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Dependency of dinoflagellate vertical migration on salinity stratification

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ABSTRACT: Increasing precipitation and surface water temperature due to global change may strengthen the salinity gradient in coastal regions, which could influence the behaviour of dinoflagellate migration. We studied diel vertical migration (DVM) behaviour in the dinoflagellates *Prorocentrum minimum* and *Heterocapsa triquetra* using vertically stratified laboratory columns with 3 different salinity gradients (difference of 6, 11 and 16 psu). With nutrient-depleted conditions at the surface, and with nutrients added below the halocline, *P. minimum* remained mainly concentrated in the bottom water, while *H. triquetra* performed DVM under all 3 salinity treatments. *H. triquetra* migrated through a salinity difference of 6 and 11 psu, concentrated at the surface at noon, then migrated to the nutrient-rich bottom water during the night. A salinity gradient of 16, however, stopped *H. triquetra* cells from moving through the gradient and resulted in a concentration of cells in the cline during the night. At midday, cells were again found at the surface. *P. minimum* and *H. triquetra* grown in 4 different salinities (10, 15, 20, 26 psu) and at 3 different temperatures (10, 15, 20°C) showed higher specific growth rates with increasing temperature only in the 2 highest salinity treatments. At 10°C, specific growth rates were not affected by different salinities.

KEY WORDS: Dinoflagellate · Stratification · Vertical distribution · Global change · Salinity · Temperature · Growth rate · *Heterocapsa triquetra* · *Prorocentrum minimum*

INTRODUCTION

Global warming is expected to increase air temperature by 1.7 to 4.9°C by 2100 (90% probability; IPCC 2001, Wigley & Raper 2001). In temperate regions of the northern hemisphere, e.g. the North Sea and the Baltic Sea, this will mean an increase in mean surface water temperature (Carstensen 2007, Conley et al. 2007) and more intense precipitation events (e.g. Jones & Reid 2001, Zhang et al. 2007). Numerous studies have shown that changes in the abundance, composition and annual succession of phytoplankton are related to changing weather patterns (Reid et al. 1998, Wasmund & Uhlig 2003, Edwards & Richardson 2004, Wiltshire & Manly 2004, Suikkanen et al. 2007) and also that increased temperature and precipitation extend thermal stratification and strengthen the salinity gradient, which may explain some of the changes in phytoplankton biomass dominance (Peperzak 2003, 2005). Moreover, a higher surface temperature stimulates growth rates (Eppley 1972, Raven & Geider 1988) and lengthens the growing season (Ilus & Keskitalo 2008) if sufficient nutrients are available.

Behavioural studies of different phytoplankton species (Amano et al. 1998, Jephson & Carlsson 2009) have demonstrated that diel vertical migration (DVM) is an important aspect of dinoflagellate ecology (Eppley et al. 1968, Kohata & Watanabe 1986). DVM is a behavioural mechanism by which dinoflagellates can access photosynthetically active radiation near the surface, and nutrients at depth (Eppley et al. 1968, Heaney & Eppley 1981, Kimura et al. 1999, Flynn & Fasham 2002). During this process, cells may need to cross both salinity and temperature gradients (halocline and thermocline, respectively), which together form a layer called pycnocline, where density changes dramatically.

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In summer, concentrations of inorganic nutrients are often very low in surface waters as compared to deeper areas, i.e. those areas below the pycnocline, or zone of maximum density differences. This may be beneficial to vertically migrating dinoflagellates. The net result may be the optimization of growth rate within environmental constraints (Cullen 1985). Dinoflagellate concentrations at certain depths under specific environmental conditions indicate that the cell growth is optimized by vertical movement in the water column. Several studies have shown that flagellates have a tendency to aggregate near a pycnocline (Nielsen et al. 1990, Bjørnsen & Nielsen 1991). Additionally, asexual reproduction (MacKenzie 1992, Persson et al. 2008), avoiding grazing (Harvey & Menden 2011) or turbulent mixing (Yamazaki & Kamykowski 1991) may have an effect on the vertical position of dinoflagellate cells in nature.

Even though many species have a wide range of tolerance for different salinities and temperatures when grown in laboratory environments (Cullen & Horrigan 1981, Kamykowski 1981, Rasmussen & Richardson 1989, MacIntyre et al. 1997, Figueroa et al. 1998, Erga et al. 2003), relatively little is known about their direct response to salinity and temperature gradients during DVM. A trade-off situation almost certainly occurs in stratified waters, especially if the temperature or salinity gradients should change dramatically with depth. There may be an acclimatizing period for species when crossing salinity or temperature gradients, and a risk of additional energy costs for organisms not growing in their optimal environment.

Prorocentrum minimum and *Heterocapsa triquetra* are 2 bloom-forming dinoflagellates that cause blooms in many eutrophic estuarine and coastal environments worldwide (Tangen 1980, Lindholm & Ohman 1995). They are widely distributed in temperate and subtropical waters (Olsson & Edler 1991, Hansen 1995, Hajdu et al. 2005), and, because some *P. minimum* produce toxins, they are potentially harmful to humans via shellfish poisoning (Heil et al. 2005). In 1998 and 2006 *H. triquetra* bloomed and formed a deep chlorophyll maximum (DCM) in the Baltic Sea (Kononen et al. 2003, Lips et al. 2010). Production in DCM layers in late summer may exceed production during spring blooms (Richardson et al. 2000).

Prorocentrum minimum and *Heterocapsa triquetra* blooms generally occur under conditions of high temperatures and low-to-moderate salinities (Heil et al. 2005), but they have also been noted at widely varying salinities and temperatures (Tyler & Seliger 1981, Hajdu et al. 2005). These species have been extensively studied (e.g. Litaker et al. 2002, Carstensen et al. 2007), but relatively little is known about the ecological importance of their DVM in stratified waters.

At some locations within the Baltic Sea, pycnoclines can create great differences between surface and bottom temperatures and salinities. For example, in October 1994, a difference in temperature and salinity of 7°C and 11 psu, respectively, was observed in the Bornholm Basin in the Baltic Sea (Jakobsen 1996). In the straits between Sweden and Denmark, the cold, salty and nutrient-rich North Sea current meets the brackish Baltic Sea water; here the salinity can go from 10 to 30 psu within 2 m depth (SMHI 2007). The vertical position of the halocline is usually between 10 and 20 m, which means that light levels at noon are between 5 and 10 µmol quanta m^{-2} s^{-1}, which is still sufficient for photosynthesis (P. Carlsson unpubl. data). Because of these factors, this coastal region forms a challenging habitat for migrating organisms.

We experimentally studied the DVM behaviour of *Prorocentrum minimum* and *Heterocapsa triquetra* at 3 different stratification regimes often found in the Baltic Sea and in waters along the southwest coast of Sweden (Brøns Hansen et al. 2003). We hypothesized that, for populations of both species, increasing stratification affects DVM behaviour, and the halocline acts as a barrier for obtaining both nutrients and sunlight. We studied specific growth rates at different salinities, combined with different temperatures, to clarify the effect of increased surface temperature and decreased salinity on the growth of organisms of both species.

MATERIALS AND METHODS

Acclimatization. Prior to the experiments, monocultures of *Prorocentrum minimum* and *Heterocapsa triquetra* (GUMACC78 and GUMACC71, respectively; Gothenburg University Marine Culture) were acclimatized by slowly increasing or decreasing salinity and temperature to adapt cells to the conditions of each planned treatment. During the acclimatization phase, cells were grown either at 10, 15 or 20 psu inside identical plastic cylinders under a 12 h light:12 h dark cycle at 15°C in f/5 medium, first under conditions of nitrogen repletion and, closer to the beginning of the experiment, of nitrogen deficiency (Guillard & Ryther 1962, Guillard 1975). Growing cells were slowly given less nitrogen until inorganic nitrogen concentration in the water was <1 µM. The medium was prepared from GF/F-filtered and autoclaved seawater from the southwest coast of Sweden, with the addition of trace metals and vitamins according to Guillard & Ryther (1962).

Nutrient measurements. The C:N ratio of the cells was calculated initially and at the end of the experiment by quantifying total organic nitrogen (TON) and total organic carbon (TOC). TON and TOC were quantified by filtering 250 ml of water with a known

cell density onto GF/F-filters, which were then analyzed in a Vario MAX elemental analyser. Detection limits were 0.06 and 0.02 mg for carbon and nitrogen, respectively.

Testing DVM in distinct salinity gradients. To test for differences in vertical migration behaviour at different halocline strengths, *Prorocentrum minimum* and *Heterocapsa triquetra* were studied over 96 h (4 days and 4 nights) in stratified water columns created in clear plastic polystyrene cylinders. Stratification treatments consisted of creating a different salinity gradient in each column (with differences of 6, 11 and 16 psu between surface and bottom). In all three columns, bottom salinity was held at 26 psu and surface salinity was fixed at 10, 15 or 20 psu. Samples were taken at the surface, within the cline and at the bottom every 6 h during the 96 h period. In a separate experiment, growth rates in waters at salinities of 10, 15, 20, and 26 psu, with temperatures of 10°C, 15°C and 20°C, were also studied.

Migratory behaviour was studied in 0.5 m high, 0.12 m diameter transparent plastic cylinders. Daylight lamps (Philips Master TL-D 90 De Luxe 36W/965) were placed 0.5 m above the cylinders, set on a 12 h light: 12 h dark cycle and turned on at 07:00 h. The light level (mean ± SD) at the surface was 14.2 ± 1.4 µmol quanta m^{-2} s^{-1}, measured using a LI-192SA underwater quantum sensor. Black, non-transparent paper was tightly wrapped around the sides of the cylinders, in order to create a vertical light gradient with decreasing light intensity towards the bottom. Light levels in the halocline varied between 4.3 and 5 µmol quanta m^{-2} s^{-1} and, at the bottom, approximately 2.6 µmol quanta m^{-2} s^{-1}.

Four replicate cylinders for each halocline treatment were prepared 12 h before the experiment. Filtered (1 µm) nutrient-depleted (<0.3 µM nitrate) seawater with salinities of 10, 15 or 20 psu was added to the cylinders. Following this, 1600 ml of nutrient-rich (20 µM nitrate) water of 26 psu was then slowly added to the bottom; a 2 mm wide silicon tube was used to siphon the water into the cylinders at the bottom. The halocline treatments are hereafter referred to by strengths of the respective gradients, viz. 6, 11 and 16 psu.

From each cylinder, using a clean 60 ml syringe connected to a thin silicon tube, 40 ml of surface and bottom water were sampled for inorganic nutrient analysis and salinity measurements. Concentrations of NO_3^- + NO_2^-, PO_4^{3-} and NH_4^+ were measured according to Valderrama (1995). At the start of the experiment, inorganic nitrate concentration in the surface water was <1 µM, while the bottom water was enriched in both nitrate and phosphate, ~20 and 5 µM, respectively (Table 1). The temperature was held at 15°C in all cylinders.

Cells were added by slowly siphoning water from the culture into the surface water of the replicate cylinders using thin silicone tubing. The concentration added to each cylinder was ~2000 cells ml^{-1}. A total of 24 cylinders, including 4 replicates of 3 salinity gradient treatments with either *Prorocentrum minimum* or *Heterocapsa triquetra* were used in the experiment. Cell addition was completed just before the first sam-

Table 1. *Prorocentrum minimum* and *Heterocapsa triquetra*. Inorganic nitrogen, phosphorus and ammonia concentrations (mean ± SD) in the surface and bottom water at start and end of experiment, at halocline strengths of 6 and 11 psu for *P. minimum* and 6, 11 and 16 psu for *H. triquetra*

Inorganic nutrient	Treatment		Surface (µM)	Bottom (µM)
Prorocentrum minimum				
$NO_3^- + NO_2^-$	11	Start	0.79 ± 0.28	19.35 ± 0.67
		End	1.3 ± 0.08	15.85 ± 2.77
	6	Start	0.46 ± 0.03	19.5 ± 0.42
		End	0.38 ± 0.02	8.65 ± 0.09
PO_4^{3-}	11	Start	0.51 ± 0.05	5.31 ± 1.33
		End	0.62 ± 0.07	5.91 ± 1.60
	6	Start	4.18 ± 1.15	5.32 ± 0.09
		End	2.22 ± 0.31	4.19 ± 0.06
NH_4^+	11	Start	0.81 ± 0.54	0.45 ± 0.06
		End	1.13 ± 1.56	0.58 ± 0.23
	6	Start	0.35 ± 0.10	0.31 ± 0.11
		End	1.13 ± 0.13	2.12 ± 0.46
Heterocapsa triquetra				
NO_3^-+NO_2^-	16	Start	0.22 ± 0.12	26.4 ± 0.83
		End	0.27 ± 0.15	19.32 ± 0.79
	11	Start	0.27 ± 0.15	19.32 ± 2.05
		End	0.41 ± 0.08	8.44 ± 0.33
	6	Start	0.36 ± 0.05	18.56 ± 0.30
		End	0.34 ± 0.04	7.7 ± 2.25
PO_4^{3-}	16	Start	0.56 ± 0.08	4.52 ± 0.12
		End	0.56 ± 0.07	4.11 ± 0.18
	11	Start	1.34 ± 0.38	5.39 ± 0.01
		End	1.04 ± 0.30	4.35 ± 0.12
	6	Start	1.01 ± 0.18	4.33 ± 1.43
		End	1.20 ± 0.05	4.49 ± 0.12
NH_4^+	16	Start	0.84 ± 0.09	0.47 ± 0.04
		End	0.34 ± 0.05	0.37 ± 0.12
	11	Start	0.35 ± 0.15	0.26 ± 0.14
		End	1.07 ± 0.37	1.24 ± 0.22
	6	Start	0.37 ± 0.12	0.38 ± 0.14
		End	1.24 ± 0.22	1.89 ± 0.21

pling at 12:00 h. Samples were thereafter taken every 6 h over a 96 h (4 d) period using a 0.6 m non-flexible clear plastic pipette with an inner diameter of 6 mm that was carefully lowered into each cylinder. The pipette was lowered until it reached a point 30 mm above the bottom and was then closed at the end above the surface after filling the pipette with water. After that, the pipette was carefully raised from the cylinder and bottom water, then halocline water and surface water were subsequently drawn and separately poured into one of three 15 ml sampling tubes, guided by markers on the pipette. The samples were fixed with Lugol's solution and cells counted using an inverted microscope (Olympus CKX 41 at 200× total magnification).

Surface and bottom-water salinity samples measured in the cylinders at the end of the experiment indicated that the gradient remained stable throughout the experiments. The sampling method had been tested prior to the experiment and the technique adjusted so as not to be affected by the decreasing water volume in the cylinders. A separate experiment to test halocline stability had also been carried out several weeks before the start of the DVM experiment by colouring the surface and bottom water with caramel colour. The separation of the water masses was then visually very clear and the stability of the haloclines could be tested visually while taking repeated samples during several weeks before the experiment began. Finally, the procedure of sampling the 3 water layers using one pipette volume and emptying the 3 water portions into different 15 ml tubes had also been tested.

Assessment of growth rates. In the second experiment, growth rates of monocultures of *Prorocentrum minimum* and *Heterocapsa triquetra* at 4 different salinities were studied at 3 different temperatures. The cultures were grown at salinities of 10, 15, 20, and 26 psu at 10, 15 and 20°C. Four replicate cell tissue culture flasks (250 ml) were filled with 200 ml of GF/F-filtered and autoclaved f/5 medium (Guillard & Ryther 1962). Aliquots of <10 ml of exponentially growing cells of *P. minimum* or *H. triquetra* were added to a concentration of 2000 cells ml^{-1} in each of the flasks and kept on a 14 h light:10 h dark cycle under a light level of 30 µmol quanta m^{-2} s^{-1}. Daylight fluorescent tubes (Philips Master TL-D 90 De Luxe 36W/965) were used for light.

Initially, 2.5 ml of water was sampled from the flasks, and again after 2 d or several weeks, depending on observed growth at various salinities and temperatures. Additionally, 4 samples were taken during the exponentially growing phase of the cells. Samples were fixed with Lugol's solution and counted using an inverted microscope (Olympus CKX 41 at 200× total magnification). Net growth rates were estimated by cell counts and measured as the change in cell number over time. The calculation was performed using the equation:

$$\mu = \frac{\ln(N_{t1}/N_{t0})}{\Delta t} \quad (1)$$

where µ is the net growth rate of *Prorocentrum minimum* or *Heterocapsa triquetra* at different salinities and temperatures. N_{t1} and N_{t0} are the cell densities at 2 different times during the exponential growth phase and Δt is the incubation time between $t2$ and $t1$.

Statistical analyses. Differences in net growth rates between salinity and temperature treatments for each species were analyzed using 2-way factorial ANOVA in the statistical package SPSS 17.0 for Windows. The salinity-by-temperature growth rate interaction effect was analyzed using a simple main effects analysis (Page et al. 2005). To test for differences in growth rates at a specific temperature we used 1-way ANOVA, followed by Tukey's post hoc test.

To evaluate the diel vertical position of cells under different stratification intensities we used a 2-way ANOVA with days (each 24 h period) as random blocking factors, vertical position (above, within and below density discontinuity) and time (6:00, 12:00, 18:00 and 00:00 h) as fixed factors, with inclusion of the interaction between vertical position and time in the model. In order to compensate for potential differences during the 4 different days of sampling, we analyzed our data according to a block design (Underwood 1997). The time of day by vertical position effect was analyzed using a simple main effects analysis (Page et al. 2005).

To test for differences in inorganic nutrient (NO_3^- + NO_2^-, PO_4^{3-} and NH_4^+) uptake within treatments throughout the experiment, a paired sample *t*-test was used. To test for net differences in NO_3^- decline in the bottom water between treatments, 1-way ANOVA with Tukey's post hoc tests were used. Halocline strength (6, 11 and 16 psu) was the independent variable (3 levels), and net decrease in NO_3^- between start and end of the experiment was the dependent variable.

Data on cell concentration were square-root transformed to make them normally distributed (as determined by the Kolmogorov-Smirnov test). All analyses were performed in SPSS 17.0 for Windows.

RESULTS

The 2 dinoflagellates *Prorocentrum minimum* and *Heterocapsa triquetra* responded differently to salinity gradients during DVM (Fig. 1). The highest densities of *P. minimum* were found in the bottom water throughout the experiment and no clear DVM pattern through the halocline was observed for this species (Fig. 2; $p < 0.05$,

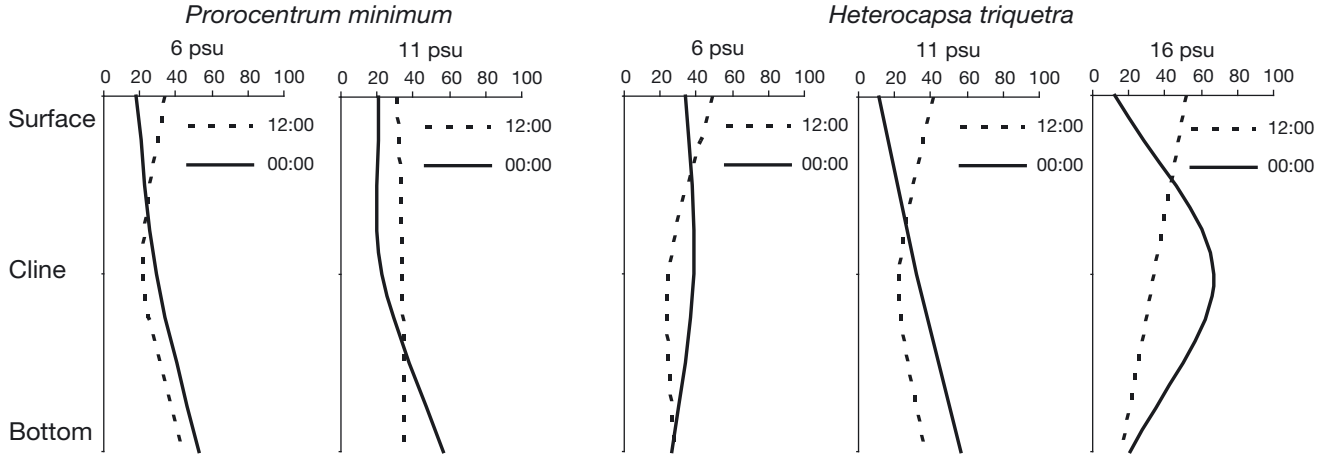

Fig. 1. *Prorocentrum minimum* and *Heterocapsa triquetra*. Means of true percentages of cell density at 12:00 and 00:00 h at halocline strengths of 6 and 11 psu for *P. minimum* and 6, 11, and 16 psu for *H. triquetra*

$F_{6,177}$ = 13.8 and $F_{6,177}$ = 4.3; salinity 6 and 11 psu, respectively). *H. triquetra*, however, showed different DVM behaviour at different halocline strengths, and an interaction was found between time and vertical position within a particular treatment (Fig. 3; $p < 0.05$, $F_{6,177}$ = 2.4, $F_{6,177}$ = 27.6 and $F_{6,177}$ = 27.8; salinity 6, 11 and 16 psu, respectively), meaning that cell-density maxima were found at different vertical positions depending on time of day. The same DVM patterns were observed every 24 h throughout the 96 h period.

DVM behaviour in *Prorocentrum minimum*

In the 11 psu treatment, the cell density of *Prorocentrum minimum* differed significantly above, within and below the halocline, depending on time of day (Fig. 2; simple effect test, $p < 0.001$, $F_{3,180} \geq 12.0$). At 12:00 h, cells were more homogeneously distributed in the water column as compared to 06:00, 18:00 and 00:00 h, when cells were mainly concentrated in the bottom water (Simple effect test, $p > 0.05$, $F_{2,180}$ = 0.2). In the 6 psu treatment, however, cell density within the halocline did not differ throughout any 24 h period (Fig. 2; simple effect test, $p > 0.05$, $F_{3,180}$ = 2.5), but differed significantly in both the surface and in the bottom water with time of day (simple effect test, $p < 0.05$, $F_{3,180}$ = 12.4, $F_{3,180}$ = 3.0, respectively). The 16 psu treatments of *P. minimum* collapsed due to cultivation problems.

DVM behaviour in *Heterocapsa triquetra*

Time of day influenced the vertical position of *Heterocapsa triquetra* cells differently, depending on

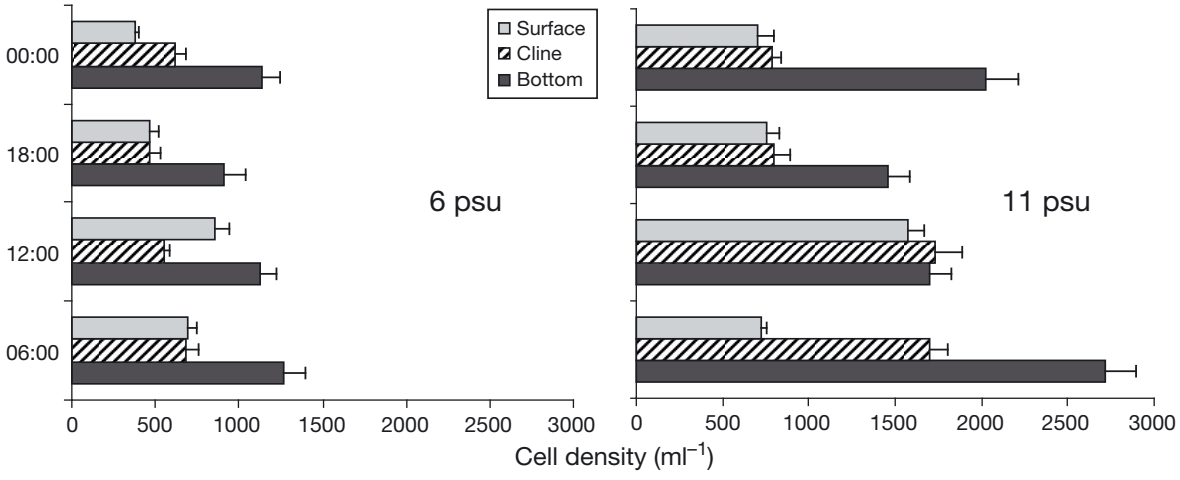

Fig. 2. *Prorocentrum minimum*. Cell density (mean ± SE of 4 replicates over 96 h period) at surface, halocline and in bottom water at 06:00, 12:00, 18:00 and 00:00 h, and at halocline strengths of 6 and 11 psu

halocline strength. The strongest salinity gradient (16 psu treatment) stopped *H. triquetra* cells from migrating downwards and resulted in the highest concentration of cells in the halocline at midnight. The cell density above and within the halocline differed according to time of day (simple effect test, $p < 0.001$, $F_{3,180} \geq 29.8$). At 00:00 h, cells aggregated mainly in the halocline; during the light period, at 12:00 h and 18:00 h, cells aggregated in the surface water. In the 11 psu treatment, however, the vertical position of maximum cell density differed significantly at all times (Fig. 3; simple effect test, $p < 0.001$, $F_{3,180} \geq 16.5$). At 00:00 h, cell density was highest at the bottom; at 06:00 h it was highest in the cline; and at 12:00 and 18:00 h highest at the surface. In the 6 psu treatment, there was a significant difference in vertical position at 06:00, 12.00 and 18:00 h (Fig. 3; simple effect test, $p \leq 0.05$, $F_{2,180} \geq 6.1$), with cell maxima at the surface.

C:N ratios and inorganic nutrients

The C:N ratio of cells decreased throughout the experiment, confirming uptake of nitrogen from the bottom water. C:N ratios were determined independently for each treatment. *Heterocapsa triquetra* showed initial C:N ratios of 7.4 ± 0.5 and 7.9 ± 0.3 (mean ± SD of the 6 and 16 psu treatments, respectively), decreasing to 4.9 ± 0.5 and 4.3 ± 0.5 by the end of the experiment. *Prorocentrum minimum* was initially 6.9 ± 0.2 and 7.8 ± 0.9 (mean ± SD of the 6 and 11 psu treatment, respectively), decreasing to 4.3 ± 0.3 and 4.6 ± 0.1 by the end of the experiment. Inorganic nitrate concentration in the bottom water decreased between the start and the end of the experiments (Table 1; paired *t*-test, $p < 0.005$, df = 7, $t = 4.5$ and $p < 0.001$, df = 11, $t = 12.6$; for *P. minimum* and *H. triquetra*, respectively), while phosphate concentration did not change significantly throughout the experiment (Table 1; paired *t*-test, $p > 0.05$, df = 7, $t = 0.6$ and $p > 0.05$, df = 11, $t = 1.4$; for *P. minimum* and *H. triquetra*, respectively). Ammonia concentration increased (Table 1; paired *t*-test, $p < 0.05$, df = 7, $t = 2.9$ and $p > 0.05$, df = 11, $t = 3.7$; for *P. minimum* and *H. triquetra*, respectively). Nitrate concentration in the bottom water showed a trend of stronger net decrease in the 6 and 11 psu treatments as compared to the net decrease in the 16 psu treatment for *H. triquetra* (Table 1; ANOVA, $p < 0.05$, $F_{2,11} = 4.5$). Tukey's post hoc test showed p-values below 0.068 when the 16 psu treatment was compared to the data from the 6 and 11 psu treatments. For comparison, the p-value for observations in the 6 and 11 psu treatments was 1.0.

Growth rates

Both temperature and salinity influenced growth of *Prorocentrum minimum* and *Heterocapsa triquetra* significantly (Fig. 4; $p < 0.001$; $F_{6,47} = 4.5$ and $F_{6,47} = 8.7$; for *P. minimum* and *H. triquetra*, respectively). Salinity-dependent differences in growth rate were found when *P. minimum* and *H. triquetra* cells were grown at 20°C (simple effect test, $p < 0.001$, $F_{3,36} = 13.9$ and $F_{3,36} = 24.1$; for *P. minimum* and *H. triquetra*, respectively). At 20°C both *P. minimum* and *H. triquetra* grew better in 20 and 26 psu treatments than in treatments of 10 and 15 psu (Fig. 3; 1-way ANOVA, $p < 0.001$, $F_{3,15} = 8.2$ and $p < 0.001$, $F_{3,15} = 23.0$; for *P. minimum* and *H. triquetra*, respectively). There was no difference in growth rates between salinities when cells were grown at 10°C. Increased temperature, however, increased growth rates of both species grown in 20 and 26 psu water (Fig. 4).

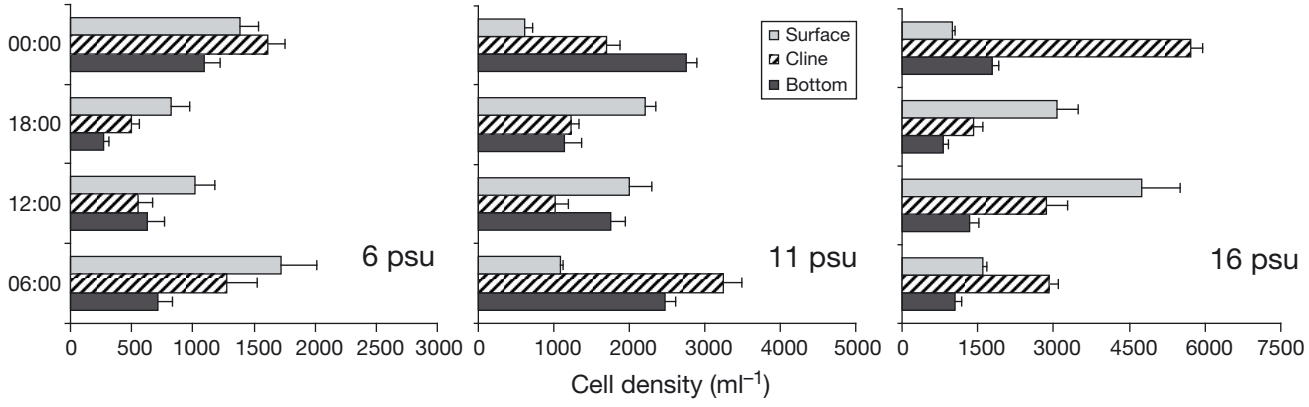

Fig. 3. *Heterocapsa triquetra*. Cell density (mean ± SE of 4 replicates over 96 h period) at surface, halocline and bottom water at 06:00, 12:00, 18:00 and 00:00 h and at halocline strengths of 6, 11 and 16 psu

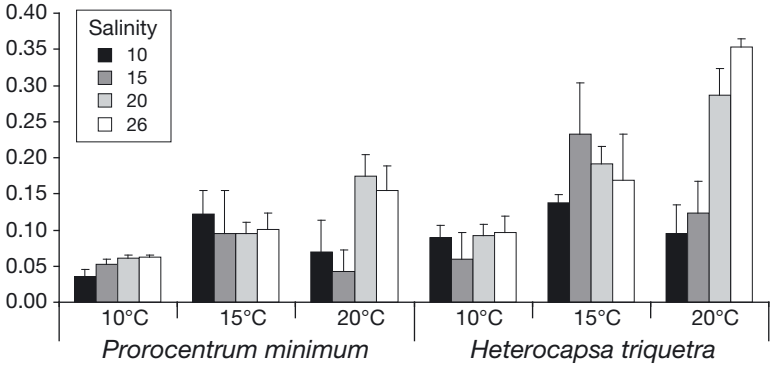

Fig. 4. *Prorocentrum minimum* and *Heterocapsa triquetra.* Growth rate (mean ± SD) at salinities of 10, 15, 20 and 26 psu and temperatures of 10, 15 and 20°C

DISCUSSION

Globally, ocean stratification is becoming significantly stronger, as surface warming increases the density contrast between the surface layer and underlying nutrient-rich waters (Behrenfeld et al. 2006). Along ocean coasts, extreme precipitation may further strengthen the barrier between surface and bottom waters. The capacity of some species to migrate vertically through strong density gradients may lead to increased growth. To better understand the mechanisms underlying bloom outbreaks of 2 dinoflagellates, *Prorocentrum minimum* and *Heterocapsa triquetra*, in the stratified temperate coastal area of the Swedish coast, we investigated DVM at different halocline strengths. Based on our observations, *P. minimum* was mainly observed to remain in the bottom water throughout all treatments while *H. triquetra* exhibited different DVM behaviour depending on the strength of the salinity gradient. A 16 psu salinity difference acted as a DVM barrier to *H. triquetra* cells, but they were able to migrate through salinity gradients of 6 and 11 psu.

Many dinoflagellates, by migrating, may reach an environment favourable for growth. The hypothesis that dinoflagellates do in fact migrate vertically through the water column is re-enforced by our data (Eppley et al. 1968, Ross & Sharples 2007), but current information on how cells respond to changing salinities during DVM is limited (but see Olsson & Edler 1991). During DVM, dinoflagellates need to adjust to new salinities and temperatures, which may result in additional energy costs (Kimura et al. 1999, Erga et al. 2003). Moreover, continuous trade-offs among light, nutrients and growth at different salinities and temperatures likely affect the DVM strategy, depending on species. Recently, in a review by Ji & Franks (2007), the possibility was discussed of unique vertical migration patterns according to inherent physiological differences. The literature on vertical distribution, whether based on field or laboratory observations, indicates a large inter-specific variation in DVM (Weiler & Karl 1979, Kamykowski 1981, Olsson & Graneli 1991, Olli & Seppala 2001, Jephson & Carlsson 2009). Anderson & Stolzenbach (1985), in a report on *Gonyaulax* (*Alexandrium*) *tamarense* and *Heterocapsa triquetra* DVM under natural bloom conditions, suggest that the pattern is different between species and may change through time.

Phototactic behaviour in dinoflagellates has been confirmed (Kamykowski et al. 1998), and nitrate depletion in the surface may trigger cells to search for nutrients at depth (Heaney & Eppley 1981). In the present study, both *Prorocentrum minimum* and *Heterocapsa triquetra* took up nutrients during the night, decreasing cellular C:N ratios significantly. Moreover, *H. triquetra* cells that concentrated in the cline during the night exhibited decreased C:N ratios, indicating an uptake of nitrogen. In nature, a concentration of phytoplankton and bacteria within pycnoclines is well-known, and field measurements in the Baltic Sea show that *H. triquetra* cell maxima were concentrated at the base of the thermocline and corresponded well with the nutricline (a layer with increased nutrient concentration; Lips et al. 2010).

By comparison, *Prorocentrum minimum* were mainly observed in the bottom water at salinity differences of 6 and 11 psu, with no clear DVM behaviour being seen. Apparently the saltier, nutrient-rich bottom water was more beneficial to *P. minimum* under these experimental conditions. Our results on growth rates confirm higher growth rates for this species in waters of 20 or 26 psu than in waters of 10 or 15 psu. Moreover, as indicated by our experiment, both *P. minimum* and *Heterocapsa triquetra* growth rates were influenced by the combination of salinity and temperature. If surface temperature increases in nature, both *P. minimum* and *H. triquetra* will experience higher growth rates, but more so if grown in salinities >20 psu. If surface salinity decreases to <15 psu, growth rates will decrease (Yamamoto et al. 1995). The best growing conditions, according to our study, were in 20 and 26 psu at 20°C. Similarly, Yamochi (1984) found that *H. triquetra* grows best at temperatures of 19 to 20°C. However, blooms of *P. minimum* and *H. triquetra* have been observed in low-salinity environments like the Baltic Sea (e.g. Kononen et al. 2003, Hajdu et al. 2005), which demonstrates the physiological flexibility of these species. There is also a possibility of clonal variation, and Baltic Sea clones may be well adapted to a low salinity (Kremp et al. 2009).

In our experiment, growth rates were higher for *Heterocapsa triquetra* than for *Prorocentrum minimum* at all salinities and temperatures, which implies that *H. triquetra* populations would increase more rapidly during similar conditions in nature. Moreover, if increasing precipitation and temperature strengthen the density gradient in coastal regions, the nutrient-rich bottom water will be inaccessible to cells unable to migrate through the gradient. This will affect growth rates negatively, as cells may eventually run out of nutrients. Also, if the surface layer increases in depth, migrating cells may reach the nutrient-rich layer only by dawn. The cells may then run out of energy instead of nutrients (discussed by Roenneberg & Merrow 2002). Apparently, according to our study, the costs of migrating through the 16 psu halocline were higher than the costs of growing in the low-salinity layer, at least for the duration of the experiment. Even so, a salinity gradient of 11 psu is relevant to the Baltic Sea or to coastal areas on the southwest coast of Sweden (e.g. Brøns Hansen et al. 2003), and *H. triquetra* can move through that gradient during DVM. Cells growing in this region may increase their fitness by shifting position during periods of surface water nutrient depletion and thus form blooms.

Even though all factors regulating the migration behaviour were not included in our laboratory experiment (e.g. turbulent mixing, prey availability or predator influence), this laboratory study leads to the conclusion that behavioural responses to stratification are species-specific. The potential range and success of DVM may also depend on species-specific swimming speed *in situ* (Throndsen 1973, Levandowsky & Kaneta 1987). However, specific laboratory studies are needed to complement field data, since the heterogeneity and fluctuating environment of the ocean makes the mechanisms affecting DVM behaviour difficult to identify. In laboratory environments one can focus on behavioural response under controlled conditions.

We have shown a clear DVM behaviour for *Heterocapsa triquetra*, with cells migrating through a salinity difference of 6 and 11 psu. The 16 psu difference, however, acted as a barrier and cells concentrated in the halocline during the night. *Prorocentrum minimum* showed no clear DVM pattern, remaining concentrated in the bottom water. Thus, the species-specific responses in DVM to stratified water found in this study increase our understanding of what to expect regarding bloom outbreaks in coastal regions in the future.

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