Species- and stratification-dependent diel vertical migration behaviour of three dinoflagellate species in a laboratory study

THERESE JEPHSON* AND PER CARLSSON
DEPARTMENT OF ECOLOGY, LIMNOLOGY AND MARINE ECOLOGY, ECOLOGY BUILDING, LUND UNIVERSITY, LUND, SWEDEN

CORRESPONDING AUTHOR: therese.jephson@limnol.lu.se

This study shows that different species of the same genus can behave differently in relation to a salinity gradient and suggests that a halocline can act as a barrier to the diel vertical migration (DVM) of dinoflagellate species. In a laboratory study, we found that the dinoflagellates Ceratium furca, C. tripos and Dinophysis acuta did not cross a salinity gradient of 5 psu, but instead exhibited DVM exclusively below and above the halocline. Furthermore, C. tripos aggregated in the halocline during the night and migrated to the surface at midday, whereas C. furca aggregated in the halocline at midday and migrated downwards to the bottom during the dark period. Dinophysis acuta was found exclusively above the halocline, with the highest density in the surface water at midday. The present results suggest that different dinoflagellate species display unique DVM behaviours in stratified waters. The species-specific DVM strategies revealed in this study are of central importance for understanding the ecology of these taxa and to further elucidate both the ecological significance and the mechanisms of DVM.

INTRODUCTION

Models are now being developed to predict algal blooms, and accordingly much interest is being focused on identifying the mode of nutrition of phytoplankton. Therefore, it is essential to correctly relate the growth of these organisms to the nutrient supply. Studies have demonstrated the importance of diel vertical migration (DVM) for nutrient uptake (Eppley et al., 1968; Cullen and Horrigan, 1981), and it is believed that migration to the nutrient rich layer may be one of the mechanisms that can promote dinoflagellate blooms in nutrient-depleted surface layers. It is assumed that vertical movements of dinoflagellates are due to positive phototactic and geotactic responses (Forward, 1976; Blasco, 1978), since dinoflagellates usually swim upward in the morning and downward in the evening. Furthermore, there is evidence that the downward movement may be triggered by inorganic nutrient depletion in the surface water (Heaney and Eppley, 1981; Flynn and Fasham, 2002). Phytoplankton may also aggregate at a certain depth for sexual reproduction (Persson et al., 2008) or as a result of differential grazing (Cullen and Horrigan, 1981, and references therein). Moreover, it seems that accumulation of plankton cells in thin layers is induced by higher concentrations or strong gradients of factors such as salinity, nutrients and oxygen (Derenbach et al., 1978). These layers are easily eroded by turbulence, but such forces act primarily above the pycnocline, and hence it appears that the layers are strongly associated with parts of the pycnocline where there is little turbulence (MacManus et al., 2003). It is not clear whether such accumulations of plankton at certain depths are attributable to passive or active processes, although the phenomenon is well known (e. g. GEOHAB, 2008).

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In the present study, we focused on light and nutrient availability related to DVM in stratified waters, because in summer the concentrations of inorganic nutrients in surface water are often very low compared with the levels found in the water mass below the pycnocline. However, light intensity is usually minimal in the nutrient-rich water below the pycnocline. Photosynthetic dinoflagellates can achieve the best possible conditions by staying close to the surface during the light period and migrating through the pycnocline and assimilating nutrients in the deep water at night (Kimura et al., 1999; Flynn and Fasham, 2002). Thus, it seems that DVM is a favourable strategy for dinoflagellates. However, a migratory behaviour entails necessary adjustments to new salinities or temperatures, which may be connected with increased energy costs (Erga et al., 2003). The situation is even more complex for mixotrophic species, for which there is an additional potential trade-off between feeding on prey and photosynthesizing (Jones, 2000). It is possible that mixotrophic dinoflagellates aggregate at the pycnocline so that they can exploit the optimal combination of prey and nutrient and light conditions available in a stable water column.

A marked pycnocline generally exists in many estuaries and in the Baltic Sea (Meier, 2007; Schulz and Hirche, 2007). The bottom current in the nutrient-rich North Sea transports water primarily into the Baltic Sea, whereas the surface water in the Baltic is moved northward. Due to this outflow of brackish water, a nearly permanent halocline exists along the Swedish west coast, and the temperature gradient is often, although not always, associated with the halocline (SMHI, 1993–2006).

Dinoflagellates belonging to the genus Ceratium are common in temperate waters, especially in late summer and autumn (Smetacek, 1981; Jansen et al., 2006). Dinoflagellate blooms occur regularly around the Swedish coast, and recurrent blooms of Ceratium and Dinophysis spp. have been reported (Nielsen, 1991; Carstensen et al., 2004). In Danish coastal waters, Ceratium spp. can constitute up to 90% of the protist biomass (Hansen and Larsen, 1992). The genus Dinophysis includes some toxin-producing species and D. acuta and D. acuminata have been reported to cause most of the toxicity ascribable to DSP (Diarrheic Shellfish Poisoning) along the Swedish west coast (Edler and Hagletorn, 1989; Godhe et al., 2002).

Vertical migration has been observed in Ceratium and Dinophysis spp. (e.g. Olsson and Granéli, 1991; Villarino et al., 1995), but the relationship between salinity gradients and DVM in this behaviour has not been fully evaluated. Therefore, we examined the response of three species of Ceratium and Dinophysis in a natural plankton community in the presence of a 5-psu halocline under controlled laboratory conditions. We hypothesized that the dinoflagellate cells would display observable diurnal migration, that the halocline would act as a barrier and that each species would choose its DVM strategy in response to the halocline.

**METHOD**

We performed a laboratory experiment to study DVM exhibited by the three dinoflagellate species Ceratium tripos, C. furca and D. acuta in natural phytoplankton communities in four replicate water columns created in PVC cylinders (height 2 m). Ten samples were taken from each cylinder at different depths every third to fifth hour over a period of 48 h.

**Experimental setup**

*Ceratium tripos*, *C. furca* and *D. acuta* were collected in waters off the Swedish west coast (N 56° 55.817′, E 12° 20.195′) on 25 October 2006, 2 days before the experiment was started. Several vertical net hauls (25 µm mesh size) were taken from a depth of 15 m to the surface, and the plankton concentrated in the net were sieved through a 100-µm mesh net and then saved in two 25-L vessels containing 100-µm-filtered surface water. The surface water at the sampling site was 12°C and received irradiance of ~500 µmol quanta m⁻² s⁻¹. Within 3 h of plankton collection, the 25-L containers were transported to the laboratory and *C. tripos*, *C. furca* and *D. acuta* cells were concentrated by reverse filtration using a 25-µm mesh net. The cells were then incubated overnight in the two 25-L containers at 12°C, during which time dead and non-motile cells accumulated on the bottom. The next day, the upper water containing live dinoflagellates was siphoned off into two other 25-L containers, and the density of the different dinoflagellates was determined by counting the cells using 10 mL sedimentation chambers and an inverted microscope (Olympus CKX 41). The densities of the three species in the two containers were found to be as follows (mean ± SD): 38 ± 6 and 19 ± 2 *C. tripos*, 78 ± 12 and 34 ± 6 *C. furca* and 77 ± 17 and 19 ± 3 *D. acuta* cells mL⁻¹.

The experiment was carried out in artificial stratified water columns created in four PVC cylinders (height 2 m, diameter 0.4 m) using 1-µm filtered seawater. During the experiment, the salinity in the surface water varied between 17.3 and 18.6 psu and the bottom water between 21.5 and 22.5 psu (Fig. 1), and the halocline was located in the middle of the cylinders at a
depth of 1.05 m. The temperature was set at 12°C. Considering the presence of nutrients, the bottom water was enriched (approximately 1 μM phosphate and 16 μM nitrate), whereas the surface water was not (approximately 0.3 μM phosphate and 2 μM nitrate).

Daylight lamps (Philips TL-D 36W/840) were positioned 0.2 m above the cylinders. The vertical light extinction in the water columns was measured using an LI-192SA underwater quantum sensor. The lights were turned on at 08:00 and off at 18:00 to simulate natural daylight and darkness. Light intensity varied between 2.7 and 3.2 μmol quanta m⁻² s⁻¹ in the halocline (Fig. 1), which means that the levels of light in the halocline were similar to the natural light conditions at midday in the pycnocline (10–12 m depth) on the Swedish west coast at the end of October (P. Carlsson, unpublished results).

Small-bore silicone tubing was used to carefully siphon off equal volumes of water (each containing about 2.5 million dinoflagellate cells) from the 25-L containers into the surface water in each PVC cylinder. The dinoflagellates were then incubated in the cylinders for 24 h before sampling was begun, and during that time dead or non-motile cells accumulated at the bottom. The equipment used to collect samples from the water in the cylinders at intervals of 0.05 or 0.1 m consisted of horizontally mounted plastic tubes that reached into the centre of each cylinder and had external valves to which a syringe could be connected. Samples (11 mL) for the determination of cell density were taken at 10 different vertical positions between 0.05 m from the surface and 0.2 m above the bottom, and this was done over a period of 48 h at 05:00, 08:00, 12:00, 17:00, 21:00 and 24:00 h. Samples were preserved with Lugol’s solution and counted using 10 mL sedimentation chambers and an inverted microscope (Olympus CKX 41). Additional water samples (50 mL) for salinity gradient measurements were taken at each depth at the beginning and the end of the 48-h experiment. Surface and bottom water samples (50 mL) for inorganic nutrient analysis were taken initially and at the end of the experiment. Salinity was measured using a Crison Basic 30 conductometer, and nutrient analysis was performed using a Technicon TRAACS 800 Autoanalyzer with detection limits of 0.1, 0.02, 0.05 and 0.02 μM for NH₄⁺, NO₃⁻/NO₂⁻, Si and PO₄³⁻, respectively.

Growth rate analyses

Photosynthetic carbon uptake at different light levels was measured in C. tripos and C. furca obtained in waters off the Swedish west coast. Unfortunately, the number of D. acuta cells collected was not sufficient for the analysis. The dinoflagellates were taken in plankton hauls (25 μm mesh size net) from a depth of 15 m to the surface. The salinity of the water at the collection site was 22 psu. Within 3 h of collection, the dinoflagellate concentrate was diluted to 25 L with the same seawater (filtered through the 25-mesh net) and was transported to the laboratory, where the cells were incubated with H¹⁴CO₃⁻ (0.5 μCi mL⁻¹) in 70-mL square Nalgene polycarbonate flasks filled with 70 mL of seawater. The flasks were positioned at six different depths in one of the cylinders used in the DVM experiment. After 5 h of incubation, the flasks were placed in darkness on ice pending further processing.

For analysis, the dinoflagellate cells were collected on a 25-μm nylon mesh and washed with 2 L of GFF-filtered seawater with a salinity of 22 psu to remove ¹⁴C-isotope that had not been assimilated. Thereafter, 20–50 mL of filtered seawater was used to back-flush the cells from the filter into a 100-mL glass jar. Subsequently, an inverted microscope and micro-capillaries were used to manually isolate 30 C. tripos and 30 C. furca cells from each sample in triplicate. These dinoflagellates were then washed four times by placing them in drops of filtered seawater on Sedgewick-Rafter slides to remove any contaminating cells, after which

Fig. 1. The salinity and light profile in the artificial water columns throughout the 48-h experiment. The lights were turned on at 08:00 and off at 18:00 to simulate natural light-dark conditions.
they were transferred to scintillation vials. Scintillation cocktail (Quicksafe) was added and radioactivity was measured as disintegrations per minute (DPM) using a multipurpose scintillation counter (Beckman LS 6500). For each depth, the background radioactivity of the rinsing water from the last wash of the cells from each triplicate sample was checked using a volume of water corresponding to the volume taken to isolate 30 cells. Carbon uptake (pg C cell\(^{-1}\) h\(^{-1}\)) was calculated as

\[
\frac{(\text{DPM}_{\text{cell}} - \text{DPM}_{\text{control}}) \times \Sigma \text{CO}_2 \times V \times k_1 \times k_2 \times 10^9}{\text{DPM}_{\text{added}} \times T}
\]

where DPM\(_{\text{cell}}\) denotes the DPM in the sample divided by the number of cells in the sample, DPM\(_{\text{control}}\) stands for the DPM in the filtered water corresponding to the water volume taken to isolate 30 cells divided by 30, \([\text{CO}_2]\) is the sum of dissolved inorganic carbon in the incubation bottle (mg L\(^{-1}\)), \(V\) is the incubation volume (L), \(k_1\) is the correction for isotope discrimination (=1.05), \(k_2\) is the correction factor for respiration of organic material during incubation (=1.06) (Ærtebjerg-Nielsen and Bresta, 1984), DPM\(_{\text{added}}\) is the DPM added to each incubation bottle and \(T\) is the incubation time (h). The highest activity was found to be 217.2 ± 13.6 DPM for \(C.\) tripos and 94.6 ± 5.3 DPM for \(C.\) furca. The background activity of the last washed control was 34.7 ± 3.4 DPM.

Species-specific growth (µ day\(^{-1}\)) based on photosynthesis was estimated as \(\mu = \ln(C + (\Delta C_h \times 12)) - \ln(C)\), where \(C\) is the carbon content per cell (9671 and 5510 pg C cell\(^{-1}\) for \(C.\) tripos and \(C.\) furca, respectively). The \(C\) value was estimated using a linear equation: \(C_{\text{cell}}\) (pg C cell\(^{-1}\)) = log \(a + b \times \log V_{\text{cell}}\), where \(a\) and \(b\) are constants (=0.119 and 0.819, respectively), and \(V_{\text{cell}}\) is the mean cell volume (102 700 and 51 667 µm\(^3\) for \(C.\) tripos and \(C.\) furca, respectively; Menden-Deuer and Lessard, 2000). Then \(\Delta C_h\) is the carbon uptake (pg C cell\(^{-1}\) h\(^{-1}\)), assuming that there is a 12-h light period (Gisselsson et al., 2002) and that one doubling of the carbon content results in cell division.

### Statistical analyses

To demonstrate that the migration pattern was the same in the four replicates despite minor differences in dinoflagellate density, the figures illustrate the densities at every sampling depth instead of mean values. Disparities in cell densities above, within and below the halocline were investigated by applying Mann–Whitney U-tests with a Bonferroni correction, reducing the critical value of alpha to 0.016. The analysed samples were designated “above” (taken from the surface to a depth of 0.95 m and pooled), “within” (one sample obtained in the pycnocline at a depth of 1.05 m) and “below” (collected from a depth of 1.2 m to the bottom and pooled). However, the \(C.\) furca samples taken at 24:00 h at depths of 1.2 and 1.4 m contained very few cells and hence were excluded from the analysis, and the samples from a depth of 1.6 m to the bottom were pooled and considered to represent the category “below”.

Repeated measures ANOVA was applied to test for differences in growth rate between species, using growth rates at different light intensities as within-subject variables and species as between-subject factors. Independent t-test was employed to investigate differences in surface and bottom water concentrations of \(\text{NH}_4^+\), \(\text{NO}_3^-\) and \(\text{PO}_4^{3-}\) in the cylinders between the start and the end of the experiment. Normality and homogeneity of data were analysed by the Kolmogrov–Smirnov test and Levene’s test, respectively. All analyses were performed in SPSS 14.0 for Windows.

### RESULTS

#### Experimental conditions

Surface concentrations of inorganic nutrients (\(\text{NH}_4^+, \text{NO}_3^-\) and \(\text{PO}_4^{3-}\)) did not decrease significantly from the start to the end of the experiment (Table I, independent \(t\)-test: \(\text{NH}_4^+, P = 0.57; \text{NO}_3^-, P = 1.00; \text{PO}_4^{3-}, P = 0.23\). However, in the nutrient-rich bottom water, it seemed that levels of \(\text{NH}_4^+\) and \(\text{NO}_3^-\) did decline at the end of the experiment (Table I, independent \(t\)-test: \(\text{NH}_4^+, P = 0.08; \text{NO}_3^-, P = 0.10; \text{PO}_4^{3-}, P = 0.19\).

#### DVM patterns

DVM was exhibited exclusively above and exclusively below a salinity gradient of 5 psu by \(C.\) tripos and \(C.\) furca, respectively (Figs 2 and 3). Dinophysis acuta showed no clear DVM behaviour and instead

**Table I: Surface and bottom concentrations of inorganic nutrients (\(\text{NH}_4^+, \text{NO}_3^-\) and \(\text{PO}_4^{3-}\)) at the start and the end (after 48 h) of the experiments**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Surface</th>
<th>Start</th>
<th>End</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{NH}_4^+)</td>
<td>1.5 ± 0.3</td>
<td>1.3 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>(\text{NO}_3^-)</td>
<td>1.5 ± 0.3</td>
<td>1.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>(\text{PO}_4^{3-})</td>
<td>12.8 ± 2.4</td>
<td>8.4 ± 3.9</td>
<td></td>
</tr>
</tbody>
</table>

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aggregated above the halocline in the area closest to the surface throughout the experiment. *Ceratium tripos* accumulated within the halocline at 17:00, 21:00, 24:00 and 05:00 (Fig. 2), and significantly more cells of this species were found there compared to above and below the halocline (Table II, Mann–Whitney $P < 0.016$). At 08:00, cell numbers were clearly lower in the halocline and simultaneously increased above this layer. At 08:00 and 12:00, no cells were found below the halocline, and there was no significant difference in cell density above and within the layer (Fig. 2; Table II). The distribution maximum of *C. tripos* cells was observed deeper in the water column, but still above the halocline, at 17:00 compared to 12:00, indicating that the cells had started their downwards migration before the lights were switched off.

For *C. furca*, DVM occurred solely below the halocline, and significantly fewer cells were found above this layer throughout the experiment (Fig. 3; Table II, Mann–Whitney, $P < 0.016$). This species clearly
aggregated at the deepest position at 24:00 and 05:00, and significantly more such cells were found at that depth compared to within and above the halocline (Table II, Mann–Whitney $P < 0.016$). At 17:00, 21:00 and 08:00, $C. furca$ cell densities within and below the halocline did not differ significantly (Table II, Mann–Whitney $P > 0.016$). At 08:00, there appeared to be an upward migration behaviour, and at 12:00 there were significantly more cells within the halocline than below it (Table II, Mann–Whitney $P < 0.016$). In comparison, $D. acuta$ did not cross the salinity gradient but instead aggregated at the top surface water throughout the experiment, with significantly more cells above than within or below the halocline (Fig. 4; Table II, Mann–Whitney $P < 0.016$).

### DISCUSSION

We studied the vertical migration behaviour of three marine dinoflagellates in artificial stratified water columns created in PVC cylinders. The results reveal that each of the species studied responded differently to the salinity gradient during their DVM (Fig. 6). DVM behaviour in situ is no doubt influenced by several factors (e.g. turbulence, prey availability or predators), and environmental conditions can change rapidly and affect the optimal growth position in the water column. Our laboratory experiment allowed us to focus on the behavioural response to stratification under controlled conditions, and thus we were able to demonstrate that two of the three species we studied exhibited continuous diel patterns, and we also found that different species do behave differently in relation to salinity gradients. These disparities in DVM may be due to inheritable physiological diversity that is activated by changes in salinity, light quality or nutrient supply. Moreover, the current findings do not confirm any simple phototactic migration behaviour, since the dinoflagellates tended to descend before the lights were turned off (Kamykowski, 1981; Olsson and Granéli, 1991), which suggests that DVM behaviour reflects an internal circadian rhythm. Earlier studies have shown that DVM in dinoflagellates can continue in complete darkness for several days (Weiler and Karl, 1979; C. Reisborg, unpublished...
results), and persistent diel rhythms of \textit{in vivo} chlorophyll fluorescence under continuous light indicate the involvement of a biological clock. It seems that persistent rhythms exist in a greater proportion of dinoflagellates compared with other plankton taxa (Brand, 1982).

It is assumed that the downward movement of dinoflagellates is triggered by inorganic nutrient depletion in the surface water (Flynn and Fasham, 2002). This hypothesis was not confirmed in our experiment, since dinoflagellate cells in the nutrient-sufficient water below the halocline in the artificial water columns continued to display a definite DVM behaviour. However, the different DVM patterns of the three species investigated indicate that each of these taxa used a strategy that allowed optimization of growth, regardless of whether it was driven by an internal biological clock and/or occurred in response to the internal physiological status of the cells (under the influence of the environmental conditions).

In any case, several different factors may be involved in regulating the migration behaviour of dinoflagellates, and these factors may vary substantially depending on the existing environmental conditions (Heaney and Furness, 1980; Cullen and Horrigan, 1981; Rasmussen and Richardson, 1989) or the phases of the cell cycle (MacKenzie, 1992). This conclusion is further supported by findings in the literature concerning vertical distribution and migration of \textit{Dinophysis} and \textit{Ceratium} spp. For example, in a study of phytoplankton in the Baltic Sea, \textit{D. norvegica} was found only near the seasonal pycnocline and exhibited very limited diurnal migration (Hajdu et al., 2007). In contrast, the \textit{D. acuta} in our experiment showed no obvious DVM, and the same behaviour has been observed by Pizarro et al. (Pizarro et al., 2008) during a bloom in the Galician Rias Baixas (NW Spain). Over a 22-h sampling period, the researchers noted that \textit{D. acuta} cells became concentrated in the colder and more brackish water at a depth of 1–4 m, where the density gradient was strongest. However, according to other investigators (Villarino et al., 1995), there is evidence that \textit{D. acuminata} exhibits DVM behaviour in the Galician Rias Baixas, where, during a period of 24 h, it was noted that cells of this species aggregated closer to the surface around noon (10:50 to 13:55) and accumulated at a depth of 10 m during most of the evening and the night. Figueroa et al. (Figueroa
et al., 1998) have described similar migratory behaviour in which maximum cell numbers occurred in surface water at noon but at a depth of 2–4 m at night. In this study, *D. acuminata* was not found below the very weak density gradient of 0.8 (24.4 above and 25.2 below the pycnocline) at night, whereas *C. furca* cells were observed below the pycnocline at dusk but were spread throughout the water column during the day, and tended to aggregate near the surface from noon to 17:00.

Velo-Suarez et al. (Velo-Suarez et al., 2008) performed in situ fine-scale vertical sampling in a stratified water column on a calm day and found large numbers of *C. furca* at the bottom of the pycnocline at a depth of 10 m. Moreover, in a laboratory study (Olsson and Graneli, 1991), it was noted that *C. furca* crossed a salinity gradient of 16.3–30.2 during DVM. Unfortunately, the conditions in many studies have varied with regard to factors such as light intensity in bottom water and halocline strength, which makes it difficult to compare the results that have been reported. Only a few investigations have examined the vertical distribution of *C. tripos* (e.g. Eppley et al., 1984; Mouritsen and Richardson, 2003), and our study is, to our knowledge, the first to demonstrate clear diel vertical behaviour in this species, including aggregation of cells within the halocline at night.

It has also been suggested that mixotrophic species adapted to low light levels do not need to migrate (Carpenter et al., 1995). Only a few dinoflagellates obtain their energy exclusively through photosynthesis, and many *Dinophysis* and *Ceratium* spp. are known to be mixotrophic (Smalley et al., 2003; Park et al., 2006). For instance, *D. acuminata*, whose feeding mechanisms have recently been described by Park et al. (Park et al., 2006), apparently requires the presence of prey in order to maintain growth over a longer period. Even so, *D. acuminata* is capable of photosynthesis, and it is still a matter of discussion whether this species has permanent plastids or kleptoplastids (Schnepp and Elbrachter, 1999; Minnhaagen and Janson, 2006). Nevertheless, these mixotrophs can compete with obligate phototrophic phytoplankton for soluble nutrients and with obligate heterotrophic dinoflagellates for prey. However, it is possible that mixotrophs have an advantage only under conditions of limited light, low concentrations of dissolved inorganic nutrients or inadequate prey (Raven, 1997; Stoeker, 1999), which has raised the question of whether mixotrophy is a better survival strategy compared to the advantages provided by growth and outcompeting other species. In this context, Smalley et al. (Smalley et al., 2003) studied *C. furca* and found that cells cultured under N- or P-depleted conditions ingested prey, whereas nutrient-replete cells did not. Moreover, the same research group (Smalley et al., 1999; Smalley and Coasts, 2002; Smalley et al., 2003) noted that *C. furca* exhibited phagotrophy, but, to our knowledge, such feeding has not yet been confirmed in *C. tripos*.

To determine growth rates, we investigated photosynthesis displayed by *C. furca* and *C. tripos* at different depths and found that both these species grew slowly (<0.13 μ day⁻¹) below the halocline, where light levels were <5 μmol quanta m⁻² s⁻¹. *C. tripos* had a higher autotrophic growth rate and, during daytime, this species aggregated at the surface and showed a growth rate of 0.8 μ day⁻¹. *C. furca* remained below the halocline throughout the 48-h experiment, which indicates...
some benefits of remaining in the poor light conditions of the bottom water. Studies have demonstrated that dinoflagellates are capable of maintaining a relatively high nutrient uptake rate in a low-light or even completely dark environment (Heaney and Eppley, 1981; Olsson and Graneli, 1991). In addition, Baek et al. (Baek et al., 2008) observed that C. furca could survive for at least 15 days in the dark which indicates the ability to tolerate poor conditions for photosynthesis. Interestingly, in our investigation, C. furca must have initially passed through the halocline at the start of the experiment, because the cells were added to the surface water. This indicates that these dinoflagellates were capable of handling a salinity gradient, but, despite this, they did not pass through the halocline during their upward migration.

The type of vertical separation displayed by the species we studied, even those belonging to the same genus, is notable and suggests that competition may occur between these dinoflagellates. However, thus far no research has been conducted to address the question of whether such competitive behaviour actually exists between phytoplankton species in natural surroundings and can result in vertical displacement of these organisms.

Our results illustrate the impact that a salinity gradient of 5 psu in an artificial water column has on the DVM of the three dinoflagellate species C. tripos, C. furca and D. acuta. DVM was clearly displayed by C. tripos and C. furca, but, due to the presence of the halocline, the movement occurred at different vertical positions in the water column. By comparison, throughout the experiment, D. acuta aggregated in the surface water and showed no clear DVM pattern. Thus, our laboratory findings lead to the conclusion that Ceratium spp. exhibit far more species-specific behavioural responses to stratification than was previously assumed.

More species-specific studies using different environmental scenarios and also fine-scale investigations of DVM in situ are needed to improve our knowledge of the mechanisms that control the behaviour and growth of dinoflagellates in their natural environments. Information regarding these responses is of interest, because it can help elucidate the competitive situations that exist between species under natural conditions in natural settings.

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