ($F = 2.011$, $df = 3$, $p = 0.142$). Among the genera documented, *Chaetoceros* sp. had the highest mean abundance at 6,695 cells $ml^{-1}$ followed by *Skeletonema* sp. with 637 cells $ml^{-1}$, both from the treatment with low SSC. The least abundant genera were *Cyclotella* sp. from the medium SSC treatment (0.1 cells $ml^{-1}$) and *Oscillatoria* sp. from the low SSC treatment (0.1 cells $ml^{-1}$). Comparing across the SSC treatments, the low SSC treatment had the highest mean abundance with 7,579 cells $ml^{-1}$, whereas the medium SSC treatment had the lowest mean abundance at 626 cells $ml^{-1}$. Phytoplankton composition between SSC treatments was significantly different, whereas there was no significant difference in phytoplankton composition between salinity and no significant two-way interaction between SSC and salinity (Table 3). The baseline phytoplankton composition was significantly different from the medium ($t = 1.976$, $p = 0.005$) and high ($t = 1.923$, $p = 0.015$) SSC concentration treatments. For the low SSC treatment, the high abundance was largely due to *Chaetoceros* sp. In the medium SSC treatment, *Leptocylindrus* sp. and *Asterionellopsis* sp. were the two most dominant genera. In the high SSC treatment, *Chaetoceros* sp. was the most dominant genus. Also, all genera that were more abundant in the medium than in the low SSC treatment declined in the high SSC treatment (*Asterionellopsis*: 85 to 8 cells $ml^{-1}$; *Leptocylindrus*: 203 to 133 cells $ml^{-1}$; *Navicula*: 25 to 20 cells $ml^{-1}$; *Karlodinium*: 30 to 19 cells $ml^{-1}$; *Protoperidinium*: 10 to 4 cells $ml^{-1}$), except for *Cylindrotheca* sp. As phytoplankton composition was not significantly different among salinity treatments, further analysis was conducted on the pooled data.

The CAP analysis produced 25 PCO axes, of which the first explained 79.8% of the variability and achieved 42.3% correct allocations (Figure 3). The phytoplankton composition had two distinct groupings, that is, the baseline and the experimental units, which was associated with silicate concentration. Among the experimental

**Table 2.** Mean Phytoplankton Composition (Cells $ml^{-1}$) for the Baseline and the Respective SSC Treatments.

| | | SSC treatments | | |
|---|---|---|---|---|
| Phylum/Genus/Organism | Baseline | Low | Medium | High |
| Diatom | | | | |
| *Asterionellopsis* | 1.37 | 15.38 | 85.48 | 8.41 |
| *Bacillaria* | | 1.20 | | |
| *Certaulina* | | | | 0.20 |
| *Chaetoceros* | 3.23 | **6,695.28** | 43.63 | **792.16** |
| *Coscinodiscus* | 0.10 | | | |
| *Cyclotella* | 0.40 | | 0.10 | |
| *Cylindrotheca* | 3.67 | 18.73 | 78.09 | 153.65 |
| *Cymbella* | | 1.53 | 1.00 | |
| *Dactyliosolen* | 0.70 | 7.98 | 4.73 | 9.99 |
| *Detonula* | | | 0.20 | |
| *Dictyocha* | | 0.50 | | |
| *Ditylum* | | 0.50 | | 0.20 |
| *Entomoneis* | 0.47 | 3.39 | 9.30 | 9.90 |
| *Eutreptiella* | | 1.00 | | |
| *Guinardia* | 0.80 | 0.50 | 0.50 | 0.40 |
| *Helicotheca* | | 0.63 | 0.30 | 8.10 |
| *Hemiaulus* | | 0.50 | | |
| *Lauderia* | 0.20 | | | |
| *Leptocylindrus* | 2.60 | 86.77 | **203.13** | 133.24 |
| *Licmophora* | 0.10 | | | |
| *Navicula* | 14.23 | 5.88 | 25.29 | 20.11 |
| *Nitzschia* | 1.60 | 1.10 | 0.90 | 1.40 |
| *Odontella* | | 1.00 | | |
| *Pleurosigma* | 3.10 | | | |
| *Pseudo-nitzschia* | 14.67 | 18.30 | 4.88 | 2.09 |
| *Rhizosolenia* | | 0.88 | 0.46 | 4.36 |
| *Skeletonema* | 181.17 | 637.64 | 66.34 | 67.96 |
| *Thalassionema* | 0.40 | | 0.35 | 0.20 |
| *Thalassiosira* | 2.60 | 21.60 | 1.80 | |
| Unknown diatom | | | 5.40 | |
| Dinoflagellates | | | | |
| *Alexandrium* cf. | | 11.03 | 0.93 | 1.40 |
| *Gymnodinium* | | 3.45 | 1.50 | 3.98 |
| *Gyrodinium* | 0.40 | 0.50 | | |
| *Karenia* cf. | 0.10 | 7.83 | 1.64 | 1.56 |
| *Karlodinium* cf. | 1.20 | 25.48 | 30.09 | 19.80 |
| *Protoperidinium* | 0.48 | 4.39 | 10.20 | 4.36 |
| *Scrippsiella* | 0.40 | 4.63 | 1.24 | 1.94 |
| Unknown dinoflagellate | 0.45 | 3.98 | 2.56 | 1.91 |
| Cyanobacteria | | | | |
| *Oscillatoria* | 0.30 | 0.10 | | |
| Others | | | | |
| *Eutreptiella* | 0.25 | | | |
| Ciliate[a] | 2.33 | 64.30 | 81.26 | 42.58 |

*Note*. Bold values indicate the dominant genus for that treatment. SSC = suspended solids concentration.
[a]Ciliophora is not classified as phytoplankton but is shown here due to the abundance in samples.

units, the spread of points to higher values on Axis 2 was due to high *chlorophyll a* concentration, particularly in treatments with low SSC which had a greater abundance of *Chaetoceros* sp.

## Discussion

Plankton are known to be sensitive to physicochemical changes in their marine environment (Hays et al., 2005).

Our study used mesocosms to examine the independent effects of three concentrations of suspended solids and three salinity levels on a natural phytoplankton community. The greatest difference in phytoplankton composition was found between the baseline and the experimental units with medium and high SSC concentrations. Among the three SSC treatments, phytoplankton abundance was the highest in the low SSC treatment, potentially due to shading effects in the medium and high treatment. Also, the change in the number of phytoplankton genera documented was likely due to their low initial density, resulting in them not being captured during the first analysis (i.e., baseline samples). Salinity did not appear to have any effect on plankton numbers or composition.

The different SSC concentrations across the experimental units affected phytoplankton abundance, but not genera richness. The SSC levels in the low treatment were lower than the baseline (i.e., 5 mg $l^{-1}$ vs. 14.31 mg $l^{-1}$) determined from the initial samples, whereas the SSC levels for the medium and high treatments (20 mg $l^{-1}$ and 80 mg $l^{-1}$) were higher than the baseline. The higher SSC in the medium and high treatments probably created a shading effect (Kirk, 1985), reducing light penetration through the water in the experimental units. Light availability is a critical control of phytoplankton dynamics in estuarine phytoplankton (Cloern, 1987), for example, Dokulil et al. (1994) found that increasing SSC in the River Danube resulted in an exponential decrease in *chlorophyll a* (biomass). Shading can also result in reduced photosynthetic rates, impacting primary productivity and phytoplankton growth (Bilotta & Brazier, 2008; Cloern, 1987).

In addition to causing a shading effect, the use of kaolin in the experiments could have had some unintended influence on the phytoplankton density and nutrient availability within the experimental units. Natural clays such as kaolin have been used in various countries such as China, Japan, and South Korea as a cost-effective method for controlling harmful algal blooms (Table 1 in Sengco & Andersen, 2004). The clays are applied to the water column and through co-flocculation, sediment algal cells out of the water column ( Anderson, 1997; Yu et al., 1995). Cell removal efficiencies as high as 95% to 99% have been reported (Yu et al., 1995). Regular homogenization of the units during the experimental period may have encouraged flocculation, forming larger particles. These larger particles would have caused cells to quickly settle out from the water column before they could be collected. Thus, the higher concentrations of kaolin (medium and high SSC) would have not only have created a turbidity regime but may have caused cells to sediment out of the water column, possibly explaining the high phytoplankton abundance in the low SSC treatment. Kaolin has also been found to be capable of adsorbing metal ions (Duursma & Eisma, 1973; Noriki et al., 1985) and

**Table 3.** PERMANOVA Results Testing for the Effects of TSS and Salinity for Phytoplankton Genus Composition.

| Factor | df | Pseudo-F | p |
|---|---|---|---|
| Suspended Sediment Concentration (SSC) | 3 | 1.861 | .026[a] |
| Salinity | 2 | 1.386 | .187 |
| SSC × Salinity | 4 | 1.4010 | .118 |

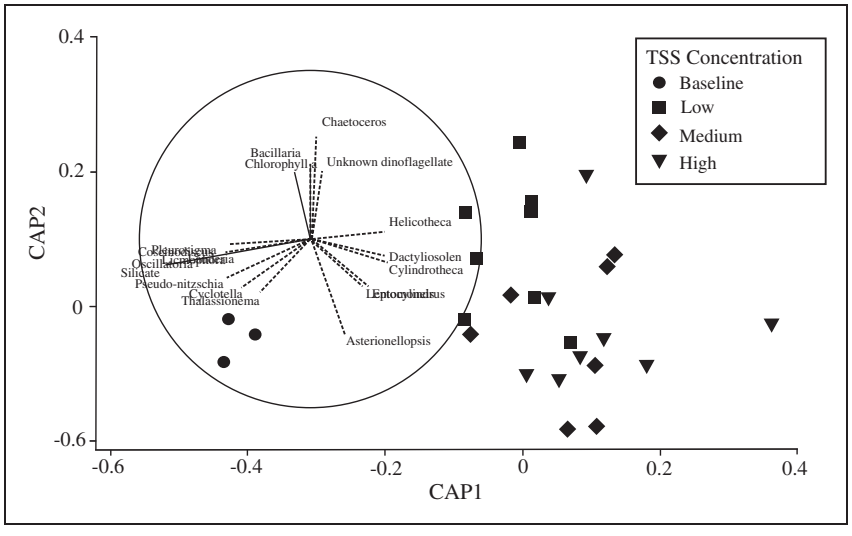


**Figure 3.** CAP Plot of Phytoplankton Composition for the Baseline Samples and Experimental Samples at Different SSC Levels at the End of Experiment. Overlayed With Phytoplankton Genera (Dotted Line) and Water Quality Parameters (Solid Lines, Bold Text) Which Have a Correlation of >.40. The Overlays Were Generated Separately but Combined in This Figure for Easy Viewing. TSS = Total Suspended Solids; CAP = Canonical Analysis of Principle Coordinates.

nutrients (Lu et al., 2015), some of which (e.g., iron, total nitrogen, total phosphate) are essential to the phytoplankton growth (Sunda, 1989). Trace metals were not tested for in this study; however, treatments with medium and high suspended sediment concentration had lower TN and TP concentrations than the treatment with low suspended sediment indicating decreased availability of nutrient uptake for the phytoplankton (Appendix, Table A3).This decreased availability of nutrients could then have further stressed the phytoplankton community. Unexpectedly, the average phytoplankton abundance was lowest in the medium SSC treatment, instead of the high SSC treatment. The kaolinite which was used to simulate the SSC is mostly made up of silicate and aluminium, thus although the higher concentration of SSC caused turbid conditions and may have adsorbed some nutrients, the silicate and aluminium within the kaolinite could have contributed toward phytoplankton growth.

The decrease in silicate concentration during the experiment (while other environmental factors such as temperature, dissolved oxygen, and pH levels varied minimally) was a strong factor separating the phytoplankton composition of the baseline and experimental units. By the end of the experiment, the mesocosms had a marked decrease (on average 20 times lower) in silicate concentration compared to the baseline, which may have resulted in the observed differences in phytoplankton composition. By Day 7, the experimental units were silicate limited (final: TN:Si = 100–5.6:1), whereas water quality measured in baseline samples were nitrogen limited (baseline: TN:Si = 0.46:1). The dominant phytoplankton genus in the baseline samples was *Skeletonema* sp. However, at the end of the experiment, the dominant genus in the experimental units for the treatments with low and high SSC concentration was *Chaetoceros* sp. Harrison and Davis (1979) observed a similar switch in dominant species with outdoor continuous cultures of a natural phytoplankton assemblage. *Skeletonema costatum* dominated under nitrogen limitation, but this changed to being dominated by *Chaetoceros* spp. with silicate limitation (Harrison & Davis, 1979). Silicon-limiting concentrations may also result in physiological changes such as the formation of thinner frustules on new cells (Paasche, 1973, 1975). This was not verified directly, but we did observe a higher mean abundance of *Cylindrotheca* sp., which is a weakly silicified diatom (Harrison & Davis, 1979), in all the SSC treatments.

Diatoms (Phylum: Ochrophyta) remained dominant in all the units despite silicate limitation. Usually, silicate limitation selects for nonsiliceous flora such as flagellates (Egge & Aksnes, 1992; Weston et al., 2008) and cyanobacteria (Rocha et al., 2002). Egge and Aksnes (1992), using floating outdoor sea enclosures, identified a minimum silicate concentration of 2 μM for diatom dominance. Both Egge and Aksnes (1992) and Weston et al. (2008) reported an increase in the flagellate *Phaeocystis* sp. in silicon-limited environments. With the exception of one unit from the treatment LI (3.49 μM), silicate concentration in the experimental units ranged from 0.17 to 1.45 μM. Besides limiting silicate concentrations, other criteria such as nutrient ratios and light have been reported in the literature for this diatom-flagellate switch. Zhou et al. (2017) found that, aside from a low Si:N ratio ($< 1$), a high N:P ratio ($> 50$) accelerated the bloom of the dinoflagellate, *Prorocentrum donghaiense*. Nitrogen concentrations in our experimental units remained low, and TN:TP ratios in the experimental units only reached a maximum of 27.3. In terms of light, flagellates have a higher requirement than diatoms (Peperzak et al., 1998). Weston et al. (2008) found that, in the Thames estuary, UK, the switch to flagellate dominance was brought about by a combination of limiting silicon concentration and higher irradiance during the spring. The (dynamic) natural light that the mesocosms were exposed to may have slowed the competitive exclusion as compared to a constant light regime. Fluctuations in light cause recurring interruptions that might reverse successional processes. These interruptions create opportunities for different phytoplankton species to occur, leading to a higher diversity and impeding competitive exclusion (Flöder et al., 2002). For example, cloud cover due to rainy weather on the last two days of our experiment would have caused a reduction in available light. The light-dependent uptake of nutrients such as nitrogen and phosphate require different optimal nutrient uptake strategies under light and dark conditions (Litchman, 2003; Litchman et al., 2004). At the time of final sampling, the experimental units may have been in a time-lag occurring before the (expected) sharp increase of flagellate abundance (Escaravage & Prins, 2002).

Regular stirring of the experimental units to maintain the kaolin in suspension may have negatively affected the growth of dinoflagellates. Among the various phytoplankton groups, dinoflagellates are the most sensitive to turbulence (Thomas & Gibson, 1990). Agitation of the water column has been found to have negative effects on dinoflagellates, some of which include disturbance of cell division, morphological changes, cell disruption and behavior interference (Estrada & Bardalet, 1997). Water column stability is needed for dinoflagellate growth (Peperzak et al., 1998), but diatoms can survive in well-mixed environments (Escaravage & Prins, 2002). In calm environments, diatoms sediment out of the surface layer, whereas more motile organisms such as dinoflagellates are able to move vertically and remain close to the surface. Mixing resuspends nonmotile cells such as diatoms and also disrupts gradients (e.g., light and

nutrients) creating a homogenous environment (Hinder et al., 2012). Diatoms have a higher growth rate, higher photosynthetic rate, and are capable of surviving at low nitrogen concentrations (Ross & Sharples, 2007); hence, they may have been able to outcompete dinoflagellates under the experimental conditions we created.

The ability of species to acclimatize to salinity changes could explain the lack of significant difference among the phytoplankton communities subjected to the different salinity treatments. Often, variation in the phytoplankton community along salinity gradients can be attributed to the fact that most species present are stenohaline (Brand, 1984; Larson & Belovsky, 2013). Sudden changes in salinity can cause osmotic stress and stenohaline organisms are be particularly vulnerable toward fluctuations in salinity (Brand, 1984). The baseline samples were collected at a river mouth that opens into the Singapore Straits. Freshwater input from drainage upstream due to heavy downpours during monsoon seasons along with overflow from the adjacent Pandan Reservoir will cause a decrease in salinity. Conversely, seawater intrusion due to tidal forces can potentially cause localized increases in salinity. Thus, phytoplankton species living in this estuarine environment are likely to have had some level of salinity tolerance (Brand, 1984). *Skeletonema* sp. was the most abundant genus recorded in the baseline samples, and a study on 10 strains of *Skeletonema* showed that all displayed euryhaline responses, with those from estuarine populations possessing the greatest range of tolerance (Balzano et al., 2011).

This experiment was carried out at a mesocosm scale, in a simplified environment, and in the absence of larger predators (>35 μm). In the natural environment, currents, tides, and runoff are potential avenues by which nutrients for coastal phytoplankton populations can be replenished. Also, connectivity to other aquatic bodies allows for the renewal of phytoplankton cells that may have been lost to processes such as mortality or sedimentation. The exclusion of predators removed the effect of grazing pressure on the phytoplankton community. Microzooplankton (<200 μm) have been shown to be key consumers of primary production (Calbet & Landry, 2004), and their grazing can significantly affect the phytoplankton community, the number of phytoplankton genera, and their abundance.

Using a manipulative mesocosm-based approach, this study tested the influence of salinity stress and SSC concentrations in combination on a natural phytoplankton community. Overall, salinity had little effect on the response variables, however, the SSC treatments probably resulted in poor nutrient and light conditions, such as suboptimal nutrient ratios and low irradiance, impacting the phytoplankton abundance and affecting community composition. Higher concentrations of kaolinite increased turbidity, leading to low irradiance thus affecting the phytoplankton primary productivity. Furthermore, the use of kaolinite likely had some additional impacts such as flocculation and nutrient absorption. Among the nutrients analyzed, silicate limitation was a prominent factor affecting the phytoplankton community, distinguishing the baseline and experimental units.

## Implications for Conservation

Even though phytoplankton perform vital functions in marine ecosystems, these roles are often overlooked when designing coastal conservation strategies. Phytoplankton are sensitive to changes in their environment, but estuaries and nearshore waters are often impacted by high levels of sediment pollution (Barton et al., 2016; Hays et al., 2005). This is likely to be compounded by climate change, especially extreme weather events that can lead to lowered salinity and increased turbidity from terrestrial run off. Although salinity in this study had no significant effect, larger changes have the potential to alter phytoplankton communities (Brand, 1984) and suboptimal salinities for resident species may allow opportunistic species to thrive (Guinder et al., 2010).

Three concentrations of kaolinite were used in this study, and changes in the phytoplankton community at higher concentrations were possibly due to various factors working in combination (e.g., nutrient limitation, cell adhesion). As shorelines undergo development and coastal cities expand, excess suspended solids due to sediment disturbance and terrestrial runoff are introduced into the marine environment. Clays similar to kaolinite occur naturally and elevated concentrations in the water column could lead to observations similar to this study. Being the foundation of most aquatic food webs, potential impacts to the phytoplankton community (e.g., changes in species composition) should not be overlooked, as these can have repercussions for higher trophic levels (Butler, 1995). Overall, this study has shown that the impacts of SSC are multi-faceted with co-varying factors that can make general predictions of sediment effects on phytoplankton challenging to formulate.

### Appendices

**Table A1.** Kaolin Particle Size Distribution Used in the Experiments.

| Particle size (μm) | Percentage composition (%) |
|---|---|
| <2 | 2.72 |
| 2–6 | 10.33 |
| 6–20 | 13.82 |
| 20–63 | 59.36 |
| 63–200 | 13.77 |

**Table A2.** Schedule of Samples Taken for Each Water Quality Parameter.

| Water quality parameters analyzed | Initial | Daily | Every 2 days | Final |
|---|---|---|---|---|
| Salinity (‰) | ✓ | ✓ | | |
| Temperature (°C) | – | ✓ | | |
| Dissolved oxygen (mg $l^{-1}$) | – | ✓ | | |
| Suspended sediment concentration (mg $l^{-1}$) | ✓ | – | – | |
| Total nitrogen (μM) | ✓ | – | – | ✓ |
| Nitrates (μM) | ✓ | – | – | – |
| Nitrite (μM) | ✓ | – | – | – |
| Ammoniacal nitrogen (μM) | ✓ | – | – | – |
| Total phosphorus (μM) | ✓ | – | – | ✓ |
| Phosphate (μM) | ✓ | – | – | – |
| *Chlorophyll a* (μg $L^{-1}$) | ✓ | – | – | ✓ |
| Silicate (μM) | ✓ | – | – | ✓ |
| Phytoplankton | ✓ | Relative fluorescence unit *Chlorophyll a* | Abundance and composition | ✓ |

**Table A3.** Mean (± *SE*) Concentration of Water Quality Parameters Tested From the Experimental Units at the End of Seven Days for Each SSC Treatment Against the Baseline.

| Parameter | Baseline (n = 3) | Low (n = 8) | Medium (n = 7) | High (n = 8) |
|---|---|---|---|---|
| Chlorophyll a (μg $L^{-1}$) | 3.60 (± 0.30) | 5.42 (± 3.31) | 1.17 (± 0.24) | 1.97 (± 0.53) |
| Silicate | 0.01296 (± 0.0004) | 0.00091 (± 0.0004) | 0.00046 (± 0.00006) | 0.00050 (± 0.00014) |
| Total nitrogen | 0.026 (± 0.002) | 0.050 (± 0.015) | 0.027 (± 0.004) | 0.032 (± 0.006) |
| Total phosphorus | 0.0018 (± 0.00009) | 0.0023 (± 0.0007) | 0.0021 (± 0.0009) | 0.0015 (± 0.0002) |

All results are in μM unless otherwise stated. ($n = X$) represents the number of replicates for each treatment.

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