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Size Dependent Competition in Centric Diatoms as a Function of Nitrogen and Silicon Availability

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ABSTRACT

Size dependent competition was examined in two marine centric diatoms, *Coscinodiscus* sp. and *Thalassiosira rotula* at various NH_4^+, NO_3^- and Si concentrations. The growth responses for both species to nutrient levels were evaluated using two forms of nitrogen (NH_4 and NO_3) and silicon in both monoculture and mixed culture conditions. Under single species culture, the impact of Si did not depend on N forms for both diatoms. The increase of NH_4-N enhanced the growth of *Coscinodiscus*, but did not affect *T. rotula*. When NO_3-N was the nitrogen source, cell densities of both species were significantly enhanced by increasing Si concentrations, but only *T. rotula* density was affected by increasing N concentration. When *Coscinodiscus* sp. and *T. rotula* grew in the same culture, *Coscinodiscus* sp. dominated in both N forms. The scale of the dominance of *Coscinodiscus* sp. over *T. rotula* increased with decreasing N and Si concentrations. In the competition experiment, when NH_4^+ was the N source, both *Coscinodiscus* sp. and *T. rotula* were significantly affected by changes in N concentration, but only *T. rotula* was affected by Si. When NO_3 was the N source, neither *Coscinodiscus* sp. nor *T. rotula* was affected by Si, but *T. rotula* was enhanced by N levels. Regardless the N form, the impact of Si on neither *Coscinodiscus* sp. nor *T. rotula* depended on N concentration. Our results indicate that large diatom species have a competitive advantage over small species, and both large and small species were sensitive to NH_4-N limitation, but the small species was more sensitive to NO_3-N limitation than the large species.

Keywords: Diatoms; Size-Dependence; Nutrient Competition; Nitrogen; Silica

1. Introduction

The paradox of plankton refers to the apparent immunity of natural phytoplankton assemblages to the principle of competitive exclusion [1]. Within natural assemblages, many species of phytoplankton are able to co-exist despite competing for relatively few limiting resources [2]. Previous studies in this area of ecology have focussed on the needs of phytoplankton in general by examining the competitive interactions occurring between phytoplankton in different taxonomic divisions [3-5]. To date, however, there has been little study of the interactions that occur within phytoplankton species that have similar resource requirements but directly compete for limiting resources. In the case of diatoms, all species require Si for growth and survival [6], but a non-limiting supply of Si alone appears to be insufficient to ensure the survival of diatom species. To adequately compete for Si, diatoms may require sufficient N for the formation of Si transporters [7,8].

Previous studies concerning competition for nutrients in diatoms have focussed on a single nutrient uptake rate for N [5,9,10], or for Si [8,11,12]. There have been few studies to simultaneously evaluate competition for both Si and N. This study attempted to understand the population dynamics of marine diatoms by examining Si and N competition in two centric diatoms of different sizes.

Diatoms will dominate phytoplankton assemblages in the presence of a sufficient supply of Si with a Si:N ratio > 25:1 [4]. There has been much investigation in the past on the role of Si in regulating the outcome of competition within phytoplankton communities. However, little is known about the influence of silicon supply on nitrogen acquisition in diatoms of different size. Si is an important nutrient for diatoms in frustule formation [13]. Without a sufficient supply of Si, frustule formation is retarded, and diatoms are unable to grow and reproduce [8].

When competing for NO_3^- as the limiting N source, the ability to take up and store excess NO_3^- for later use would constitute an immense competitive advantage, allowing larger algal species to more efficiently compete for NO_3^- under fluctuating conditions. In a study on NO_3^- uptake in diatoms, Stolte and Riegman [14] observed that

larger cells were able to take up NO_3^- at a higher rate for a longer period of time than small cells. Thus, the ability to store NO_3^- gives algal species with larger vacuoles a competitive advantage when competing for NO_3^- as the limiting N source under fluctuating nitrate supply. The difficulty in storing NH_4^+ gives the uptake rate, rather than the cell size, more importance in competition for a limiting source of this nutrient [5]. Hence the species with the highest uptake rate will dominate when competing for NH_4^+ as the N source [15].

The objective of this study was to examine the possible influence of nitrogen source and silicon availability on competition for nitrogen between two species of marine diatom. The competitive interactions between a large and a small species of centric diatoms were evaluated when different levels of Si and different forms N were presented to the algae.

2. Methods

2.1. Culture Conditions

Two centric diatoms, *Coscinodiscus* sp. (strain CS-342) and *Thalassiosira rotula* were used in this study, and were obtained from the CSIRO collection, Hobart, Australia. There were two reasons for choosing these two species. Firstly, both species were collected from Jervis Bay, NSW in 1995, and were grown at the CSIRO in similar culture conditions to those proposed for use in this study (in f/2 medium at 17.5˚C). Thus any lag phase in growth was minimised when the specimens were cultured for this experiment. The second reason was their morphology and their size relative to each other. *Coscinodiscus* sp., (100 μm ± 2.3 μm in diameter, 35 μm ± 0.9 μm thick) is much larger than *T. rotula* (20 μm ± 1.3 μm in diameter, 13 μm ± 0.7 μm thick). To minimise any lag phase in growth that may have occurred when the species were exposed to the different N sources, specimens were grown in both NO_3^- and NH_4^+ as N sources. Cultures of each species for the experiments with NO_3^- as the sole N source were grown in f/2 medium enriched with Si according to the CSIRO modification of the method of [16]. Cultures for the experiments with NH_4^+ as the sole N source grown in enriched f/2 with N provided by NH_4Cl. Prior to experimentation, cultures were maintained separately at 17˚C - 20˚C under fluorescent light (12:12 h light:dark). All experimental work was conducted using 250 ml polypropylene bottles, since diatoms have been reported to etch Si directly from glassware [7].

2.2. Growth Response of a Single Species to Changes in N and Si Concentrations

Two experiments were conducted on each species in monoculture to examine the effect of limited supplies of Si and N on algal growth. These experiments were designed to test the growth of *Coscinodiscus* sp. or *T. rotula* under various N and Si concentrations, and to confirm that each species could survive by itself in all treatment conditions, thus ensuring that any detrimental growth was due to competition, rather than conditions in the treatment that were unsuitable for growth. A 3 × 3 factorial experiment was used and each species was tested under three N levels of either NH_4-N or NO_3-N and three Si levels. Nutrient concentrations were achieved by manipulating the amount of N and Si in the f/2 medium to provide different percentages of the full f/2 nutrient concentrations. The nutrient treatments included 0% N, 50% N and 100% N, each of which was prepared with 0% Si (0 μM), 25% Si (142 μM) and 100% Si (562 μM). Thus, three Si levels were prepared at each N level, providing nine treatment combinations, with three replicates per combination. The response of each species to these treatment combinations was examined firstly with NO_3^- as the sole N source at three concentrations (0, 605 and 1210 μM NO_3^-), then again using NH_4^+ as the sole N source at three levels (0, 28 and 56 μM NH_4^+).

Each culture vessel used for the monoculture experiments contained 160 ml of media inoculated with 40 ml of either *Coscinodiscus* sp. or *T. rotula*, with a cell density of 100 ± 15 cells per ml. Vessels were thoroughly swirled twice daily to re-suspend cells, and were maintained at constant temperature (22˚C ± 1˚C). A volume of 60 ml of culture was replaced with fresh medium once a day (semicontinuous culture), giving a dilution rate of 0.3 d^{-1}, which is an acceptable approximation of steady state algal growth [3,4]. Light was provided by fluorescent tubes (12:12 h light:dark). Cell numbers were measured for each species every 2 - 3 d using a palmer counting chamber and a light microscope at 100× magnification. The number of cells present in 100 μl of suspension taken from a 1 ml sample was estimated from the average of two separate counts.

2.3. Competition between *Coscinodiscus* Sp. and *T. rotula* for N

Coscinodiscus sp. and *T. rotula* were used to examine competition for N with different N forms, and different levels of nitrogen and silicon. A 3 × 3 factorial experiment was conducted including three N levels and three Si levels. Nutrient concentrations were achieved by manipulating the amount of N and Si in the f/2 medium to provide different percentages of each nutrient, and were identical to those used in the monoculture experiments. Thus, nine combinations of N and Si were again used with three replicates. The competition response of each species to each nutrient combination was examined firstly with NO_3^- as the sole N source, then again using NH_4^+ as the sole N source.

Each vessel used for the competition experiment contained 120 ml media with 40 ml of *Coscinodiscus* sp. cells, and 40 ml of *T. rotula* cells, each with a density of 100 cells·ml^{-1} ± 18 cells·ml^{-1}. Vessels were swirled twice daily to resuspend cells, and were maintained at constant temperature (22˚C ± 1˚C). A volume of 60 ml of culture was replaced with fresh medium once a day, to give a dilution rate of 0.3 d^{-1}. Light was provided by fluorescent tubes (12:12 h light:dark). The impact of competition was measured directly by removing a 1ml sample from each flask and counting species numbers using a palmer counting chamber and a light microscope at 100× magni- fication.

2.4. Statistical Analysis

SPSS statistical analysis software was used for all statistical tests. The effect of the different treatment combinations on cell numbers was examined for both the monoculture and mixed culture experiments via an analysis of variance using the derived variables method [17]. The results for each replicate within the treatment were averaged over time, providing a derived variable that was indicative of the overall effect of the different treatments on cell densities over the experimental period. Univariate ANOVAs were performed on derived data from the monoculture experiments, since the cell densities of the separated species were the single dependent variables in each experiment. When the two species were combined for the competition study, the cell densities of both species were dependent variables, requiring the use of a multivariate analysis of variance. Bonferroni post-hoc multiple comparisons were used to further examine the treatment effects detected in the ANOVAs. Assumptions of normality and homoscedasticity were examined prior to all statistical tests. All data were natural log transformed. In the competition experiment, a t-test was used to detect the competitive outcome between the two diatoms. The difference between the two species densities at the final time point was calculated for each treatment level, and then tested against zero. A significance level of 0.05 was used for all tests.

3. Results

3.1. Growth Response of a Single Species to Changes in N and Si Levels

When grown in NH_4^+ as the N source, *Coscinodiscus* sp. abundance remained relatively constant throughout the course of the experiment in all treatments (**Figure 1**). The exception was the zero NH_4^+ treatment, where the abundance declined by approximately 30% over time. *Coscinodiscus* sp. was significantly affected by changes in NH_4^+ level ($P < 0.001$, **Table 1**), with the abundance increasing across all NH_4^+ treatments. Post-hoc tests revealed that cell density in zero NH_4^+ concentration was significantly lower than in 28 µM NH_4^+ and 56 µM NH_4^+ treatments ($P = 0.001$). There was no significant difference between cell densities in the 28 µM NH_4^+ and 56 µM NH_4^+ treatments ($P > 0.40$). Cell densities of *T. rotula* decreased by approximately 50% for all treatment

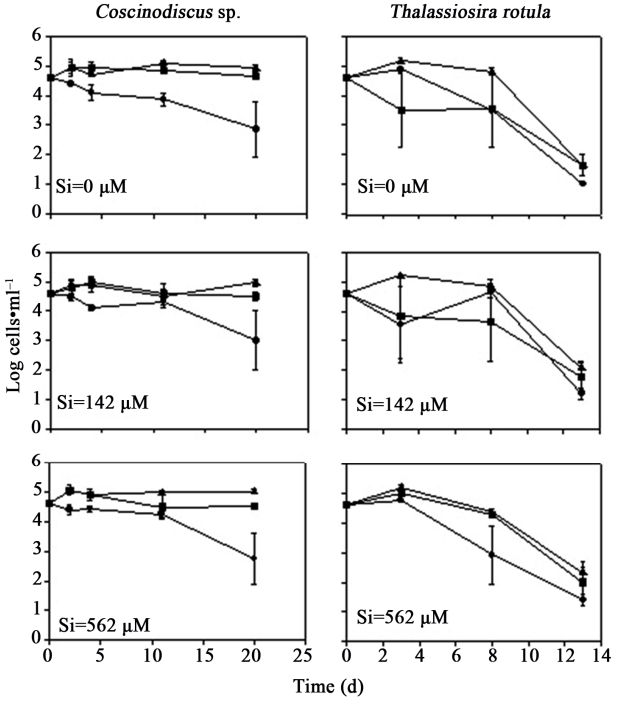

Figure 1. Growth of *Coscinodiscus* sp. (left) and *Thalassiosira rotula* (right) in monoculture over time, with three Si and three NH_4^+ levels: Nil (●), 28 µM (■), and 56 µM (▲). Values are mean ± SE ($n = 3$). Error bars are shown only when larger than the size of the symbol.

Table 1. Univariate ANOVA results for the effects of NH_4^+ and Si, and NO_3^- and Si on the cell densities of *Coscinodiscus* sp. and *Thalassiosira rotula* in monoculture.

	Coscinodiscus sp.				*Thalassiosira rotula*		
Source	df	MS	*F*	*P*	MS	*F*	*P*
NH_4^+	2	0.792	41.094	0.001	0.180	3.370	0.06
Si	2	0.006	0.311	0.74	0.017	0.319	0.73
NH_4^+ × Si	4	0.013	0.687	0.61	0.021	0.389	0.81
Error	18	0.019			0.053		
Total	26						
NO_3^-	2	0.039	2.492	0.11	0.261	6.907	0.01
Si	2	0.29	18.666	0.001	0.154	4.082	0.04
NO_3^- × Si	4	0.007	0.473	0.76	0.016	0.423	0.79
Error	18	0.016			0.038		
Total	26						

combinations over the duration of the study. No signifycant difference in *T. rotula* cell densities was detected between NH_4^+ treatments ($P > 0.06$). The cell densities of *Coscinodiscus* sp. and *T. rotula* were not significantly affected by Si concentration when NH_4^+ was the N source ($P > 0.73$). The impact of NH_4^+ on population abundance did not depend on Si concentration for both *Coscinodiscus* sp. ($P = 0.61$) and *T. rotula* ($P = 0.81$).

When NO_3^- was the N source, *Coscinodiscus* sp. densities remained relatively unchanged for all treatment combinations over time. However, an initial decline in the cell density for both species was observed in all treatments (**Figure 2**). No significant difference in *Coscinodiscus* sp. density was detected between NO_3^- treatments ($P = 0.11$, **Table 1**), but cell densities were significantly enhanced by increasing Si concentration ($P = 0.001$). Post-hoc tests showed cell densities in the zero Si treatment were significantly lower than densities observed in the 142 µM Si and 562 µM Si treatments ($P < 0.001$). There was no detectable difference between the 142 µM Si and 562 µM Si treatments ($P = 0.99$). Cell densities of *T. rotula* also remained relatively unchanged for the duration of the monoculture experiment, with an initial decline 3 - 5 d after the experiment began, though not as obvious as *Coscinodiscus* sp. (**Figure 2**). Changes in both NO_3^- and Si concentration had a significant effect on *T. rotula* cell density when NO_3^- was the N source ($P = 0.01$ and $P = 0.04$, respectively; **Table 1**). Cell densities increased with increasing NO_3^- and Si concentrations (**Figure 2**). Post-hoc test showed that cell numbers in the zero NO_3^- treatment were not significantly lower than those in the 605 µM NO_3^- treatment ($P = 0.09$), but were significantly lower than those in the1210 µM NO_3^- treatment ($P = 0.005$). Cell densities in the 605 µM NO_3^- treatment were not significantly different from those observed in the1210 µM NO_3^- treatment ($P = 0.65$). *Thalassiosira rotula* densities in the zero Si treatment were not significantly different from those in the 142 µM Si treatment ($P > 0.05$), but were lower than densities in the 562 µM Si treatment ($P = 0.04$). There was no significant difference in cell numbers between the 142 µM Si and 562 µM Si treatments ($P = 0.99$). No interactions between NO_3^- and Si were observed for either species ($P > 0.76$).

3.2. Competition between Two Algal Species for N

When grown in the same culture, *Coscinodiscus* sp. dominated *T. rotula* in low N and Si concentrations (**Figures 3-4**). When NH_4^+-N was the N source, *Coscinodiscus* sp. density was significantly greater than the cell density of *T. rotula* by the end of the experimental period in all treatment levels except the 56 µM NH_4^+ and 562 µM Si treatment ($P = 0.39$; **Table 2**, **Figure 3**). However, as the Si concentration decreased, *T. rotula* initially dominated for the first 3 days - 4 days before *Coscinodiscus* sp. became dominant. This initial dominance of *T. rotula* occurred in the nil Si and 142 µM Si treatments for all NH_4^+ treatments and was also the case in the 28 µM NH_4^+ and 562 µM Si treatments (**Figure 3**). When NO_3^- was the N source, the cell density of *Coscinodiscus* sp. was significantly greater than the cell density of *T. rotula* at the end of the experiment in all treatment levels except the 1210 µM NO_3^- treatments ($P > 0.49$; **Table 2**, **Figure 4**). The scale of the dominance of *Coscinodiscus* sp. over *T. rotula* increased with decreasing NO_3^- and Si concentrations.

The outcome of competition between *Coscinodiscus* sp. and *T. rotula* for N differed for each N source. *Coscinodiscus* sp. was significantly affected by changes in NH_4^+ concentration, with numbers increasing across all NH_4^+ levels ($P = 0.001$; **Table 3**, **Figure 3**). Post-hoc tests revealed that cell densities in the zero NH_4^+ treatment were significantly lower than those observed in the 28 µM NH_4^+ and 56 µM NH_4^+ treatments ($P = 0.001$). No significant difference in cell densities was detected between the 28 µM NH_4^+ and 56 µM NH_4^+ treatments

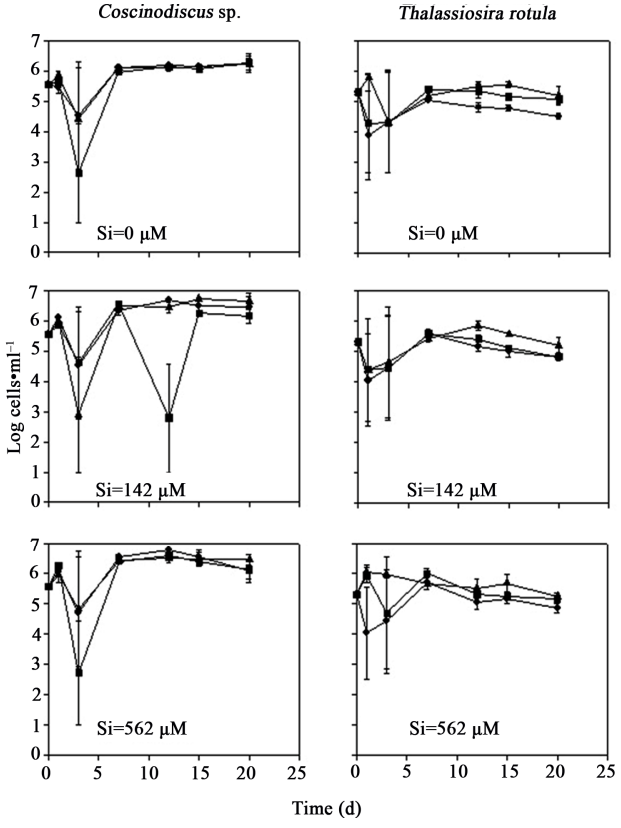

Figure 2. Growth of *Coscinodiscus* sp. (left) and *Thalassiosira rotula* (right) in monoculture over time, with three Si and three NO_3 levels: Nil (●), 605 µM (■), and 1210 µM (▲). Values are mean ± SE ($n = 3$). Error bars are shown only when larger than the size of the symbol.

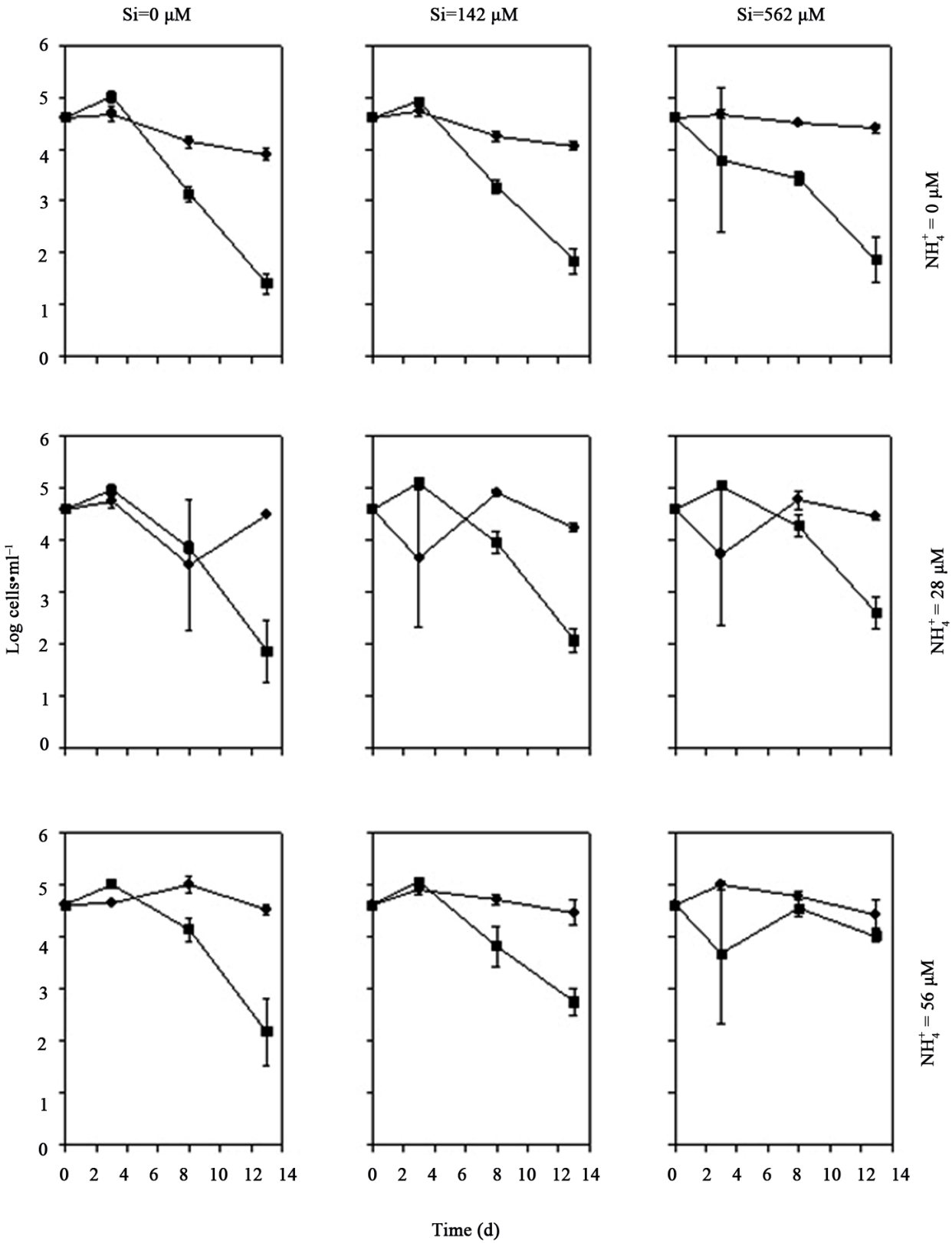

Figure 3. The effect of increasing NH_4^+ concentration on the cell densities of *Coscinodiscus* sp. (●) and *Thalassiosira rotula* (■) over time in different Si concentrations. Values are mean ± SE (*n* = 3). Error bars are shown only when larger than the size of the symbol.

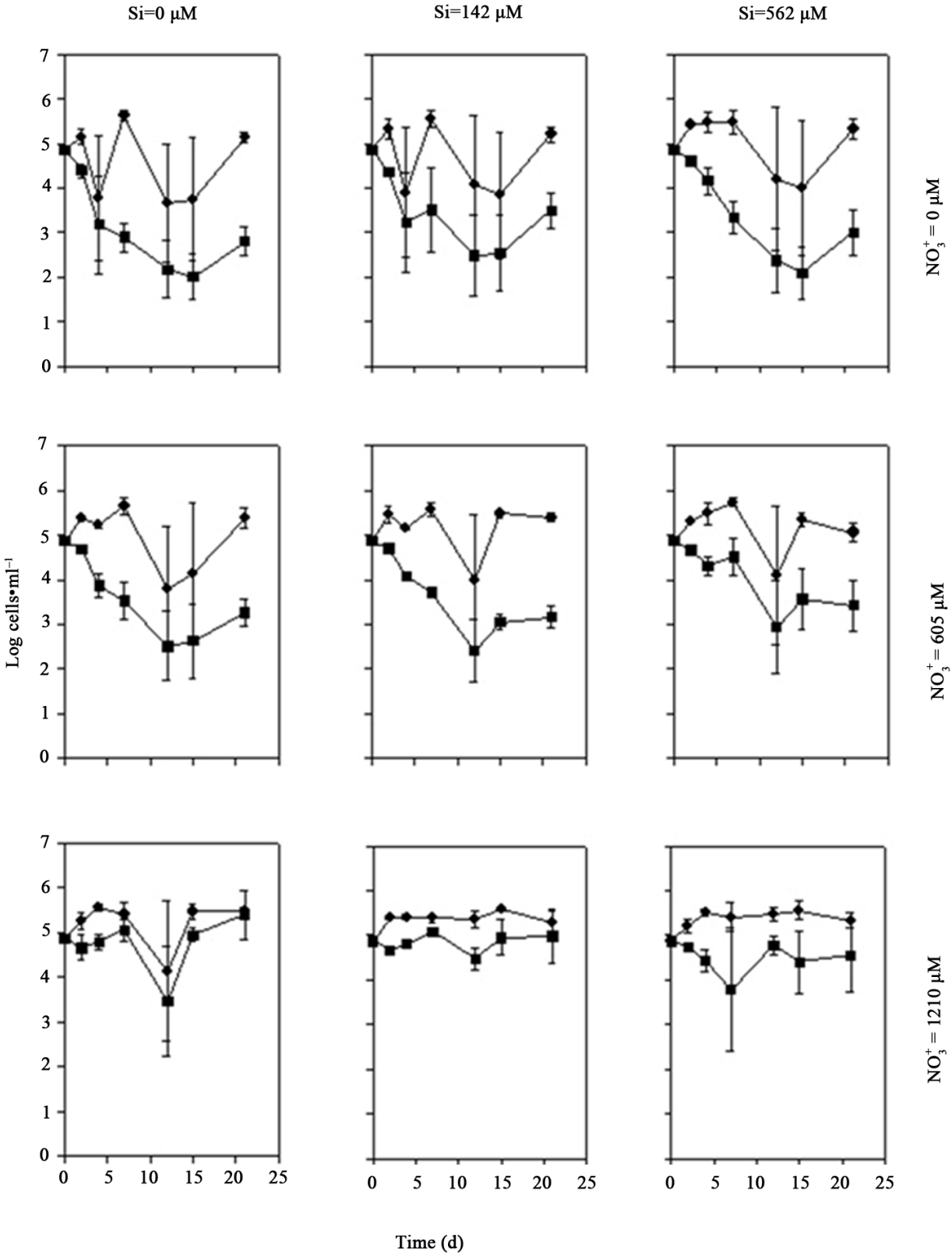

Figure 4. The effect of increasing NO_3^- concentration on the cell densities of *Coscinodiscus* sp. (●) and *Thalassiosira rotula* (■) over time in different Si concentrations. Values are mean ± SE (*n* = 3). Error bars are shown only when larger than the size of the symbol.

Table 2. Comparisons between *Coscinodiscus* sp. and *Thalassiosira rotula* abundances at different NH_4-N (Nil, Low = 28 µM, and High = 56 µm), NO_3-N (Nil, Low = 605 µm, and High = 1210 µM) and Si (Nil, Low = 142 µM, and High = 562 µM) combinations.

Treatment comparisons	*T*	df	*P*
NH_4-N and Si combinations			
Nil NH_4^+ vs Nil Si	10.37	2	0.01
Nil NH_4^+ vs Low Si	7.25	2	0.02
Nil NH_4^+ vs High Si	6.93	2	0.02
Low NH_4^+ vs Nil Si	4.53	2	0.05
Low NH_4^+ vs Low Si	7.13	2	0.02
Low NH_4^+ vs High Si	5.83	2	0.03
High NH_4^+ vs Nil Si	4.22	2	0.05
High NH_4^+ vs Low Si	4.27	2	0.05
High NH_4^+ vs High Si	1.09	2	0.39
NO_3-N and Si combinations			
Nil NO_3-N vs Nil Si	7.75	2	0.02
Nil NO_3-N vs Low Si	3.01	2	0.09
Nil NO_3-N vs High Si	7.35	2	0.02
Low NO_3-N vs Nil Si	24.49	2	0.002
Low NO_3-N vs Low Si	7.11	2	0.02
Low NO_3-N vs High Si	2.65	2	0.10
High NO_3-N vs Nil Si	0.16	2	0.89
High NO_3-N vs Low Si	0.46	2	0.69
High NO_3-N vs High Si	0.84	2	0.49

Table 3. Multivariate ANOVA results for the effects of NH_4^+ and Si, and NO_3^- and Si on the cell densities of *Coscinodiscus* sp. and *Thalassiosira rotula* in competition.

		Coscinodiscus sp.			*Thalassiosira rotula*		
Source	df	MS	*F*	*P*	MS	*F*	*P*
NH_4^+	2	0.183	21.681	0.001	0.089	9.531	0.002
Si	2	0.020	2.310	0.13	0.040	4.324	0.03
$NH_4^+ \times$ Si	4	0.004	0.496	0.74	0.004	0.411	0.80
Error	18	0.008			0.009		
Total	26						
NO_3^-	2	0.016	0.469	0.63	1.681	22.080	0.001
Si	2	0.009	0.272	0.77	0.003	0.035	0.97
$NO_3^- \times$ Si	4	0.012	0.358	0.84	0.081	1.059	0.41
Error	18	0.035			0.076		
Total	26						

(P = 0.99). *Coscinodiscus* sp. cell densities were not significantly impacted by changes in NH_4^+-N was the N source (P = 0.13; **Table 3**, **Figure 3**). In contrast to *Coscinodiscus* sp., *T. rotula* densities in all treatment combinations decreased by approximately 50% over the course of the experiment, with the exception of the 56 µM NH_4^+ and 562 µM Si combinations in which numbers declined by approximately 10% (**Figure 3**). Changes in NH_4^+ concentration had a significant impact on *T. rotula* cell numbers (P = 0.002; **Table 3**). *Thalassiosira rotula* abundance increased with increasing NH_4^+ concentration (**Figure 3**). Post-hoc tests revealed that cell densities in the nil NH_4^+ treatment were significantly lower than those in the 28 µM NH_4^+ treatment (P = 0.02), and the 56 µM NH_4^+ treatment (P = 0.001). No significant difference in cell densities was detected between the 28 µM NH_4^+ and the 56 µM NH_4^+ treatments (P = 0.71). Cell densities of *T. rotula* were also signifycantly enhanced by increasing Si concentrations (P = 0.03; **Table 3**, **Figure 3**). Post-hoc tests revealed that no significant difference in cell numbers was detected between the nil Si and 142 µM Si treatments (P = 0.99). However, cell numbers in the zero Si treatment were significantly lower than those observed in the 562 µM Si treatments (P = 0.04). There was no significant difference in cell densities between the 142 µM Si and 562 µM Si treatments (P = 0.10). Interactions between NH_4^+ and Si levels were not detected for either species (P > 0.74).

When examining NO_3^- as the N source, *Coscinodiscus* sp. densities declined for all treatments from day 7 to day 12 of the experiment (**Figure 4**), before returning to initial levels. When competing for NO_3^- as the N source, *Coscinodiscus* sp. densities were not significantly impacted by changes in either NO_3^- (P = 0.63) or Si concentration (P = 0.77). However, the numbers of *T. rotula* were significantly impacted by changes in NO_3^- concentration (P = 0.001, **Figure 4**). Post-hoc tests showed that cell densities in the zero NO_3^- treatments were not significantly different to those observed in the 605 µM NO_3^- treatments (P = 0.99), but were significantly lower than the cell densities in the 1210 µM NO_3^- treatments (P = 0.001). Cell densities in the 605 µM NO_3^- treatment were significantly lower than densities observed in the 1210 µM NO_3^- treatments (P = 0.001). Changes in Si concentration had no significant effect on *T. rotula* cell densities (P = 0.97; **Table 3**). Interactions between NO_3^- and Si were not observed for *Coscinodiscus* sp. (P = 0.84) or *T. rotula* (P = 0.41; **Table 3**).

4. Discussion

4.1. Single Species Responses to Changes in N and Si Levels

In monoculture, the impact of NH_4-N on algal abundance was size dependent. The increase of NH_4-N concentra-

tion significantly enhanced the abundance of the large species, but did not significantly affect the small species. While still able to survive initially in NH_4^+, both *Coscinodiscus* sp. and *T. rotula* did not increase in abundance over the duration of the experiment when NH_4^+ was the N source. Indeed, the *T. rotula* density decreased over the experimental period. Since NH_4^+ is difficult to store, and is assimilated more quickly than it can be taken up [15], cells of both *Coscinodiscus* sp. and *T. rotula* may not be able to satisfy N requirements with NH_4^+ alone. *Coscinodiscus* sp. was significantly affected by changes in NH_4^+ concentration, with cell densities in the nil NH_4^+ treatment lower than those in the other treatments, and no difference in cell density between the 28 µM and 56 µM NH_4^+ treatments. At zero NH_4^+, no N can be gained from the medium, and cells must rely on N stores to ensure their survival. This would result in slow cell division as algal cells usually reduce reproduction to reduce metabolism of stored N when the ambient nutrients are low [18]. *Thalassiosira rotula* was not significantly affected by changes in NH_4^+ concentration, but cell densities decreased over the experimental period when NH_4^+ was the N source, a phenomenon observed previously in other diatoms by Stolte *et al*. [15]. This decrease may be due to the exhaustion of supplies of stored N in the smaller *T. rotula* cells.

Compared with NH_4-N, NO_3-N concentration significantly enhanced the small species, but not the large species. When NO_3^- was used as the N source, there was no change in *Coscinodiscus* sp. cell densities over the duration of the experiment, even in the zero NO_3^- concentration, which suggests that *Coscinodiscus* sp. is still able to survive on stored N after three weeks without an N supply. Sommer [19] reported that in times of N shortage, algal cells could utilize stored N to sustain survival. In the present study, the absence of a significant difference in cell densities between the 0 µM NO_3^- and 1210 µM NO_3^- concentrations suggests that total N stores had not yet reduced to a level in *Coscinodiscus* sp. cells where a slowing of metabolism was necessary. In contrast to *Coscinodiscus* sp., *T. rotula* densities were significantly affected by changes in NO_3^- concentration. The cell densities of *T. rotula* in the zero NO_3^- level were significantly lower than densities in 1210 µM NO_3^-, but cell densities did not significantly decrease over the course of the experiment, suggesting that a reduction in cell metabolism and division may be a strategy for algal cells to adapt to low N [20]. By slowing metabolism when no N is available in the culture medium, cells of *T. rotula* may be able to prolong survival by using stored NO_3^- [18]. The resulting reduction in cell division may account for the significantly lower cell densities in zero NO_3^- when compared to 1210 µM NO_3^-.

A change in Si concentration had no significant effect on cell densities of either *Coscinodiscus* sp. or *T. rotula* when NH_4^+ was the N source. When grown in NO_3^- the cells did not appear to be limited by the 142 µM Si treatment, since densities were not significantly different to those observed in the 562 µM Si treatment. However, cell densities of *Coscinodiscus* sp. were significantly lower in the absence of a Si supply. The cells were unable to get any Si from the medium when the Si concentration was zero, thus conditions were not suitable for regular growth, and cell densities were significantly lower than treatments where Si was available. However, when grown in NO_3^- without Si, *Coscinodiscus* sp. cell densities did not decrease over the duration of the experiment. There are two possible explanations for this result. Firstly, the cells could redirect Si usage to ensure their survival in the shortage of Si supply [21]. In addition to its requirement for frustule formation, diatoms need Si for DNA, protein and chlorophyll synthesis, and the production of thymidilate kinase and DNA-polymerase [13]. Without sufficient Si for these necessities, normal cellular function would cease. The survival strategies to limited Si supplies include changes in frustule valve morphology [22] and frustule thickness [23]. These responses require less Si for frustule formation, which leaves it available for other cellular requirements, thereby prolonging the life of the cell. In this study, however, no Si was available in the zero Si treatment. For *Coscinodiscus* sp. cells to survive in the environment without an external Si supply they might obtain Si from internal storage. Binder and Chisholm [13] reported the internal formation of Si pools in Si limited diatom populations, which could prolong the life of the cell if coupled with a slowing of the cell cycle. However, prior to exposure to the Si concentrations in this study, *Coscinodiscus* sp. cells were not Si limited, and thus any Si stored before the experiment was not stored as a survival response. The second possible explanation involves bacterial digestion of the frustule. Bidle and Azam [24] suggested that bacterially mediated Si regeneration could be achieved via the hastening of diatom frustule dissolution by marine bacteria. Thus it may be possible for diatoms to obtain a Si supply through bacterially mediated recycling in the absence of Si in the environment. A decrease in *Coscinodiscus* sp. densities was observed in all NO_3^- concentrations on the third day of the NO_3^- monoculture experiment. The dead cells resulting from this decrease might have provided a Si supply for the remaining *Coscinodiscus* sp. population if bacteria present in the non-axenic cultures were accelerating the dissolution of the unoccupied frustules. *Thalassiosira rotula* densities were also affected by changing Si concentrations in the NO_3^- medium. However, in contrast to *Coscinodiscus* sp., the effect of Si limitation was found in 0 and 142 µM Si treatments. This result may be explained by the size of *T. rotula* relative to *Coscinodis-*

cus sp. The smaller *T. rotula* may have less Si available for regeneration in the form of changing frustule morphology and thickness, thereby becoming Si deficient more quickly than *Coscinodiscus* sp.

4.2. Nutrient Competition between Two Algal Species

In the competition experiment between *Coscinodiscus* sp. and *T. rotula* for N, the large species was dominant over the small one in both N forms when nutrients were limiting. In NH_4^+ medium, *Coscinodiscus* sp. dominated by the end of most treatment combinations. This pattern of dominance showed that small diatom was out competed for nutrients in all treatment levels, except when both NH_4^+ and Si were present in maximal levels. In NO_3^- medium, *Coscinodiscus* sp. also dominated the assemblage at the end of most treatments. This indicates that small species was unable to compete with large species for NO_3^- when NO_3^- concentrations are less than 1210 μM NO_3^-.

The effects of changing N and Si on *Coscinodiscus* sp. densities, when grown in competition with *T. rotula*, were essentially the same as those observed when *Coscinodiscus* sp. was growing in monoculture. This suggests that *T. rotula* has no effect on the growth of *Coscinodiscus* sp. in the competition studies. However, the effects of changing nutrient concentrations on *T. rotula* densities, when grown in competition with *Coscinodiscus* sp., were different from the effects observed when *T. rotula* was grown in NO_3^- and NH_4^+ in monoculture. When grown with NH_4^+-N, *T. rotula* abundances were significantly affected by changes in NH_4^+ and Si concentration in the competition study. These effects were not observed when *T. rotula* was growing by itself. When grown in NH_4^+ in monoculture, *T. rotula* densities began to decrease between day 8 and day 13 of the study. When competing with *Coscinodiscus* sp. for NH_4^+, this decrease occurred more quickly, between day 3 and day 13 of the experiment. Similar results were observed when comparing monoculture and competition studies for cells growing in NO_3^--N. These findings suggest that the large species is a stronger competitor for N and can out compete the small species.

Ammonia (NH_4^+) is a positively charged or neutral (NH_3) molecule, and can therefore easily diffuse over biological membranes, making it difficult to store [9]. In NH_4^+ medium, the competitive superiority of *Coscinodiscus* sp. may be due to a higher NH_4^+ uptake rate (*i.e.* a higher affinity for NH_4^+), because assimilation of NH_4^+ is a faster process and nitrogen is more rapidly incorporated into amino acids than it is taken up at the cell surface [14]. In NO_3^- medium, dominance of *Coscinodiscus* sp was probably due to its large size, which enables to store a greater amount of NO_3^- in a larger vacuole [15]. Stored NO_3^- can be utilized when NO_3^- levels are low, which allows the cell to survive when sufficient N cannot be taken up from the medium. This advantage allowed *Coscinodiscus* sp. to more efficiently make use of the available nutrients when NO_3^- was the N source, and thus dominate the community. These results agree with previous studies, which have reported the dominance of larger diatoms when NO_3^- is the sole N source [14].

Results from the monoculture experiments showed that both *Coscinodiscus* sp. and *T. rotula* were unable to satisfy N requirements with NH_4^+ alone, and probably used whatever NH_4^+ they could take up from the medium together with stored NO_3^- to ensure N requirements were met. A higher affinity for NH_4^+ uptake, together with a large capacity for NO_3^- storage, might have provided *Coscinodiscus* sp. with a competitive advantage when NH_4^+-N was the N source in this study, by enabling the cells to more efficiently make use of total available N (*i.e.*, stored NO_3^-, and NH_4^+ in the culture medium). The disadvantaged *T. rotula*, with a smaller storage capacity were possibly unable to secure a sufficient N supply when NH_4^+-N was the N source. As stored NO_3^- was used up, *T. rotula* were unable to survive on NH_4^+ alone and cell densities decreased as the experiment progressed. This result was more pronounced in the lower Si concentrations, which may imply that cells that are unable to obtain a sufficient N supply are less competitive for low Si levels. If N supplies were insufficient, cells may prioritize their N requirements, addressing the needs of cellular functions essential to survival first. The selective loss of non-essential N compounds in response to N deficiency, including the reduction of cellular protein content has been reported in a previous study on marine diatoms [18]. This loss might include the proteins required for the completion of Si metabolism [12]. This may explain why cells that were unable to acquire an adequate supply of N might have been prevented from meeting their Si requirements via an incomplete Si transport system.

When NH_4^+-N was the N source, *T. rotula* was out competed for NH_4^+, and was therefore out competed for Si. When NO_3^--N was the N source, *T. rotula* was outcompeted for NO_3^-, and was therefore out-competed for Si. These results provide evidence for the key dilemmas outlined in the introduction. Firstly, we show that competition for Si is N source dependent, since in both NH_4^+ and NO_3^-, the species that was best able to utilize the N source dominated the assemblage. Secondly, we have demonstrated that competition for N is dependent on N source in the environment, with large species dominating in NO_3^-, and the species with the highest affinity for the nutrient dominating in NH_4^+. The larger *Coscinodiscus* sp. dominated when NO_3^--N was the N source, and the species with the highest affinity for NH_4^+, also *Coscinodiscus* sp., dominated when NH_4^+-N was the N source. However, it should be noted that the affinity of *Coscino-*

discus sp and *T. rotula* for a particular nitrogen source was not directly examined in this study. A comparison of uptake rates and K_s values for the different forms of nitrogen would provide further valuable information on the nature of competition between these two species.

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