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Neoceratium growth and survival under simulated nutrient-depleted conditions

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ABSTRACT

The dinoflagellate *Neoceratium* is common in oligotrophic regions of the oceans, where nutrient concentrations are often extremely low. Using culture isolates, we investigated whether two *Neoceratium* species (*N. hexacanthum* and *N. candelabrum*) can grow autotrophically at low nutrient concentrations found in surface waters of oligotrophic subtropical gyres. No autotrophic growth (indicated by changes in cell numbers, the presence of dividing cells, or cellular protein increase) was observed when *N. hexacanthum* and *N. candelabrum* were grown in low nutrient seawater. In separate experiments, to determine survival time under oligotrophic nutrient conditions, 68 % of *N. hexacanthum* cells were able to re-establish growth after spending 1-10 days in North Atlantic gyre seawater; 40 % recovered after 11-20 days and only 3 % recovered after 21-30 days. The longest period any single cell survived, and then went on to divide, was 26 days. These findings demonstrate that *Neoceratium* cells could remain viable for >3 weeks in surface waters of oligotrophic subtropical gyres, but to sustain growth nutrients must be obtained periodically from an alternative source: possibly via phagotrophy, vertical migration, or a combination of the two.

KEYWORDS: Dinoflagellates; *Neoceratium*; Subtropical oligotrophic gyres

INTRODUCTION

In oligotrophic subtropical gyres (OSGs), globally important ecosystems that cover over 30% of the surface of the Earth, primary productivity is limited by low standing stocks of nutrients, usually nitrogen (Moore et al., 2013). The low nutrient concentrations found here are predominantly sustained via microbial regeneration in the surface mixed layer, rather than by inputs from below the nutricline (Karl 2002). Organisms that have low sinking velocities and high nutrient uptake rates are, therefore, ideally positioned to thrive (Fuhrman et al., 1989; Zubkov et al., 2007), which explains why these environments are typically dominated by prokaryotes and small eukaryotic algae (Cho and Azam, 1988; Zubkov et al., 2000; Zubkov and Tarran, 2008; Hartmann et al., 2012).

Dinoflagellates in the genus *Neoceratium*, are commonly found, although not dominant, in OSGs (e.g. Graham 1941; Graham and Bronikovsky 1944; Weiler 1980). In the North Pacific central gyre, for example, where *Neoceratium* is estimated to contribute <1% of total phytoplankton carbon, concentrations range from 0.8 to 3 Cells L^{-1} (Weiler, 1980; Matrai, 1986). Although cell concentrations are significantly lower than in coastal waters (typically 4-14 %; Weiler, 1980; Matrai, 1986), growth rates are relatively high based on one *in situ* study in the North Pacific subtropical gyre: 26-38 % of the maximum rates observed for this genus (Weiler, 1980). This is curious, as large cells, with low surface area to volume ratios, should be at a disadvantage due to their relatively low nutrient uptake rates (Aksnes and Egge, 1991).

The presence of *Neoceratium* in OSGs may reflect an ability of cells to optimally position themselves in the water column for nutrients and light (Weiler and Karl, 1979; Heaney and Furnass, 1980; Taylor et al., 1988; Baek et al., 2009), or supplement their nutrient demand via phagotrophy (Bockstahler and Coats, 1993; Li et al., 1996; Smalley et al., 1999, 2003, 2012; Smalley and Coats, 2002). However, it is possible that *Neoceratium* do not require an alternative source of nutrients: cells may be able to survive and grow at the low nutrient concentrations typically found in OSGs. Physiological adaptations to low nutrient concentrations may allow cells to survive and grow in nutrient impoverished surface waters of OSGs: for example, low Ks values (for N and P) and specific characteristics for nutrient uptake – such as luxury consumption – have been suggested to give *Neoceratium* an advantage over other algal species growing at low nutrient concentrations (Baek, et al., 2008), and the production of the enzyme alkaline phosphatase may allow for the exploitation of dissolved organic phosphorus pools (Girault et al., 2012; Mackey et al., 2012). These adaptations may account for the previous observation of *Neoceratium fusus* growing in low nutrient culture medium (Baek et al., 2007; $NO_3^- \leq 1.0$ μM and $PO_4^{3-} \leq 0.1$ μM).

Here we use cultures of two *Neoceratium* spp. (*N. hexacanthum*, and *N. candelabrum*) commonly found in OSGs, to determine if (and for how long) cells can survive and grow at nutrient concentrations typically found in surface waters of these environments. Cell growth, under these conditions, was investigated by monitoring changes in cell numbers, dividing cells, protein per cell and *Fv/Fm* in low nutrient seawater (LNSW) compared to nutrient replete seawater (RSW). Changes in other cell growth properties (survival time, viability, time-lag of growth response and growth rate) were determined by monitoring cells in embryo dishes, exposed to North Atlantic gyre seawater (NAGSW) for varying periods of time before addition of nutrients.

METHOD

Maintenance of *Neoceratium* cultures

Neoceratium strains (*N. hexacanthum*, strain number P10B2; *N. candelabrum*, strain number P37C2) were obtained from the Gulf of Villefranche (Point B, 0-80 m), in The Mediterranean Sea between 2007 and 2008. They were cultured in K/5 medium (Keller et al., 1987), minus silicate, made from NAGSW (Table I) – collected from the surface of the central gyre in October 2009 during Atlantic Meridional Transect (AMT) 19, onboard the Royal Research Ship (R.R.S.) James Cook. All cultures were maintained in 100 ml borosilicate Erlenmeyer flasks and kept at 18^{o}C in a temperature controlled incubator with a 12:12 h light/dark photocycle, with a photon flux density of 60 μmol quanta m^{-2} s^{-1}.

Isolation into experimental flasks

Prior to all experiments, *Neoceratium* were isolated from their culture medium into experimental medium using the following method. The concentration of cells in the stock culture (late exponential/stationary growth phase) was determined in order to calculate the volume required to achieve desired experimental concentrations (~10-30 cells ml^{-1}). Prior to transference to experimental Erlenmeyer flasks (acid washed with HCL and autoclaved), this predetermined volume of stock culture was collected on a 20 μm filter and rinsed with filtered NAGSW, using a pipette, in order to remove excess nutrients. Cells were rinsed from the filter into one of the experimental flasks, and then evenly divided (by volume) between all the flasks. All experiments were performed in triplicate.

Experiments were conducted in 100 ml Erlenmeyer flasks, using 50 ml of LNSW or RSW (Table I). Experiments were incubated at 18^{o}C on a 12:12 h light/dark cycle at a photon flux density of 60 μmol quanta m^{-2} s^{-1}. Every four days (starting on day one) sub-samples from each experimental treatment were taken, from which: cell numbers, number of dividing cells, estimated protein per cell, photosynthetic efficiency of photosystem II (PSII; *Fv/Fm*), and dissolved inorganic nutrient concentrations (NO_3^- + NO_2^-, and PO_4^{3-}; only for *N. candelabrum*) were determined.

Cell numbers

Cell numbers were determined via enumeration of 1 ml of experimental medium in a 1 ml plastic Sedgewick-Rafter (S-R) chamber, under an inverted microscope. In addition to cell numbers, observations on motility were made and the number of dividing cells was recorded.

Protein content of cells

1.5 ml of experimental medium was frozen (-80^{o}C) in 1.5 ml cryoviles for analysis at a later date. The average protein content of *Neoceratium* cells was measured using the bicinchoninic acid (BCA) method (Smith et al., 1985), using bovine serum albumin (fraction 5) as a standard. BCA reagents

were added in an 8:1 ratio (reagents: sample), and samples were left at room temperature for 24 hrs, prior to analysis on a Nanodrop 1000 Spectrophotometer. Data values more than two standard deviations from the mean were omitted from the final analysis (*N. hexacanthum*: 1 of 54 samples; *N. candelabrum*: 2 of 54 samples).

Fast repetition rate fluorometry (FRRf)

Fast repetition rate fluorometry (FRRf) was used to assess the maximum PSII photochemical efficiency (F_v/F_m), which typically decreases under periods of 'stressful' growth such as nutrient starvation (Kolber et al. 1988; Kolber and Falkowski 1993). 2 ml of each experimental medium was transferred to a 15 ml darkened Falcon tube for at least 1 hr prior to FRRf analysis, allowing the primary receptor molecules of PSII (quinones) to be fully oxidised and ready to receive electrons (Suggett et al., 2009). FRRf measurements were made using the 'standard' protocols described by Suggett et al. (2003) and Moore et al. (2005). A Chelsea Instruments Fast^{tracka} FRR fluorometer was programmed to deliver single photochemical turnover saturation of PSII from 100 flashlets of 1.1 μs at 1 μs intervals. Fluorescence transients generated were then fitted to the Kolber-Prasil-Falkowski (KPF; Kolber et al. 1998) model to yield values of F_v/F_m (see Moore et al. 2006).

Nutrient measurements

Dissolved inorganic nutrients (nitrate + nitrite and phosphate) were analysed from subsamples taken from LNSW experimental flasks (*N. candelabrum* experiments only). 10 ml subsamples were filtered through 0.2 μm Millipore filters into 15 ml Falcon tubes; subsamples were the result of combining 3.5 ml subsamples from triplicate experimental flasks – this was done in order to provide sufficient volume for analysis. Samples were stored at -80^{o}C prior to analysis within 1 month of collection. Nitrate and phosphate measurements were analysed using a Skalar SanPlus Autoanalyser according to Sanders and Jickells (2000).

Changes in cell growth properties over 30 days of exposure to NAGSW

N. hexacanthum cells (exponential growth phase) were reverse filtered in order to remove excess nutrients from the culture medium. Following this, approximately 10 cells (average: 9; range: 5-14) were transferred to 30 embryo dishes containing 2.5 ml of NAGSW, and covered with a glass lid to prevent evaporation (the mass of each dish was monitored to ensure no evaporation occurred). Exact cell numbers were confirmed, and every day for 30 days (starting on day 1) nutrients were added (0.5 ml of K-medium) to a single dish. Following nutrient addition cell numbers and dividing cells were monitored every 2 days, allowing for the estimation of the following:

Maximum viability time: The longest period that any single cell survived and went on to divide.

Changes in growth rate: Growth rate was estimated by fitting an exponential growth model [Stirling Model; *f=y0+a(exp(b*x)-1)/b*] to the data and then using the slope of the line to estimate growth rate in divisions per day (d^{-1}). Several different exponential models were tested for how well they fitted the data (in SigmaPlot). The Sterling model was used as it had the highest R^2 values.

Change in the percentage of viable cells (cells capable of growth): For each embryo dish, on the day of nutrient addition, we attempted to calculate the number of viable cells present that contributed to the increase in cell numbers. To do this we removed the first two data points from each of the growth curves and then fitted the Stirling growth model (discussed above) to the data in order to estimate how many cells were viable on day 1 (y=0). For dishes where no growth was observed, the number of viable cells was taken to be zero. By combining data together into three groups (day 1-10, day 11-20 and day 21-30), and comparing the number of viable cells from each group to the total number of cells upon nutrient addition, it was possible to estimate the percentage of viable cells during each of these three periods.

Changes in time-lag of growth response: The growth time-lag for each dish was estimated from the number of days it took, after nutrient addition, for the first sign of growth to occur: either an increase in cell number, or the presence of dividing cells.

RESULTS

Growth at nutrient concentrations typical of OSGs – *N. hexacanthum*

In experiments on *N. hexacanthum*, in LNSW, cell numbers showed little variation over 21 days (Fig 1A: range, 17.7 to 20 cells ml^{-1}). Cell numbers increased in RSW up until day 13, from 28 to 49 cells ml^{-1} respectively; after this point cell numbers decreased from 49 to 33. The percentage of dividing cells observed broadly mirrored the above pattern (Fig. 1C): no dividing cells were observed in LNSW; in RSW dividing cells increased from 0 to 4.8 % between day 1 and 9, before decreasing to 0.69% by day 17. Motile cells were observed throughout the 21 day experiment in RSW, but no motile cells were observed after day 17 in LNSW.

Average protein content per cell (Fig 1E) remained stable in LNSW between day 1 and 13 (8.4 to 9.1 ng $cell^{-1}$), before decreasing to 4.3 ng $cell^{-1}$ by 21. In RSW average protein per cell fluctuated between 6.9 and 11.3 ng $cell^{-1}$, but was similar on day 1 and day 21 (8.7 and 9.9 ng $cell^{-1}$ respectively).

After remaining fairly constant, from day 1 to 5, *Fv/Fm* decreased under both nutrient scenarios until day 9 (Fig. 1G); this decrease was greatest in LNSW (-0.073) and least in RSW (-0.039). From day 9 to day 21 *Fv/Fm* decreased in RSW (from 0.265 to 0.235), and increased in LNSW (from 0.221 to 0.245).

Growth at nutrient concentrations typical of OSGs – *N. candelabrum*

In experiments on *N. candelabrum*, in LNSW, cell numbers (Fig. 1B) steadily decreased over 21 days, from 21 to 15 cells ml^{-1}, whereas cell numbers increased in RSW over the course of the whole 21 day period, from 16 to 156 cells ml^{-1}. The percentage of dividing cells (Fig. 1D) mirrored the above pattern: the percentage of dividing cells on day 1 was roughly similar across both scenarios (9 and 12 %). Beyond day 5 no dividing cells were observed in LNSW; in RSW there was an increase in dividing cells, between day 1 and 5, from 12-19 %, followed by a decrease to 2 % by day 21. Motile cells were observed throughout the experiment under both nutrient scenarios.

Apart from one low protein measurement on day 5 for LNSW (3.2 ng $cell^{-1}$), the average protein content per cell (Fig 1F) remained relatively stable in both nutrient scenarios between day 1 and 13 (4.4 to 6.35 ng $cell^{-1}$). This was followed by a decrease down to 2.7 (LNSW) and 4.0 ng $cell^{-1}$ (RSW) by day 21.

Fv/Fm values (Fig. 1H) closely mirrored changes in cell numbers (Fig. 1B). In LNSW values stayed relatively constant up until day 9 (range, 0.089 to 0.116); this was followed by a decrease, to 0.014, by day 21. In RSW there was a rapid increase between day 1 and 9 (from 0.107 to 0.325), followed by a gradual increase until day 17 (to 0.394), before falling to 0.319 by day 21

In LNSW PO_4^{3-} concentrations remained between the detection limit of <0.2 μM and 0.4 μM. NO_3^- concentrations ranged between 0.41 μM and 0.86 μM.

Changes in cell growth properties over 30 days of exposure to NAGSW

In almost all embryo dishes cell numbers either remained the same or decreased before nutrients were added (Fig. 2). The longest time any single cell survived in NAGSW, and then went on to re-grow after addition of nutrients, was 26 days (Fig. 2). Motile cells were observed after 28 days, but none of these cells were able to re-establish growth when nutrients were added.

The percentage of viable cells decreased over the course of the experiment (Fig. 3A): between 1-10 days, the percentage of viable cells was 68 % (71/105 cells); this dropped to 40 % between day 11-20 (44/110 cells), and 3 % (2/64 cells) between day 21-30.

The time-lag between nutrient addition and the first sign of growth appearing generally increased over the course of the experiment (Fig. 3B): the time-lag was 2 days up until day 10; with

the exception of day 13, from day 11 onwards the time-lag was always greater than 2 days (between 4-8 days).

Once growth did commence, the growth rate of cells appeared to be unaffected by the length of exposure to NAGSW (Fig. 3C). The mean growth rate (once time-lag had been accounted for) observed was 0.16 d^{-1} (range: 0.07-0.31 d^{-1}), and for most embryo dishes observed (12 out of 17) the growth rate was within a fairly narrow range of 0.11-0.19 d^{-1}. Although the lowest growth rate (0.07 d^{-1}) was observed on day 26, the growth rate just one day before (day 25) was close to the mean growth rate observed (0.15 d^{-1}).

DISCUSSION

Our observations, on *N. hexacanthum* and *N. candelabrum*, revealed that ontogenetic growth (cell division) did not occur at the low nutrient concentrations used here (starting concentrations: NO_3^-, 0.58 μM; PO_4^{3-}, 0.04 μM), and cells became more nutrient stressed with time, as evidenced by decreasing *Fv/Fm* values. We can eliminate the possibility that cells were slowly increasing in size (somatic growth), and therefore potentially dividing on timescales longer than the experimental period (21 days), as average protein content per cell roughly halved over the course of the experiment in both species. This suggests that *Neoceratium* could not survive in OSGs if they were entirely dependent on the nutrient concentrations found in surface waters (above the nutricline), much less achieve the high growth rates (0.09-0.13 d^{-1}) that have been previously observed in the North Pacific subtropical gyre (Weiler, 1980). We cannot rule out the possibility that *Neoceratium* exploit nutrient micropatches in OSG surface waters (Lehman and Scavia, 1982). However, given the theoretical low nutrient uptake rates of *Neoceratium* spp., compared to other smaller species of phytoplankton found in OSGs (Aksnes and Egge, 1991), this strategy would seem to be ineffective as nutrients would likely be used up by other phytoplankton before *Neoceratium* could exploit them. Increases in swimming speed, sinking rate and turbulent shear can all reduce nutrient diffusion limitation in larger cells to some extent (Chisholm, 1992). In our experimental set-up using culture flasks, sinking rate and turbulent shear would have been negligible compared to the natural environment. However, it has been pointed out that these two factors could, at most, scarcely double nutrient uptake rates in a cell with a diameter of 200 μm (Chisholm, 1992). Therefore, it seems unlikely that this factor would account for growth of *Neoceratium* in OSG surface waters.

Results from experiments on *N. hexacanthum* in embryo dishes containing NAGSW, where growth did not occur until the addition of nutrients, support our conclusion that cell growth is not possible in OSGs. These experiments, however, did demonstrate that cells can potentially survive in oligotrophic surface waters, and remain viable, for a long period of time (>3 weeks; Fig. 3). This

survival time is consistent with the measurements of *Fv/Fm* on *N. candelabrum*, which showed that after 21 days in LNSW the value for this parameter had fallen to almost zero, indicating severe nutrient stress. Few other studies have focussed on the long term survival of phytoplankton under oligotrophic conditions. However, one study on the dinoflagellate *Gymnodinium splendens* demonstrated that this species could survive for 65 days under oligotrophic conditions (Dodson and Thomas, 1977) – nearly three times longer than *N. hexacanthum* in the present study. In addition to inter-specific nutrient tolerances between *Gymnodinium* and *Neoceratium*, this significantly longer survival time is probably the result of the higher nutrient concentrations that were used in that study (NO_3^-, 0.08 μM; PO_4^{3-}, 0.46 μM) compared to our own (NO_3^-, <0.02 μM; PO_4^{3-}, <0.02 μM). It is likely that this ability to survive long periods of time in the absence of sufficient nutrients may partly explain the success of *Neoceratium* in oligotrophic regions, and also, their success in stratified coastal regions during summer.

Due to the sampling resolution (2 day intervals), time-lags in growth response, upon nutrient addition, could only be resolved within 2 day intervals. Even so, a clear pattern emerged. The time-lag in growth response increased beyond 10 days of exposure to NAGSW. This increase in time-lag is consistent with studies that have found the delay in growth response to be positively related to duration of nutrient 'starvation', especially in species that are capable of storing nutrients internally – a strategy that is proposed to be ecologically advantageous in environments where nutrients are only encountered periodically (see review by Collos, 1986). Once cells began to grow, however, there did not appear to be a detrimental impact on growth rates. This suggests that the adverse effects of prolonged exposure to surface oligotrophic conditions, of greater than 10 days, do not persist once cells overcome the initial delay in growth.

There are several important implications of these findings. *Neoceratium* cells must periodically access an external source of nutrients in order to survive and grow in surface waters of OSGs. In order to maintain a healthy population in a steady state of growth this nutrient source would ideally need to be accessed, at most, every 10 days, but could be accessed over a longer timescale (10-20 days) whilst still allowing for a relatively high chance (~40%) of survival. Under extreme circumstances, a small number of cells (~3%) could survive nutrient starvation for longer than 20 days, enabling a 'seed population' to survive. In the long-term, however, this strategy would not be ecologically successful as cell losses would be too high to maintain a population. It is for this reason that we rule out the possibility that *Neoceratium* may be solely reliant on ephemeral processes that can introduce nutrients into surface waters, such as internal and tropical instability waves (Gregg et al., 2003; Jochum et al., 2004), and mesoscale processes (Falkowski et al., 1991; Karl, 1999; Gonzalez et al., 2001). The increase in nutrient concentrations associated with these events would appear to be great enough to support the growth of *Neoceratium* (McGillicuddy et al.,

1998; Baek et al., 2007), but their occurrence is likely to be too infrequent to solely account for the success of *Neoceratium* in OSGs. Therefore, we foresee that *Neoceratium* must, additionally, use at least one of the following strategies.

The first strategy may be to obtain nutrients via phagotrophy: a tactic that is thought to be widespread amongst small eukaryotic algae (<5µm) in the OSGs of the Atlantic Ocean (Zubkov and Tarran 2008; Hartmann et al. 2011, 2012), and one thought to be commonly used by a number of *Neoceratium* species, which commonly feed on ciliate prey (e.g. Bockstahler and Coats, 1993a; Li et al., 1996; Smalley et al., 1999, 2003; Smalley and Coats, 2002). Some important questions here would be: how much prey would *Neoceratium* need to ingest in order to obtain sufficient nutrients for survival and growth, and whether encounter rates with prey species are high enough to support this method of nutrient acquisition. It is possible, by making a few assumptions to calculate approximately how much prey (in the form of ciliates) would be required for survival and growth. Our measurements of intracellular protein show that, in *N. hexacanthum* exposed to LNSW, protein concentrations decrease by 5 ng (±2 ng) over 21 days. Therefore, to maintain their cells, *Neoceratium* need approximately 0.14-0.33 ng day^{-1} of protein to supplement their nitrogen demands. Assuming a nitrogen assimilation efficiency of 75 % for type II mixotrophs feeding on microzooplankton (Stickney et al., 2000), *Neoceratium* would need to ingest 0.19-0.44 ng of protein per day. Using previously published protein contents of a number of ciliate species (1-15 ng; (Zubkov and Sleigh, 1996; Zubkov and Sleigh, 2000), it would appear that an ingestion rate of 0.01-0.44 ciliates day^{-1} would be required to survive in OSGs. For *Neoceratium* to divide at the rate observed by Weiler (1980) (0.09-0.16 d^{-1}), adding the further assumption that the complete asexual division process requires the ultimate doubling of cellular protein concentrations, we calculate that *Neoceratium* would need to consume 0.9-1.8 ng protein d^{-1}, or 0.06-1.8 ciliates d^{-1}. Is this ingestion rate possible, based on the number of predator/prey collisions that may occur in the gyres? Although there are many considerations to take into account when considering predator/prey collision rates, it is possible to make a conservative estimation based on likely predator and prey cell densities, swimming speeds and feeding behaviour (Kiørboe, 2008). Using general predator/prey encounter equations discussed by Kiørboe (2008), we conservatively estimate that encounters between a single *Neoceratium* cell and its ciliate prey are at least 60 d^{-1}. Therefore, under the worst case scenario, only 0.1 to 3 % out of 60 encounters each day would need to be successful in order for *Neoceratium* to achieve the growth rates observed by Weiler (1980), and only 0.02 to 0.7 % of these encounters would need to be successful for *Neoceratium* to survive in OSGs.

The second strategy may involve vertical migration to the nutricline. Villareal and Lipschultz (1995) have previously suggested that all large phytoplankton (>100 µm) in the Sargasso

Sea are capable of vertical migration, and that they use this mechanism to acquire nutrients from the nutricline. A number of studies indicate that *Neoceratium* undergo cell division at depth around dawn (Weiler and Chisholm, 1976; Weiler and Eppley, 1979; Baek et al., 2009), followed by upward migration before the transition from dark to light (with downward migration occurring before dusk). However, this strategy would seem to be ill-suited for survival in OSGs, where the nutricline is often many tens of metres deeper than the euphotic zone and, therefore, inaccessible in a single diurnal migration. Therefore, it is possible that migrations take place over periods greater than a single day – a phenomenon that has been observed in the dinoflagellates *Pyrocystis noctiluca* and *P. fusiformis* (Rivkin et al., 1984). Unfortunately, the proximate triggers of this behaviour are not well understood and need to be elucidated.

CONCLUSION

Our study demonstrates that *Neoeceratium* cells are unable to grow at low nutrient concentrations representative of surface waters in OSGs. Nevertheless, cells can survive and remain viable for over 3 weeks, suggesting that the presence of *Neoceratium* in OSGs may be explained by them enduring long periods in surface waters above the nutricline, with periodic exploitation of alternative nutrient sources. Mixotrophy and/or vertical migration appear to be the most feasible mechanisms by which nutrients may be obtained. Knowing the relative contribution of these two strategies to *Neoceratium* nutrient acquisition is necessary not just from the point of view of obtaining a more complete understanding of *Neoceratium* ecology, but also for gaining a better appreciation of how microbial food webs function within OSGs.

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