Diel vertical migration in marine dinoflagellates

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Fakultetens opponent: Associate Professor Tammi L. Richardson,
Dept. of Marine Science Program and Biological Sciences
University of South Carolina, USA

Avhandlingen kommer att försvaras på engelska

Dissertation
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List of papers

This thesis is based on the following papers, referred to in the text by their roman numerals:

I. Jephson, T., Carlsson, P. 2009. Species- and stratification-dependent diel vertical migration behaviour of three dinoflagellate species in a laboratory study. *Journal of Plankton Research* 31:1353–1362.

II. Jephson, T., Carlsson, P., Fagerberg, T. 2012. Dominant impact of water exchange and disruption of stratification on dinoflagellate vertical distribution. *Estuarine, Coastal and Shelf Science* 112:198–206.

III. Jephson, T., Fagerberg, T., Carlsson, P. 2011. Dependency of dinoflagellate vertical migration on salinity stratification. *Aquatic Microbial Ecology* 63:255–264.

IV. Bresolin, K., Jephson, T., Hasper, T., Carlsson, P. 2012. Dinoflagellate diel vertical migration in stratified waters: species-specific behavior in relation to thermoclines of different strengths. Submitted.

V. Jephson, T., Rengefors, K., Mittag, M., Carlsson, P. 2012. How does blue- and red light influence vertical migration and rhodopsin gene expression in the dinoflagellate *Alexandrium minutum*? Submitted.

Preface

When people ask me about the topic of my PhD project, I simply answer “algal blooms”. Algal blooms are nowadays a well-known phenomenon and most people are interested in hearing more about it. When I continue explaining that the species I work with are able to both photosynthesize and ingest prey, are unicellular toxic organisms, and can swim at a speed roughly comparable to if you would swim about 70 km/h, people are deeply impressed and ask where you find them. The answer to that question is; it depends!

The following pages will give you a view of the results from my research on vertical distribution and diel vertical migrations in a microalgae group called dinoflagellates. I reveal species-specific behavior, altering behavior depending on environmental conditions and unchanged expression levels of the photoreceptor rhodopsin at varying light spectra’s.

Introduction

As light is the main energy source for most of the phytoplankton community, the greatest fitness of photosynthetic organisms in the sea ought to be obtained by staying close to the surface. However, the surface water in the summer is often depleted in nutrients due to the fast and efficient uptake by phytoplankton and limited vertical mixing of the water column. More nutrients may be found in deeper parts of the sea for those organisms that can move.

Most phytoplankton are non-motile, changing their position in the water mass passively through turbulence and sedimentation, but some phytoplankton groups, like the dinoflagellates, have the ability to move using their flagella. Behavioral oriented research in different phytoplankton species have demonstrated that diel vertical migration (DVM) is an important aspect of dinoflagellate ecology. DVM is a behavioral mechanism by which dinoflagellates access photosynthetically active radiation near the surface during daytime and swim towards more nutrient rich waters at the deep during night. In estuarine areas these species may need to cross both salinity (halocline) and temperature (thermocline) gradients, also called pycnoclines.

The pycnocline may constitute a barrier for migrating organisms, considering the need to adjust to the new salinity and temperature which may result in extra energy costs. Even so, the benefit of being able to collect and store nutrients at night may exceed the costs, and a trade-off situation between photosynthesis and uptake of nutrients most certainly occurs in stratified waters (Yoshiyama et al. 2009). The concept of trade-off implies that there is an optimal strategy whereby an organism may maximize its growth or fitness, and that all organisms probably strive towards this strategy (MacArthur 1972). Dinoflagellate cells need to choose the optimal position in the water column, where the balance between abiotic and biotic factors is best matched for growth. How dinoflagellates optimize these factors is highly dependent on the existing environmental conditions and during favorable conditions blooms may form. Increased knowledge of the ecological importance of DVM, and how dinoflagellates cope with stratification, is important to be able to understand the impact of environmental changes on the phytoplankton community. Therefore, the aim of my research has been to further clarify the DVM behavior of dinoflagellates in stratified waters.

Background

Stratification

Stratified waters exist both globally and along the coastline of marine waters as well as in lakes. A pycnocline is formed as a result of differences in salinity and/or temperature between water masses and is generally an area with low turbulence. The formation of a strict salinity gradient is called a halocline and may occur in estuaries where the salty seawater meets freshwater. As density increase with salinity, the more saline seawater will appear closest to the bottom. Temperature gradients are called thermoclines and during summer solar radiation produces a warm surface layer with low mixing, which drastically turns into colder deeper water (Lalli and Parsons 1997).

Thermal stratification of marine waters is significantly increasing in strength (Behrenfeld et al. 2006), as a result of global warming. If we continue to release an overload of carbon dioxide into the atmosphere we may expect an increase in surface water temperature of about 0.2°C per decade (IPCC, 2007). This may affect the phytoplankton community in the upper zone, which in turn may affect CO^2 uptake of the ocean. In the tropics and mid-latitude, where there is limited vertical mixing as the water column is stabilized by thermal stratification, an increase in surface temperature may decrease plankton productivity (Bopp et al. 2001, Boyd and Doney 2002, Sarmiento et al. 2004). A decrease in productivity may be the result of a shift in the plankton community structure when surface nutrients are exhausted earlier in the season. At higher latitudes however, phytoplankton are often light-limited because intense vertical mixing carries them hundreds of meters down into darkness. Here, mixing may be reduced when the surface temperature increases which may lead to an increase in productivity as light conditions becomes more stable (Polovina et al. 1995). Also in temperate regions of the northern hemisphere, i.e. the North Sea and the Baltic Sea, an increase in mean surface water temperature (Carstensen 2007, Conley et al. 2007) and intensifying extreme precipitation events (e.g. Jones and Reid 2001, Zhang et al. 2007) is expected.

In coastal regions, increased temperature and precipitation extend thermal stratification and strengthen the salinity gradient which seems to change phytoplankton biomass dominance towards more harmful algae blooms, including dinoflagellates (Peperzak 2003, Peperzak 2005). A dramatic pycnocline generally exists along the Swedish coast and is strongest in the Öresund/Kattegatt area in the southwest of Sweden (Fig. 1. T, Jephson unpublished data). The nutrient rich North Sea bottom current transports water into the Baltic Sea, while the brackish Baltic Sea water is transported out. As the cold salty North Sea current meets the Baltic Sea water in the narrow area of Öresund a distinct separation of the two water

masses occur. The northgoing current speed is sometimes very high in the surface waters (2–4 knots) and during summer seasons the nutrient concentrations are less than 0.5 µmol/l (inorganic nitrogen) (SMHI 2012). The difference in salinity and temperature between the currents forms a pycnocline all year round and the salinity gradient may vary from 10 to 30, sometimes in less than two meters depth (Fig. 1, T. Jephson unpublished data). The vertical position of the pycnocline in Öresund/Kattegatt is usually at a depth between 10 and 20 meters (SMHI 2012), which means that light levels are quite low (between 5 and 10 µE $m^{-2}s^{-1}$ at noon, but still enough intensity to allow photosynthesis (Fig. 1, T. Jephson unpublished data). In the Baltic Sea, the halocline is situated deeper than in the Öresund/Kattegatt area and is found permanently at 60–70 meter depth, while the thermocline is seasonal and found at about 15–20 meters in summer. The deep water in the Baltic Sea has increased nutrient levels compared to surface waters, particularly inorganic phosphorus (Carpenter et al. 1995, Hällfors et al. 2011).

Dinoflagellates

Phytoplankton are the dominating primary producers of the pelagic system converting inorganic materials (inorganic carbon, nitrate and phosphate) into new organic compounds (e.g. lipids, proteins) by the process of photosynthesis. They use solar energy to fix carbon dioxide and form particulate organic carbon. Most of the organic carbon is thereafter consumed and respired in the upper ocean by zooplankton and bacteria but part of the organic matter is exported down where it enriches the inorganic nutrient pool of the deeper zone of the oceans (Lalli and Parsons 1997). Marine phytoplankton accounts for 50% of the global primary production (Longhurst et al. 1995).

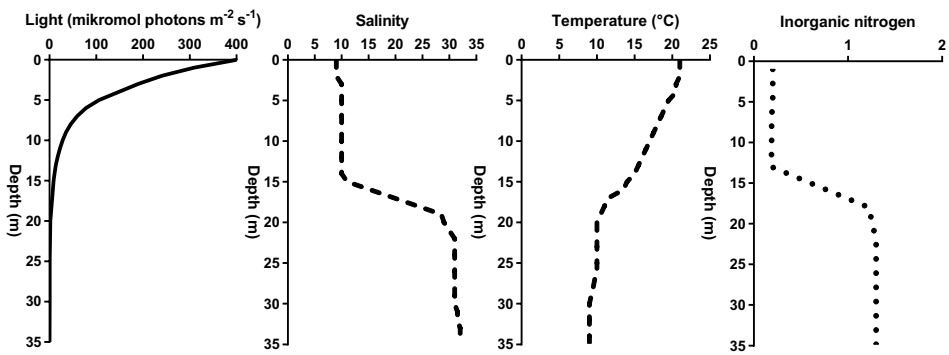

Fig. 1. An example of a summer situation in the stratified Öresund area.

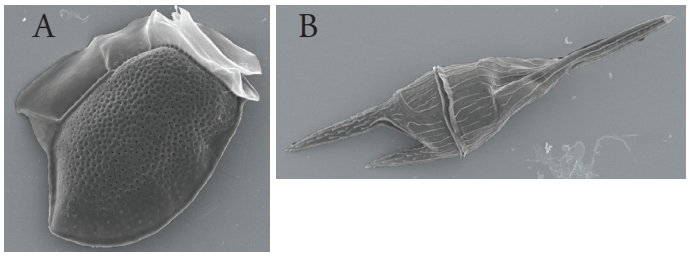

Fig. 2A. Picture of *Dinophysis acuta* (50 µm long).
Fig. 2B. *Ceratium furca* (85 µm long).
These species are studied in paper I and II. Photo: Therese Jephson.

The two largest groups of marine phytoplankton are the diatoms and the dinoflagellates. About 2000 marine species of dinoflagellates are described. Dinoflagellates are often abundant in summer or autumn and several are harmful, known as HAB-species (Harmful Algae Bloom) (Taylor 1987). Some genera that occur along the Swedish west coast and in the Baltic Sea are; *Dinophysis* (Fig. 2A), *Ceratium* (Fig. 2B), *Heterocapsa*, *Prorocentrum*, *Protoperidinium* and *Gonyaulax*. Some of these species are harmful because they produce toxins and others because they form high biomass blooms disrupting food webs, e g by causing oxygen depletion during breakdown. High densities of dinoflagellates may visibly color the water, producing so-called red-tides with their reddish-brown pigment (peridinin) (Hallegraeff 1994, Smayda 1997). Some species (i.e. *Alexandrium* spp., *Pyrodinium* spp. or *Gymnodinium* spp.) produces a neurotoxin called saxitoxin (Bates et al. 1978, Andersson et al. 1990, Heil et al. 2005), which is responsible for the paralytic shellfish poisoning (PSP) that could be lethal to people eating mussels/clams that have accumulated the toxin. Other dinoflagellates are the main contributor of an additional mussel toxin called DST (Diarrheic Shellfish Toxins), commonly found on the west coast of Sweden (Godhe et al. 2002). The genera *Dinophysis* include several toxin-producing species that are known to cause DSP (Diarrheic Shellfish Poisoning) (Yasumoto et al. 1980, Carmody et al. 1996, James et al. 1997). The problem with toxic mussels has been highlighted in several studies and is a major problem for the shellfish industry around the world (Lee et al. 1988, Cembella et al. 1989, Pavela-Vrancic et al. 2002, Morono et al. 2003).

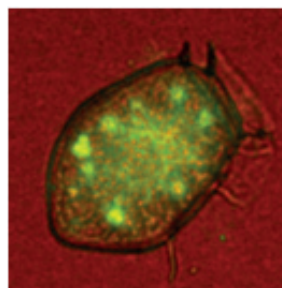

Fig. 3. The presence of possible food vacuoles in *Dinophysis acuta* using epifluorescence microscope and SYTOX-labeling. Photo: Therese Jephson.

Nutrient uptake strategies

Different species of dinoflagellates utilize different energy sources and both strict autotrophy and strict heterotrophy exists (Jones 1994, Stoecker 1998), as well as mixotrophy, which is the strategy combining autotrophy and heterotrophy (Taylor 1987). Mixotrophs ingest bacteria or eukaryotic prey to gain both organic substrates and inorganic nutrients (Arenovski et al. 1995, Caron et al. 2000). Most dinoflagellates are believed to be mixotrophic with some species being more autotrophic and some more heterotrophic (Stoecker 1998). Only few species are believed to exclusively obtaining all their energy from photosynthesis and many species that are considered autotrophic may be proven mixotrophic in the future (Jones 1994).

Several techniques have been used to demonstrate ingestion of prey by dinoflagellates. One of the techniques involves the findings of what has been interpreted as food vacuoles inside the dinoflagellate cell (Hansen 1998, Hansen and Calado 1999). Food vacuoles are observed in *Dinophysis fortii* (Koike et al. 2000) and the feeding mechanism has been reported for *D. acuminata* (Park et al. 2006)*)*. The mechanism was observed using microscope, revealing that *D. acuminata* uses a peduncle to extract the cell contents of the marine ciliate *Myrionecta rubra*. The peduncle is first used to connect prey and predator. The ingested material is then broken up into several small food vacuoles within the cells (Park et al. 2006). During my studies I observed the presence of possible food vacuoles in *D. acuta* using epifluorescence microscope and SYTOX-labeling (Fig. 3). These vacuoles can, however, easily be confused with protozoan parasites or accumulation bodies (Gisselson et al. 2002). I therefore used thin section transmission electron microscopy (TEM) to search for fractions of the ciliate prey within these cells. Unfortunately, I was not able to identify possible prey structures due to low quality of the samples (T. Jephson unpublished data).

The potential advantages of being mixotrophic rather than auto- or heterotrophic has been studied with variable results. Supposedly, mixotrophic flagellates may have the possibility to ingest prey when light is insufficient and use photosynthesis when light is sufficient (Arenovski et al. 1995, Christaki et al. 1999), meaning that they can compete with obligate phototrophic phytoplankton for soluble nutrients and with obligate heterotrophic flagellates for uptake of food particles. However, there may be a high cost for synthesizing both the photosynthetic and the heterotrophic apparatus. Mixotrophs may only have an advantage if light or prey density is limited (Raven 1997, Stoecker 1998), which has raise the question of mixotrophy as a survival strategy more than advantages for growth and to outcompete other species.

Motility

Dinoflagellates have the ability to move using their two flagella. They have one longitudinal flagellum directed posterior and one transversal flagellum (Taylor, 1987). The flagella are powered by adenosine-triphosphate (ATP). While actively photosynthesizing, ATP molecules are being produced during the light reaction and ATP is used to fuel the building of carbohydrates in the dark reactions. In the dark, photosynthesis ceases and ATP must be produced from other processes such as lipid hydrolysis (McKay 2006).

Swimming allows dinoflagellates to use light and nutrients throughout the water column and may be vital to their success in aquatic ecosystems. The swimming velocity may vary, depending on environmental conditions, species and cell size, and has been estimated to be between 50 and 500 μm s^{-1}. Some species can theoretically swim up to 1.8 m in one hour (Hasle 1950, Hand et al. 1965, Levandowsky and Kaneta 1987, McKay 2006). However, the net swimming velocity can be lower because of randomness in the swimming direction and, as mentioned, influenced by environmental conditions such as salinity, temperature and light (Hand et al. 1965, Kamykowski et al.1998). There is also evidence that heterotrophic dinoflagellates adapt their swimming strategy to increase their encounter rate with prey when the prey density decreases (Cosson et al. 1988, Sheng 2007). Furthermore, there are indications that swimming rate increase during light periods compared to dark ones (Baek et al. 2009) and the speed of descent is faster than that of ascent (Park et al. 2001), probably owing to gravity. Moreover, larger cells tend to swim faster than small ones due to larger carbon reserves (Kamykowski and McCollum 1986, McKay 2006), and a decrease in swimming velocity has been observed when salinity and temperature decreases below a salinity of 20 and a temperature of 14°C (Hand et al. 1965, Kamykowski and McCollum 1986).

Many dinoflagellates perform diel vertical migration (DVM) (Blasco 1978, Olsson and Graneli 1991), where cells typically swim downwards at night and back towards surface during daytime (Eppley et al. 1968, Heaney and Eppley 1981, Kimura et al. 1999, Flynn and Fasham 2003). Swimming capacity makes DVM possible and when performing this behavior cells may need to cross density gradients of both salinity and temperature.

Aim

Main focus has been on the ecological importance of diel vertical migration (DVM) in stratified environments (Fig. 4).

Specific questions have been:

- What are the causes behind vertical migration and varying vertical distribution patterns?
- How do dinoflagellates respond to temperature and salinity gradients during DVM?
- Do different species have different DVM strategies?
- How do dinoflagellates respond to different wavelengths of light during DVM?

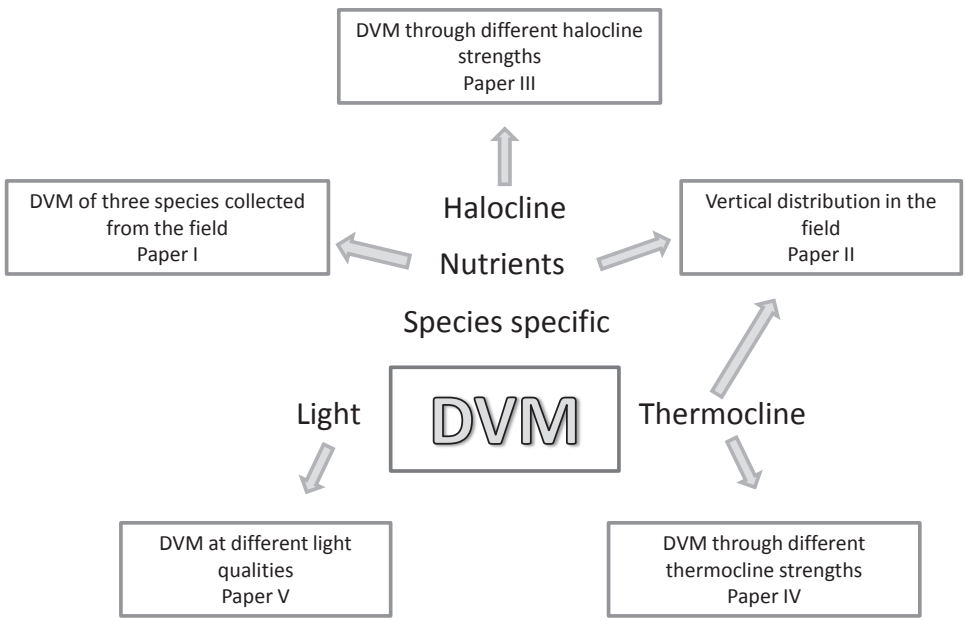

Fig. 4. A schematic representation of DVM behavior studied in Paper I-V.

Methods

In this thesis, I studied the responses to halo- and thermoclines in dinoflagellate species generally found along the Swedish coast and in the Baltic Sea. Experimental data are needed to complement field data, since the heterogeneity and fluctuating environment of the marine waters makes the causes affecting DVM behavior difficult to identify. Consequently, I have mainly performed my DVM experiments in the laboratory, although one field study is included.

Paper I

In paper I the aim was to observe DVM behavior in three dinoflagellate species commonly occurring in the natural phytoplankton. *Ceratium tripos*, *C. furca* and *Dinophysis acuta*, were collected from the Swedish west coast two days before the experiment started. The experiment was carried out in an artificial stratified filtered (1 µm mesh) water column inside four PVC cylinders (2 m high and 0.4 m in diameter) with a weak salinity gradient. The salinity in the nitrogen depleted surface water was 18 and salinity in the nutrient enriched bottom water was 22. Light levels in the halocline were similar to *in situ* light levels in the cline at midday. The DVM behavior of the three species was studied by estimating cell density from water samples collected from the cylinders at ten vertical positions during a 48-hour period. The sampling times were 05.00, 08.00, 12.00, 17.00, 21.00 and 24.00. Additionally, we measured photosynthetic carbon uptake by the cells at different depths inside the experimental cylinders to evaluate the importance of photosynthetic capacity in relation to the vertical position chosen by the species.

Paper II

In this paper, we studied a natural dinoflagellate community in late summer in the stratified Gullmar Fjord on the west coast of Sweden. Here we aimed to observe the vertical distribution and altering DVM behavior of dinoflagellates around the pycnocline *in situ*. The Gullmar Fjord has an entrance sill at 43 m depth and a maximum depth of 120 m. The water below the sill is composed of Atlantic saline water, which enters the fjord via the North Sea and the Skagerrak, whilst the upper water column is derived from surface Kattegat and Skagerrak waters, together with freshwater inflows to the fjord. The upper water column is often stratified with respect to both temperature and salinity (Arneborg, 2004). We performed three separate 48-hour surveys to study the natural dinoflagellate community. Cell density of the dominating *Ceratium* and *Dinophysis* species was estimated, using an inverted microscope, in samples taken at five vertical positions. Vertical profiles of chlorophyll *a* fluorescence, light, salinity, temperature and inorganic nutrients were also measured. Samples were taken at 12:00, 18:00, 24:00 and 06:00.

Paper III

In paper III we carried out an experiment to test for differences in vertical migration behavior at different halocline strengths. Here we studied monocultures of *Prorocentrum minimum* and *Heterocapsa triquetra* isolated from the west coast of Sweden and the Baltic Sea area, respectively. DVM behavior during three separate stratification situations was studied separately in the laboratory. Stratification treatments consisted of three different salinity gradients: 6, 11 and 16 salinity differences between the surface and bottom water. Bottom salinity was always 26 and surface salinity was 10, 15 or 20. Migratory behavior was studied in 0.5 m high plastic cylinders (0.12 m in diameter) at 12:00, 18:00, 24:00 and 06:00 over 96 hours. Nitrogen was depleted in the surface water during the experiment to trigger the nutrient deficient cells to swim down towards the nutrient rich bottom water. We measured the initial C:N ratio of the cells and at the end of the experiment by using total organic nitrogen (TON) and carbon (TOC).

In this paper we also studied growth rates of *P. minimum* and *H. triquetra* at 4 different salinities at 3 different temperatures. The monocultures were grown at salinities; 10, 15, 20, and 26 at 10ºC, 15ºC and 20ºC. Net growth rates were estimated by cell counts during the exponentially growing phase of the cells and calculated as the change in cell number over time.

Paper IV

In paper IV we aimed to observe the DVM pattern of *H. triquetra* and *P. minimum* in temperature stratified conditions. Here we used artificial temperature stratified water columns created in Plexiglass cylinders (2 m high, 0.15 m diameter). Stratification treatments consisted of creating two different temperature gradients (with differences of 10°C and 17°C between upper and lower water masses) in separate cylinders. Vertical position of cells was estimated from counting cells from water samples taken from each cylinder at 13 different vertical positions at 12:00 and 24:00 over a period of 96 hours. In this paper we also hypothesized about decreasing swimming velocities in the colder bottom water. Swimming velocity was studied in the 8°C bottom water and the 18°C and 25°C surface waters, using a video system consisting of a TV/video recorder Philips (model 14PV-203/01) and a video camera (Nikon Digital Sight) fitted to an inverted microscope.

Paper V

In this study, we aimed to investigate the effect of light quality (daylight, blue- and red light) on DVM behavior in *Alexandrium minutum.* We hypothesized that a pulse of red or blue light will alter the phase of the DVM rhythm resulting in that cells will ascend/descend earlier/later depending on if they are exposed to blue- or

red light. We also hypothesized that cells would continue migrating in darkness due to an internal clock. Once again, DVM behavior was observed by estimating cell density at different vertical depths in artificial water columns inside plastic cylinders. Sampling occurred at 09:00, 13:00, 17:00, 22:00, 01:00 and 06:00 over 72 hours. During the first sampled 24 hours, light conditions were identical in all treatments (12:12 light:dark) and light was turned on at 07:00. After this, in one treatment, cylinders were henceforth exposed to the previous light:dark cycle and in another treatment light was kept off. In the two remaining treatments, cells were exposed to red and blue light pulses (2 hours) at 07:00.

In this paper, we also aimed to study differences in gene expression of the light-driven rhodopsin proton pump by using real-time quantitative PCR. Cells were exposed to a 2-hour red/blue light pulse, normal daylight or darkness. Cells were harvested and good quality RNA was extracted.

Results and discussion

DVM in stratified waters

The ability to cross halo- and thermoclines during DVM may be important for the survival and growth of dinoflagellates (Rasmussen and Richardson 1989). The results in paper I–IV indicate variation among species in their behavioral responses when migrating through density gradients. Some of the earliest studies and most cited papers that address dinoflagellate DVM in stratified waters include the results by Kamykowski and Zentara 1977, Blasco 1978, Cullen and Horrigan 1981, Heaney and Eppley 1981, Kamykowski 1981. These studies include several species within different dinoflagellate genera's and many species are able to migrate through weak density gradients, which were also observed in Paper I-III. However, my results also indicate that both halo-and thermoclines act as barriers during DVM (paper III and IV). Blasco (1978) showed that even weak density gradients may act as barriers. Moreover, some species may cross weak gradients but change their DVM behavior when stratification strengths increase (paper III and IV). For example, the dinoflagellate *Cachonina niei* crossed a 10ºC gradient but not a 15ºC gradient (Kamykowski 1981). *Ceratium furca* was shown to cross a 2ºC (Eppley et al. 1968) and 7ºC temperature gradient (Kamykowski 1981). In addition, *Prorocentrum micans, P. minimum* and *C. furca* cross a 7ºC temperature gradient and a strong salinity gradient (salinity difference of 14) during their DVM (Olsson and Graneli 1991). Based on my observations, *P. minimum* had difficulties in migrating though both salinity and temperature gradients and was mainly observed in the bottom water when exposed to both weak and strong gradients (paper III and IV). *Heterocapsa triquetra,* however, exhibited different DVM behavior depending on the strengths of the salinity gradient. Cells migrated through salinity gradients of 6 and

11 but the salinity difference of 16 acted as a barrier for *H. triquetra,* and resulted in a concentration of cells in the cline during the night. At midday, cells were again found at the surface. A salinity gradient of 16 is often found in the Öresund area, but also a salinity difference of 11 is relevant in the Baltic Sea or coastal areas on the west coast of Sweden (e.g. Brøns Hansen et al. 2003). *H. triquetra* growing in this region may increase its fitness by shifting position during periods of surface water nutrient depletion and thus form blooms, maybe faster than *P. minimum*.

In the experiments in paper IV, we focused on thermoclines and the temperature difference was 10°C and 17°C. These differences in temperature match to a density gradient similar to what you will find at halocline strengths of 11 or 16, studied in paper III. As mentioned, *P. minimum* was found mainly in the bottom water while *H. triquetra* was mainly observed in the warmer surface water. Salinity was 28. The warmer surface water ought to have been more beneficial for growth in relation to temperature and light. However, nutrient concentrations were low compared to the bottom water, which may be one reason for *P. minimum* cells to aggregate in the colder bottom water. Light was still sufficient for photosynthetic growth below the thermocline. Moreover, the surface temperature of 25˚C is a high temperature for these coldwater species and not commonly reached in temperate regions (paper IV).

The thermocline may be associated with the halocline in estuarine areas, meaning that motile plankton need to cope with both salinity and temperature differences simultaneously. From the results in paper III, I show that the combination of salinity and temperature is important for growth rates (Matsubara et al. 2007). My results indicate that *P. minimum* and *H. triquetra* grow faster in higher temperature, but only when grown in salinities higher than 15. At 10°C, growth rates were not affected by different salinities (Fig. 5, paper III). As mentioned earlier,

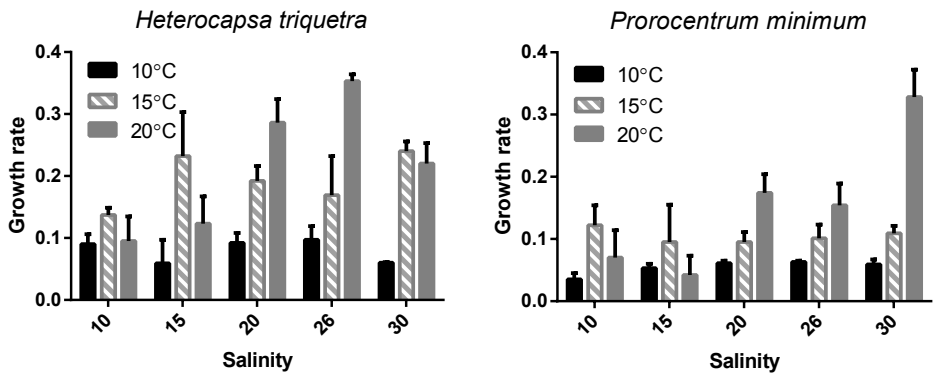

Fig. 5. Growth rates (mean and SD) of *Heterocapsa triquetra* and *Prorocentrum minimum* grown in five different salinities and at three different temperatures. The figure is redrawn from data included in paper III.

P. minimum were mainly observed in the bottom water during my experiments, where salinity was 26 in both studies and temperature was 8°C (paper IV) and 15°C (paper III). Apparently, cells prefer not to migrate through either haloclines or thermoclines during DVM to reach the surface water with higher light intensities. The more saline, nutrient-rich bottom water was likely more beneficial for *P. minimum* during existing environmental conditions. Comparing growth between if cells are growing in surface water conditions with if they are growing in bottom water conditions indicate that there are no significant difference in growth rates when grown at rather low light levels, as in my DVM experiments.

Blooms of both *P. minimum* and *H. triquetra* are observed in low salinity environments (e.g, Kononen et al. 2003, Hajdu et al. 2005) which demonstrates the physiological flexibility of these species. There is a possibility for clonal variation within a species and the Baltic Sea clones may be well adapted to a low salinity (Kremp et al. 2009). In my experiment, growth rates were higher for *H. triquetra* than for *P. minimum* at all salinities and temperatures (Fig. 5), which implies that *H. triquetra* will have an advantage during similar temperature and salinity conditions in nature.

In addition, studies have shown that the extent of DVM is dependent on species-specific swimming velocities *in situ* (Throndsen 1973, Levandowsky and Kaneta 1987). Our results on swimming velocities of *P. minimum* and *H. triquetra* at different temperatures indicate that cells swim faster in higher temperature, at 18°C and 25°C, but the observed swimming rate at 8°C still enable the cells to perform a vertical migration in the experimental cylinders. The swimming velocities calculated in paper IV indicate that cells may perform DVM back and forth to a nutrient rich layer.

Even though many species have high tolerance for different temperatures and salinities, there may be additional energy costs for these species when crossing strong gradients and not growing in their optimal environment (Nakamura and Watanabe 1983, Matsubara et al. 2007). Lower swimming speed, time lags or aggregation below the pycnocline may be the result of extra energy costs (Erga et al. 2003, Kimura et al. 1999). There may also be an acclimatizing period for species when the environmental conditions change rapidly (Nagasoe et al. 2005) which means that it takes a longer time for cells to pass the cline. According to the results in paper III, the costs of migrating through the salinity gradient of 16 were higher than the costs of growing in the low salinity layer. In theory, each species (or each cell) would act to optimize the balance between costs and benefits of their behavior. Species that are best adapted to fast adaptation and have a wide range of tolerance may have an advantage over other species during stronger shallower stratification regimes and earlier nutrient depletion of the surface water.

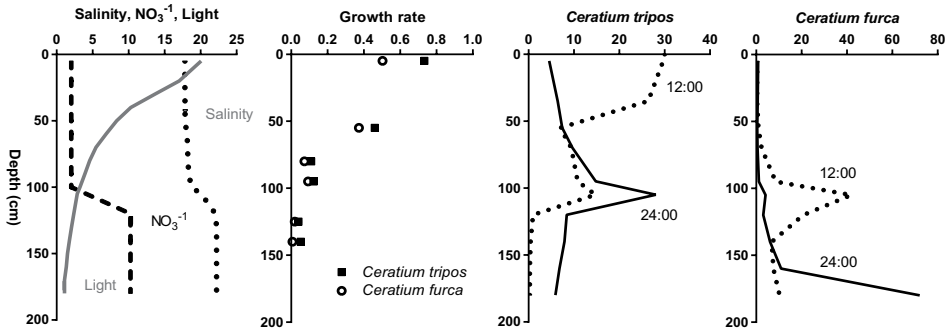

Fig. 6. Schematic picture of the different DVM behavior (cell density) at 12:00 (dotted line) and 24:00 (solid line) between species within the same genera of *Ceratium* spp. The salinity, inorganic nitrogen (µM), light levels (µmol photons m^{-2} s^{-1}) and growth rate at each depth in the 2 meter high cylinders used in the experiment in paper I are also shown. The figure is redrawn from mean data from paper I.

Interestingly, my results show that a difference in DVM behavior exists not only between genera's, but also between species within the same genera (Fig. 6, paper I). I observed a clear DVM pattern of *C. furca* and *C. tripos* where they did not cross a salinity gradient of five, but instead exhibited DVM exclusively below (*C. furca*) and above (*C. tripos*) the halocline (Fig. 6, paper I). Such an obvious vertical separation in distribution patterns of two closely related species, just collected from the field before the experiment, shows that the species cope with the existing environmental condition in unique ways, demonstrating different trade-off strategies.

Vertical distribution *in situ*

Even though the DVM behavior is an acknowledged strategy for many dinoflagellate species, few studies have repeatedly observed the behavior in stratified waters *in situ*. Instead, several field observations report on vertical heterogeneity and a distribution of phytoplankton where high densities is typically found within or near a pycnocline (Nielsen et al. 1990, Kononen et al. 2003, Velo-Suárez et al. 2009, Lips et al. 2010). These subsurface, high cell concentrations are often called deep chlorophyll maxima (DCM). Production in these layers in late summer may exceed surface production during spring blooms (Richardson et al. 2000). DCM is formed by aggregation of a variety of photosynthetic plankton, including dinoflagellates (Fee 1976). Aggregation of toxic-producing dinoflagellates may produce a highly toxic area and the toxins may later spread to the entire water column. Maximum density of *Dinophysis norvegica* was observed below the surface, at 22 meters (i.e. around

the thermocline) in the Baltic Sea (Gisselsson et al. 2002) which indicate that there is a need for replicated sampling both in time and in vertical position during monitoring in this area. Especially since toxicity may differ within cells between populations with high density compared with cells in low-density populations. It has been suggested that a population with high density of *D. acuminata* has less toxins per cell, while a low-density population has higher toxin content per cell. Thus, 100 high-toxicity *D. acuminata* cells in a low-density population at surface may lead to the same accumulation of toxin in a mussel as the ingestion of 1500 low-toxicity cells from a high-density population (Lindahl et al. 2007).

Heterocapsa triquetra bloom and form DCM in the Baltic Sea (Kononen et al. 2003, Lips et al. 2010) and according to Setala et al. (2005), light levels at DCM may not always support efficient autotrophic growth. This indicates that supplementary nutrient intake is needed to support growth at these depths. The pycnocline may include potential prey species (e.g. bacteria, other phytoplankton or ciliates) which benefits mixotrophic algae and lead to high concentrations of mixotrophic cells at specific depths. Areas with high densities of phytoplankton and/or bacteria are also called biological stratification or thin layers and are often correlated with thermoclines (Steinbuck et al. 2009) and haloclines (Rines et al. 2002). The formation of thin layers occurs in the oceans and in estuarine environments. Several layers, a few mm wide, may be visibly very clear during calm weather along the Swedish west coast (pers. obs. T. Jephson). These very fine layers of increased concentration of cells indicate the need for representative resolution when sampling and searching for DVM behavior and subsurface blooms. Unfortunately, few replicated studies with more representative resolution exist (but see; paper II, Velo-Suárez et al. 2009, Hällfors et al. 2011, Katano et al. 2011). The vertical resolution in these studies may still not be enough to detect very fine thin layers of mixotrophic dinoflagellates.

In the field study (paper II) I focused on evaluating DVM behavior and vertical distribution in a Swedish stratified fjord. During this survey environmental conditions changed rapidly, disturbing DVM behavior. During a period of stable abiotic conditions, preceded with period of calm seas, DVM behavior of *Dinophysis* spp. and *Ceratium* spp. was observed. However, no DVM was noticed just after a gale with strong impact of hydrological forces and incoming water masses passed the area. Also, Chl a maxima changed vertical position during calm weather situation and the peak in Chl a fluorescence was significantly deeper and closer to the halocline at midnight and early morning compared to midday and early evening (Fig. 7, paper II). The vertical movement of Chl a maxima was disrupted by the gale. In summary, under calm weather conditions dinoflagellates may actively migrate, but as turbulence increases, dinoflagellates may be unable to maintain their swimming possibilities and the vertical trajectories becomes less distinct due to the inability of the cells to determine their location in the water column (Thomas and Gibson 1990, Kiørboe 1993, Smayda 2002). There are, however, indications

of dinoflagellates coping very well with moderate turbulence, and swimming rate of many species exceeds *in situ* vertical current velocities, which may allow migration behavior to be maintained (Smayda 2002). Moreover, laboratory studies show that swimming direction recovers after 6 days in no-turbulent conditions after cells were exposed to high turbulent mixing (Thomas and Gibson, 1990). This indicates that the dinoflagellates studied in paper II may carry on their migration behavior when the environmental situation once again stabilizes.

The results from paper II suggest that *Dinophysis* species mainly occurred above the pycnocline, in contrast to *Ceratium* species (Mouritsen and Richardson 2003, Lindahl et al. 2007). These results agree with previous observations on the vertical distribution of *D. acuta* from the Gullmar Fjord (Lindahl et al. 2007), and recent observations in the Baltic Sea which showed that *D. acuminata* was restricted to the upper 9 meters and did not perform DVM (Sjöqvist and Lindholm 2011). In addition, during no-turbulent conditions, *D. acuta* aggregate above the halocline in the area closest to the surface, while *C. furca* aggregated in the cline during midday and migrated towards bottom during the dark period (paper I). This vertical niche separation found in both paper I and paper II is hard to explain but I exclude light as the main explanation since *Dinophysis* spp. and *Ceratium* spp. seem to have similar carbon fixation rates (Graneli et al. 1997). In paper I, I observed that carbon uptake differ somewhat between *C. furca* and *C. tripos* (Fig. 6) at low light intestines,

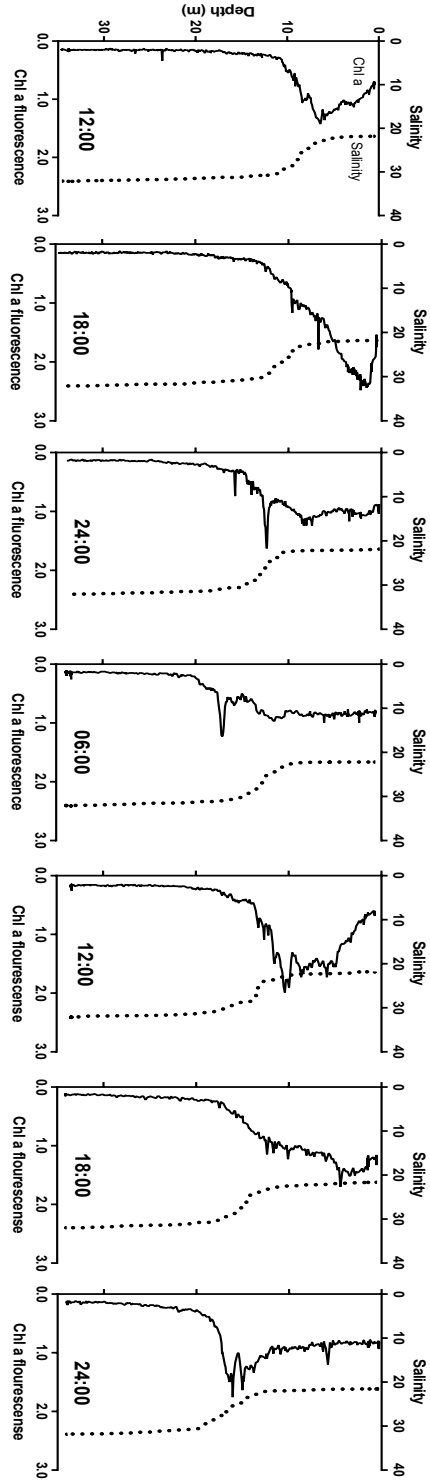

Fig. 7. Chl a fluorescence and salinity measured during two days (starting Aug 23 12:00 and ending at Aug 24 24:00) in the Swedish Gullmarn fjord, during calm weather conditions. The figure is redrawn from data included in paper II.

but this is likely not enough to explain the vertical niche separation between the species. Possible coexistence of phytoplankton species by vertical niche separation is suggested from models (Huisman et al. 2006) and have been shown during field observations (Sommer 1982, Olli and Seppala 2001). Information on vertical niche separation among dinoflagellates is important when we interpret data from sampling and monitoring *in situ*.

The mechanisms and causes behind DVM

The strategy of diel vertical migration (DVM) is believed to be important for the survival and ecology of dinoflagellates (Eppley et al. 1968, Kohata and Watanabe 1986) and the factors regulating migration behavior is most likely primary linked to light- and nutrient conditions. However, several hypotheses exist and although DVM has been widely documented in various dinoflagellate species (Eppley et al. 1968, Kamykowski and Zentara 1977, Cullen and Horrigan 1981, Salonen et al. 1984), the ultimate cause behind the strategy is not clear.

Optimization of photosynthesis

Phototaxis is generally thought to be the most important mechanism behind the vertical position of dinoflagellates in the water column and the diel vertical migration of dinoflagellates has long been considered a positive phototactic response (Hasle 1950, Schaefer et al. 1994). The downward movement during nighttime may be because of negative phototaxis or passive sinking (Taylor 1987). The DVM pattern observed within my thesis indicates that cells swim towards light during daytime (paper I-V), which is likely the result of a phototactic response. In paper V, I observed increased cell densities closer to the light source when cells were exposed to both white, blue, or red light. When light was turned off, cells were mainly found in the bottom water, also at midday. In the 12:12 light:dark treatment cells were observed to follow the cycle of staying closer to the surface during the light period and closer to the bottom at night.

Light intensity fluctuates on a regular basis *in situ* and when light intensities are too strong, at midday, phytoplankton may remain at a subsurface location to avoid photoinhibition (Flynn and Fasham 2003). Such a subsurface location of phytoplankton (measured as Chl a) at midday was observed in paper II (Fig. 7, Katano et al. 2011). However, light at the very surface also includes ultraviolet radiation (UVR) that may be harmful to the plankton species if exposed to high concentrations (Williamson et al. 1994, Bancroft et al. 2007). Nevertheless, many phytoplankton species seem to handle the natural UV-levels by synthesizing and accumulating the natural sunscreens mycosporine-like amino acids (MAAs). A

rapid increase in MAAs was revealed in the dinoflagellate *H. triquetra* when exposed to UV-light, which indicates that there is a plastic response in order to handle UVR exposure (Hylander and Jephson 2010).

To be able to detect light, dinoflagellates need photoreceptors. Three families of photoreceptors have been identified in photosynthetic organisms: phytochromes, cryptochromes, and phototropins (Fankhauser and Staiger 2002). Phytochromes absorb in the red/far-red region of the visible spectrum (600–800 nm wavelength), while the cryptochromes and phototropins absorb in the UV-A/blue region (350–500 nm wavelength; Ahmad 1999). Both phytochromes and cryptochromes have been characterized in algae (Hegemann 2008), but it was only recently that the first blue-light receptor was identified in a dinoflagellate. The blue-light receptor, called KbCRY (Brunelle et al. 2007), is a 55kDa protein located in the chloroplast of the dinoflagellate *Karenia brevis*. We hypothesize that a blue-light receptor exists also in *Alexandrium minutum* since a phototactic response to blue light was observed in paper V.

Additionally, Hartz et al. (2011) have recently suggested that rhodopsin plays a role in phototactic behavior in dinoflagellates. They found that phototaxis in the heterotrophic *Oxyrrhis marina* decreased significantly when an inhibitor of rhodopsin (hydroxylamine) was added to the culture (Hartz et al. 2011). Rhodopsins are light-driven proton pumps. Their specific function is to harvest light energy non-photosynthetically (Spudich and Jung 2005) and it remains to be determined what physiological processes that benefits from the rhodopsin generated energy in dinoflagellates. Transcripts of rhodopsin or homologs to rhodopsin have recently been identified from dinoflagellates grown in the laboratory, including, *Pyrocystis lunula*, *Polarella glacialis, Alexandrium catenella* and *O. marina* (Okamoto and Hastings 2003, Lin et al. 2010, Slamovits et al. 2011), which suggests that rhodopsin genes are ubiquitous in dinoflagellates. Rhodopsin in dinoflagellates may enable these organisms to harvest and convert solar energy to ATP. Supposedly, the protons may be pumped across the cell membrane to generate power to drive nutrient transport or flagella motion. In paper V, we observed that *A. minutum* express rhodopsin genes by using real-time quantitative PCR. The results showed unchanged transcript levels in *A. minutum* at daylight, blue, and red light intensities, as well as in cells grown in darkness. With my expression data, and since a phototactic response was observed in all light treatments except in darkness, I cannot determine if rhodopsin acts as the gene that mediates phototaxis in *A. minutum*. Many genes seem to be regulated post-transcriptionally in dinoflagellates (Erdner and Anderson 2006, Moustafa et al. 2010) and rhodopsin may be constantly expressed in the cells so that they are prepared to react and move immediately when exposed to a light-pulse.

I find it interesting and essential to find out more about photoreceptors and their relevance for DVM at different wavelengths since light quality varies vertically in the water column because of the absorptive and scattering processes of the water (Kirk 1994). Water strongly absorbs light in the red and infrared wavebands. Much of the red light is therefore extinct at rather shallow depths, but low intensities of red light is likely present throughout the photic zone (Ragni and D'Alcala` 2004).

Circadian rhythm

Even though many studies imply that the DVM behavior is strictly linked to some type of physical response, such as phototaxis, this view is debated since modeling studies indicate the possibility for DVM to continue in complete darkness for several days without running out of energy (Flynn and Fasham 2003). In addition, cells tend to start to descend before sunset and often ascend before dawn (partly observed in paper I, Eppley et al. 1968, Weiler and Karl 1979, Cullen and Horrigan 1981, Kamykowski 1981, Olsson and Graneli 1991, Katano et al. 2011). This suggests the possibility of an internal clock that controls the rhythm. Circadian clocks are common in phytoplankton but not well studied in dinoflagellates. The exception is the circadian rhythm of bioluminescence in *Gonyaulax polyedra* that has been studied for several years (Roenneberg and Morse 1993, Mittag et al. 1994). The circadian expression of luciferin-binding protein (LBP) has served as a model to study the mechanism of such rhythms, and LBP synthesis is controlled at the translational level (Morse et al. 1990). Bioluminescence continues for several weeks in cultures kept at constant conditions. However, the rhythm is not always 24-hours, but a little bit shorter. Light has an auxiliary role in the phenomenon since the clock may need a light pulse at a certain clock time to control the phase of the rhythm during the 24-hour period (Kondo et al. 1991). Moreover, increasing intensities of continuous blue light will cause the period to become shorter, whereas higher intensities of constant red light will result in longer periods (Roenneberg and Hastings 1988). In my experiments, phase shifting during DVM was not observed when I exposed the cells to blue- or red light (paper V).

Nutrient acquisition

A discussed and supposed stimulus for dinoflagellates to migrate vertically is the need for nutrients (Cullen and Horrigan, 1981, Heaney and Eppley 1981). Some researchers hypothesize that cells only need to migrate when the cells are nutrient depleted. For example, modeling studies indicate that dinoflagellates begin to migrate when nitrate is depleted in the surface (Carpenter et al. 1995, Flynn and Fasham 2003). During nitrogen depletion in the surface water, cells increased their N:C ratio by crossing the halocline at nighttime and increasing their nitrogen uptake (paper III). Thus, increased growth rates after performing DVM is probable.

The results in paper III indicate that the nutrient depleted surface water was the trigger for migrating downwards at nighttime, but in paper I, IV and V there is no evidence that nutrients are the most important reason for migrating. I observed that cells migrated exclusively within the nutrient-free surface layer or in the nutrient-rich bottom layer in paper I and IV. During exclusively nutrient-rich conditions in the water column, cells tended to concentrate more in the bottom water during night and at the surface during day (paper V). When light was turned off during daytime, cells started to move towards the bottom. The reason why cells moved towards bottom, despite high nutrients levels at the surface may be due to negative phototaxis or passive sinking.

Predation

The concept of "cascading migration" has recently been introduced as a possible phenomenon in planktonic behavior. There is broad consensus that avoidance of predators is the primary driver of DVM in zooplankton (Lampert 1989, Bollens and Frost 1989), but recent experiment also indicate that dinoflagellate vertical distribution and migration is influenced by their grazers, and not only by light or nutrients (Quenette 2010). For example, the dinoflagellate, *Akashiwo sanguinea* migrated vertically (up during daytime and down during night) both in presence and in absence of a copepod predator, however the amplitude of the DVM was enhanced by the presence of predators (Bollens et al. 2012). This implies that migrations that occur at one trophic level can affect the vertical migration of the next lower trophic level, and so on, throughout the food web (Bollens et al. 2011). Grazers were not included in any studies within this thesis and the trade-off during DVM, between gaining enough energy and being eaten, will be interesting to pursue further.

Concluding remarks and future perspecitives

The results from my research show evidence for species-specific DVM behavior and that dinoflagellate cells alter their DVM behavior depending on the strengths of haloclines and thermoclines. The pycnocline can thus be viewed as a barrier between the nutrient rich bottom water and the surface water with high light radiation. This suggests that an increase in stratification, both stronger temperature gradients in the open ocean and salinity gradients in estuarine areas will benefit migrating species able to cross the gradient during DVM. These species will increase their fitness if they are able to access the deep nutrient pool. Moreover, if different species have different optimal growth conditions they may have different trade-off strategies when choosing the most suitable position in the water column and a geographical separation among species is to be expected. However, during powerful mixing of the water column i.e. during strong winds, the direction and possibility for the dinoflagellate cells to perform DVM may be limitied and result in that the cells may be unable to continue the behavior.

In addition, the results from my studies indicate that the primary trigger for vertical migration is light in combination with an internal clock controlling the behavior. However, nutrients cannot be excluded as a trigger since the reason for maintaining DVM behavior is likely to benefit from the accumulation of nutrients in the deep during night.

Today, harmful algal blooms (HABs) affect nearly every coastal region of the world and most blooms occur in coastal marine environments. This thesis adds information on the behavior of dinoflagellates during their DVM in stratified waters, but further knowledge is needed to draw definitive conclusions of the importance of the behavior in a changing world. I agree with Doney (2006), that if we are going to be able to detect the impact of climate change we need increased and more sophisticated monitoring and not only of variables such as chlorophyll concentration and productivity, but also of plankton taxonomy and physiology. Today, climate models predict that global warming will strengthen stratification and reduce vertical mixing in the oceans, which according to the results from my studies, will generate more variability in dinoflagellate vertical migrations.

Efforts are made into designing accurate models that predict harmful algae blooms and these models need to be derived from reliable experimental and observational data. High resolution sampling and repeated measurements in time is needed to be able to detect DVM behavior in the field and species-specific data may need to be coordinated and integrated in the models (e.g. Ji and Franks 2007, Ralston et al. 2007). If monitoring is restricted to the upper ten meters (for example if monitoring is done by using transport ships or ferries) we may fail to notice an important subsurface bloom. To predict harmful algal blooms of vertically migrating species the migration patterns and the growth rates in the natural environment needs to be further clarified for each species.

Interestingly, vertically migrating organisms that feed or photosynthesize at one depth and then move to another depth to respire, excrete, or be preyed upon, serve to actively transport material and energy through the water column. Understanding the role of the migration-driven biological pump in carbon cycling is becoming more relevant, considering the increasing input of CO^2 to the oceans (Palacios et al. 2004).

Another interesting concern when it comes to the discussion on the responses and behavior in dinoflagellates is that there may be physiological differences not only between closely related species, but also within a population. The genetic diversity within a population have been revealed in diatoms (Rynearson and Armbrust 2000), raphidophytes (Lebret et al. 2012) and dinoflagellates (Rengefors et al. 2012), indicating that each cell in a population may be genetically unique individuals. Genetic differences may cause physiological differences (Rynearson and Armbrust

2000), and it is suggested that daughter cells receive unequal shares of the parental resources. The differential allocation may influence their vertical migration behavior, since the nutrient poor daughters may be more positively phototactic (Janowitz and Kamykowski 2006). This may result in different DVM behavior among cells within a population. There is however no evidence for cell specific DVM behavior yet.

Finally, studies on gene expression (including transcriptomics, proteonomics) will continue to be an important approach to address behavior ecology in dinoflagellates. Increased knowledge on which genes that are expressed and which proteins that are synthesized in the cells at specific times of the day may be crucial to really understand the processes that control DVM.

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