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Influence of salinity on seasonal germination of resting stages and composition of microplankton on the Swedish west coast

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ABSTRACT: Surface sediment from Gullmar Fjord, Sweden was cultured in the laboratory to assess the influence of different salinities on the germination of benthic resting stages and subsequent vegetative growth. Sediment cultures were grown in media with salinities of 15, 25, and 35‰ in both spring and summer conditions. Many microplankton species grew in the cultures. Dominant taxa were the diatoms *Chaetoceros*, *Detonula*, *Skeletonema*, and *Thalassiosira*, and the dinoflagellates *Diplopsalis*, *Scrippsiella*, and *Oblea*. Growth of *T. minima* and *T. pseudonana* after more than 2 yr of storage provides new evidence of a dormant stage in these species. Salinity significantly influenced germination only in *D. confervacea* and *O. rotunda*, but it showed significant effects on growth for all the dominant taxa. Salinity optima of the microplankton in the experiments were compared to salinity ranges of these species in fjords on the Swedish west coast. In the fjords, *D. confervacea* grows poorly at salinities >27‰ and may be outcompeted by halotolerant species, such as *C. socialis* and *T. minima*. Because salinity is most variable in spring, a wide salinity tolerance is particularly advantageous during this season. The dominant dinoflagellates were well adapted to salinities between 22 and 35‰, and *O. rotunda* is favored at the high end of this range. Results illustrate how climate-related changes in sea-surface salinity may alter the microplankton community on the Swedish west coast through direct effects on resting-stage germination and planktonic growth.

KEY WORDS: Resting stages · Cysts · Spores · Germination · Diatoms · Dinoflagellates · Salinity · pH

INTRODUCTION

Growing concern about harmful algal blooms has furthered recent study into the ecological role of resting stages in microplankton life cycles. Germination of resting stages and successful growth of newly germinated vegetative cells can play a significant role in coastal microplankton dynamics and bloom formation (Ishikawa & Taniguchi 1997, Itakura et al. 1997, Kremp 2001). For microplankton in coastal areas, some sudden increases in population cannot be explained by vegetative division alone. Environmental cues that control resting stage germination and growth may determine the seasonal patterns of a species (e.g. when a species appears in the plankton) and the overall success of a population. It is, therefore, important to understand how environmental factors regulate germination and growth on a species-by-species basis.

Seasonal cues, such as temperature and light, regulate resting-stage germination in both diatoms (Eilertsen et al. 1995, McQuoid & Hobson 1995) and dinoflagellates (e.g. Binder & Anderson 1987, Kremp 2001). For some dinoflagellates, a required maturation period may also restrict germination to a particular season (Anderson & Keafer 1987, Nuzzo & Montresor 1999, Kremp & Anderson 2000). In contrast, nutrients appear to play a minor role in the revival of resting stages (e.g. Cannon 1993). Salinity influences microplankton productivity and growth (Brand 1984, Rijstenbil et al. 1989, Ellegaard et al. 1993, 2002, Jensen & Moestrup 1997), and freshwater influx has been linked to some harmful blooms in coastal areas (Taylor & Haigh 1993, Hallegraeff et al.

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1995, Weise et al. 2002). Although salinity is known to affect resting-stage formation and morphology (Oku & Kamatani 1997, Ellegaard et al. 2002), the influence of salinity on germination is unknown for diatoms; for dinoflagellates, it is known for only 2 species of *Alexandrium* (Cannon 1993, Kim et al. 2002).

The objective of this study was to examine the influence of salinity on the germination and subsequent growth of diatom and dinoflagellate resting stages from natural sediments. The Swedish west coast was selected for this study because it has a wide range of salinity and a diverse microplankton community. The outflow of low salinity water from the Baltic Sea, together with the inflow of higher salinity water from the North Sea, has a major influence on salinity along the Swedish west coast. In this region, salinity can vary in response to large-scale climatic forcing patterns, such as the North Atlantic Oscillation (NAO). Analyses of time-series data suggest that climate variations, such as NAO, may be important in structuring coastal microplankton communities (Belgrano et al. 1999, Irigoien et al. 2000). Moreover, climate models suggest that strengthening of the polar front, and thus intensification of NAO, will lead to altered salt exchange and content in the North Sea and the Baltic Sea (Schrum 2001, Edwards et al. 2002). Given the strong influence of these seas on local hydrography, we can expect that the Swedish west coast will also experience climate-related changes in salinity.

Along the Swedish coast, many microalgae show an affinity for either low or high salinity (Snoeijs et al. 1993–1998, Godhe & McQuoid 2003), but the role of resting stage germination in these distributions is unclear. In this study, germination of resting stages and subsequent vegetative growth were examined under controlled salinities. The salinity effects observed in the laboratory were compared with the seasonal distribution of microplankton in Swedish fjords. The use of 'resting stages' in this paper includes sexually formed dinoflagellate cysts, non-sexual diatom resting spores, and physiologically resting diatom cells (McQuoid & Hobson 1996, Montresor 2001).

MATERIALS AND METHODS

Sediment sampling. Sediment cores were sampled from Gullmar Fjord (58° 17′ N, 11° 30′ E) on the Swedish west coast (Fig. 1). Cores taken in November 2000 and May 2003 were used in 2 separate experiments. The top centimeter of each core was sampled immediately and stored in darkness at 5°C. The surface sediment collected in November 2000 was used to inoculate experimental cultures in November 2001, and surface sediment collected in May 2003 was used for experiments in January 2004. After at least 8 mo of storage in darkness, it was assumed that all dinoflagellate cysts had completed their dormancy requirements and that non-resting stages were no longer viable.

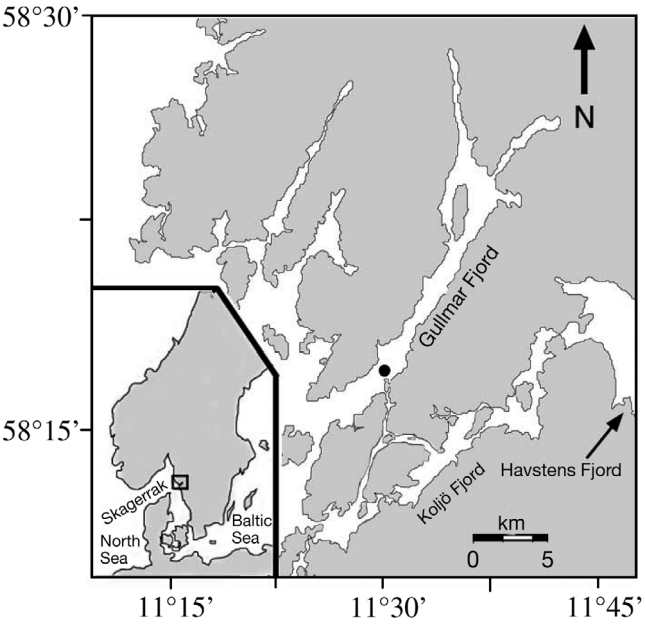

Fig. 1. Gullmar Fjord on the Swedish west coast. Sediment sampling station is marked (●)

Seasonal germination and growth at different salinities. Sediment collected in 2000 was cultured in November 2001 for 20 d to monitor the germination of resting stages and subsequent vegetative growth. Natural seawater collected from a depth of 35 m in Gulmar Fjord was diluted with deionized water to salinities of 15, 25, and 35‰. In Gullmar Fjord, surface water (0 to 5 m) ranges from ca. 11 to 33‰ and deep water (>50 m) is most often between 32 and 35‰ (Fig. 2). The 3 treatments were selected to represent the natural range of salinity. Water of each salinity was autoclaved after enrichment with f/2 medium (Guillard & Ryther 1962) – silica + selenium (0.01 µmol l^{-1}) for dinoflagellate culture, and f/2 + selenium (0.01 µmol l^{-1}) for diatom culture. For each of the 3 samples of surface sediment, 1 Nunc flask was filled with 150 ml of medium having a salinity of 15‰. For diatom samples, 0.5 g wet weight of the sediment was mixed into each flask. For dinoflagellate samples, sediment was sieved between 25 and 100 µm mesh and the equivalent of 2 g wet sediment from the 25 to 100 µm fraction was added to each flask. Although sieving excluded cysts <25 µm from the experiment, this was necessary to remove small diatoms which outcompete dinoflagellates in this type of culture. The inoculation procedure was repeated for salinities of 25 and 35‰, giving 9 diatom

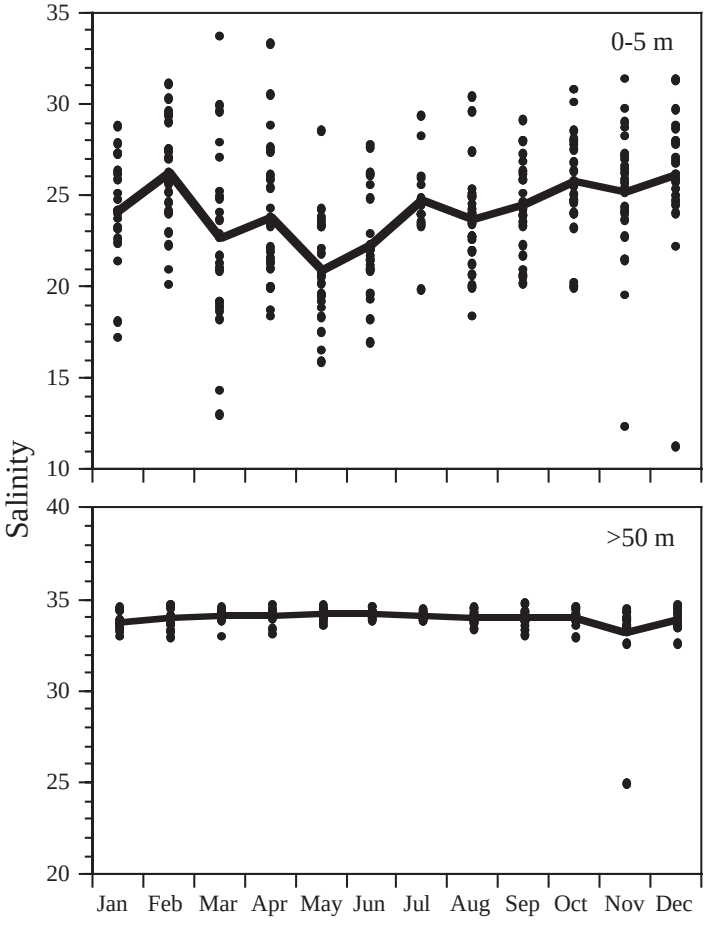

Fig. 2. Monthly records of sea surface (0 to 5 m) and deep water (>50 m) salinity in Gullmar Fjord from 1964 to 1996 and 2000 to 2003

and 9 dinoflagellate samples per experiment. Flasks were incubated at 90 µmol m^{-2} s^{-1} with daylight fluorescent lamps (OSRAM L 36W LUMILUX Plus). Because some species germinate only in a particular season, the experiment was run under both 'spring' (6°C and 14:10 h light:dark cycle) and 'summer' (18°C and 16:8 h light:dark cycle) conditions. Control flasks with growth medium and no sediment were also incubated and showed no growth throughout the experiments.

Diatom samples were shaken prior to sampling to resuspend settled vegetative cells. Although this mixed resting stages in with the germinated vegetative cells, the resting stages were greatly outnumbered by vegetative cells after a few days and were not a major component of the sample. If samples had not been mixed, a very poor selection of diatom vegetative cells would have been sampled, since in batch culture, these cells tend to accumulate at the bottom of the flask. Dinoflagellate samples were removed prior to shaking since the germinated cells tend to swim up in the flask. Dinoflagellate samples were extracted while gently swirling the pipette to homogenize the sample without resuspension of settled sediment. Samples of 5 ml were removed and preserved with Lugol's solution every second day. Diatom taxa were enumerated in settling chambers, using a Zeiss Axiovert inverted microscope, from 4 transects scanned at 400× magnification. Dinoflagellate cells were enumerated from the entire chamber at 200 to 400× magnification. All vegetative cells were presumed to have arisen from resting stages present in the sediments

Germination and growth at different salinities with controlled pH. In addition to changing the salinity, dilution of seawater in the experiment in 2001 also diluted the carbonate alkalinity and pool of dissolved inorganic carbon (DIC). Alkalinity and DIC were determined by acid titration of dissolved carbonate (Parsons et al. 1984). After autoclaving, the medium contained 0.6, 1.0, and 1.5 mM DIC for salinities of 15, 25, and 35‰, respectively. As a consequence, a second experiment using sediment collected in 2003 was set up in January 2004 to confirm the results obtained in 2001, and to begin to address the importance of pH and DIC in germination. Changes in pH and DIC vary with salinity because the concentration of salt influences equilibrium constants and the components of sea salt are involved in the acid-base reactions of seawater (Hinga 2002).

Cultures were set up with spring conditions as in the first experiment except that there were 4 replicates at each salinity, and $NaHCO_3$ was added to give initial DIC concentrations of 1.5 mM in all treatments. After inoculation with sediment, the pH of each culture was adjusted to 8.0 by adding drops of 1.0 N HCl in artificial seawater. HCl solutions had a salinity corresponding to the treatment salinity. The pH of 2 replicates was allowed to increase similar to the first experiment, whereas the pH of the other 2 replicates was regularly adjusted. Samples were taken every 2 d for 20 d and examined as described above. After sampling, the pH of each culture was recorded. For 2 of the 4 replicates, the pH was adjusted between 8.0 and 8.1 using a 0.5 N solution of NaOH in artificial seawater over the first 6 d and the acid solutions described above for Days 8 to 20. Because pH showed no effect in the spring incubation, the experiment was not repeated under summer conditions.

Calculations and statistical analyses. In all experiments, 2 growth parameters — germination time and growth rate — were calculated for species that had sufficient data. Germination time was estimated as the day that a species began to increase in number (McQuoid & Hobson 1995). Growth rates of the resulting vegetative cells were calculated by a linear regression of the $\log_2$-transformed cell concentrations during early exponential growth. Regressions were based on

3 to 6 data points, except in a few of the cultures at 18°C which were based on 2 data points. Comparisons of these growth parameters at different salinities were made with single-factor analysis of variance (ANOVA) in the first experiment. Season was not included as a factor in the ANOVA since incubations were made only once in spring and once in summer, and thus there is no replication for season. In the second experiment, differences in the growth parameters were tested with a 2-factor (salinity and pH) ANOVA. Significance was defined as $p < 0.05$. All data were tested for heterogeneous variances with Cochran's *C*-test, and log-transformed when necessary.

Microplankton composition. Microplankton assemblages and salinities from Gullmar Fjord were sampled monthly by the Swedish Meteorological and Hydrological Institute, and periodically by myself from 2000 to 2003 (Fig. 2). Because some species were not well represented in these samples, I also used monthly data collected by the Plankton Monitoring Group, Kristineberg Marine Research Station, in Havstens Fjord and Koljö Fjord (Fig. 1) from 1993 to 1998. Data were compiled into winter (December to February), spring (March to May), summer (June to August), and autumn (September to November) distributions, and plotted against corresponding measurements of sea-surface salinity.

RESULTS

Taxonomic considerations

Vegetative cells, presumably germinated resting stages, of many diatom and dinoflagellate taxa were observed in the sediment cultures (Table 1). The dominant dinoflagellate taxon was *Scrippsiella*. Many *S. trochoidea* cysts were present in the sediments, and a few *S. lachrymosa* cysts were observed. Although it is very difficult to distinguish between the vegetative stages of these 2 species, Lewis (1991) gives cell length ranges of 16 to 30 µm for *S. lachrymosa* and 23 to 37 µm for *S. trochoidea*. The vegetative cells that I observed were rarely smaller than 30 µm long. This, together with *S. trochoidea* cysts being more abundant in the sediment inoculum, indicates that *S. trochoidea* was the dominant *Scrippsiella* species in the cultures. It is possible, however, that a small number of the cells identified as *S. trochoidea* were *S. lachrymosa*.

Some diatom species could not be quantified. Diatom samples in the summer treatment contained numerous cells of a small (diameter = 4.5 µm) unicellular *Thalassiosira* species. Examination by scanning electron microscopy suggested that the species was *T. pseudonana*. Unfortunately, the small size and delicate features of this species made it difficult to quantify in the sedimentation chambers. Although no extra silicate was added to dinoflagellate cultures, silicate in the natural seawater allowed some growth of diatoms, and *Chaetoceros didymus*, *T. rotula*, and *Ditylum brightwellii* were more noticeable in the dinoflagellate cultures compared to the diatom cultures. These diatoms could not be counted in the dinoflagellate samples, however, because the removal of many diatom cells by sieving did not allow accurate quantification.

In the first few days after inoculation, *Thalassiosira minima* cells still had sediment grains attached to their cell walls, suggesting that they were original resting stages from the sediment. Once this species was growing exponentially, the cells appeared clean, indicating that they were new vegetative cells. Similarly, during

Table 1. Germination (+) of microplankton resting stages from Gullmar Fjord sediment cultured under different salinities

	Salinity		
	15	25	35
Diatoms			
Amphora spp.	+	+	+
Attheya septentrionalis	+	+	+
Chaetoceros diadema		+	+
Chaetoceros didymus	+	+	+
Chaetoceros simplex	+	+	+
Chaetoceros socialis	+	+	+
Cylindrotheca closterium	+	+	+
Detonula confervacea	+	+	+
Ditylum brightwellii	+	+	+
Entomoneis sp.	+	+	+
Leptocylindrus danicus		+	
Melosira nummuloides	+	+	+
Navicula spp.	+	+	+
Nitzschia spp.	+	+	+
Odontella aurita		+	+
Paralia sulcata	+	+	+
Skeletonema costatum	+	+	+
Surirella spp.	+	+	+
Thalassiosira eccentrica	+	+	+
Thalassiosira minima	+	+	+
Thalassiosira nordenskioeldii	+	+	+
Thalassiosira pseudonana	+	+	+
Thalassiosira rotula	+	+	+
Dinoflagellates			
Alexandrium spp.	+	+	+
Diplopsalis spp.	+	+	+
Gonyaulax digitalis	+	+	+
Gonyaulax spinifera	+	+	+
Gymnodinium nolleri		+	+
Lingulodinium polyedrum	+	+	+
Oblea rotunda	+	+	+
Polykrikos schwartzii	+	+	+
Protoceratium reticulatum	+	+	+
Protoperidinium denticulatum	+		+
Protoperidinium oblongum	+		+
Scrippsiella trochoidea	+	+	+
Scrippsiella/Pentapharsodinium spp.	+	+	+

the early days of incubation, *Skeletonema costatum* often looked like resting cells because cells had condensed cytoplasm. These observations suggest that initial growth in the cultures was from resting stages and not vegetative cells.

Seasonal germination and growth at different salinities

Species that showed a consistent increase in cell numbers were used to calculate germination time and growth rate; this was possible for 9 taxonomic groups. Although cell numbers for other species were too erratic to calculate these parameters, some patterns were noted. *Chaetoceros diadema*, *Odontella aurita*, and *Gymnodinium nolleri* were observed only in the 25 and 35‰ treatments; *Leptocylindrus danicus* was observed only at 25‰; and *Protoperidinium* species were observed only in the 15 and 35‰ treatments. Presumably, the *Protoperidinium* species were present in the 25‰ treatment, but their numbers were too low for them to be observed in the samples.

Distinct differences were observed between the seasonal treatments (Figs. 3 & 4). All diatoms except *Chaetoceros simplex* were more abundant in the spring treatment, and *Detonula confervacea* did not grow at all in the summer treatment (Figs. 4 & 5). *Chaetoceros simplex* was 2 or 3 times more abundant in summer. The dinoflagellate *Oblea rotunda* also showed higher concentrations in summer.

The influence of salinity could be seen on all of the 9 dominant taxa (Figs. 6 & 7). *Skeletonema costatum* grew well at all salinities. *Thalassiosira nordenskioeldii* grew only moderately well in the cultures. This species had faster growth at low salinities in spring conditions and high salinities in the summer treatment. *Chaetoceros simplex* grew slowest at 25‰. *Detonula confervacea* grew fastest at 15 and 25‰, whereas *Diplopsalis* spp. and *T. minima* grew faster at 25 and 35‰ in some treatments. Only *C. socialis* and *Oblea rotunda* showed the fastest growth at 35‰.

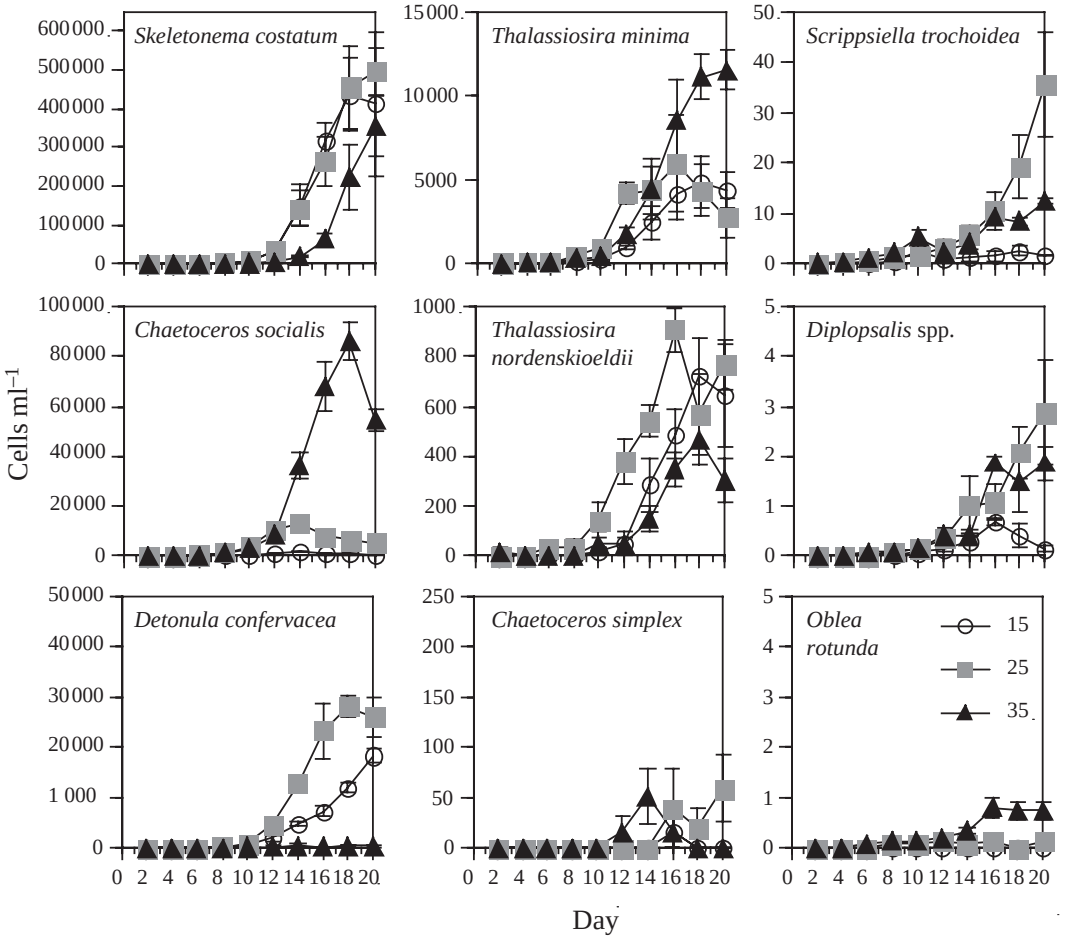

Fig. 3. Growth curves (average ± SE, n = 3) of 9 dominant taxa at salinities of 15, 25, and 35‰ incubated in spring conditions. Sediment used in cultures was collected from Gullmar Fjord in 2000

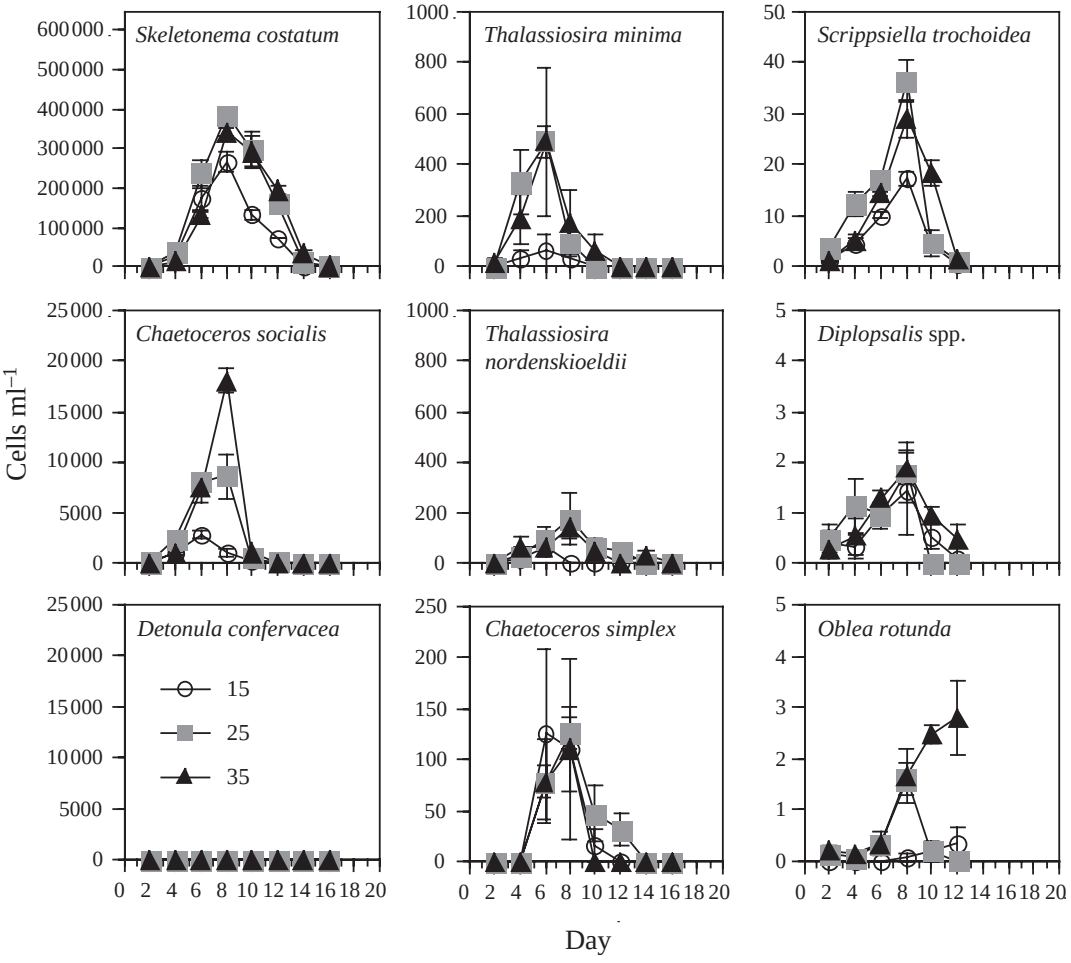

Fig. 4. Growth curves (average ± SE, n = 3) of 9 dominant taxa at salinities of 15, 25, and 35‰ incubated in summer conditions. Sediment used in cultures was collected from Gullmar Fjord in 2000

In the spring treatment, salinity significantly influenced germination time for *Detonula confervacea* and *Oblea rotunda* (Fig. 5). *Scrippsiella trochoidea*, *Skeletonema costatum*, and *Thalassiosira minima* germinated first (Days 2 to 8). *D. confervacea* and *Chaetoceros socialis* were slower to start at some salinities. *T. nordenskioeldii* usually germinated by Day 10. *C. simplex*, *Diplopsalis* spp., and *O. rotunda* were the slowest to germinate, especially at lower salinities. Spring growth rates ranged from 0.4 to 1.3 divisions d^{-1}, with fastest growth by *D. confervacea*, *S. costatum*, and *T. minima* (Fig. 6). Reliable growth estimates could not be calculated for *C. simplex* in spring conditions. Salinity significantly influenced growth of all species except *S. trochoidea* and *T. nordenskioeldii* in spring (Fig. 6).

The time between inoculation and germination was generally shorter under summer conditions (Fig. 5). *Diplopsalis* spp. and *Scrippsiella trochoidea* had already germinated by Day 2. Most other species germinated by Days 3 to 6, but *Detonula confervacea* did not germinate under summer conditions. Salinity had a significant effect on the germination of *Oblea rotunda* only (Fig. 5). Growth rates were also higher in summer treatments, generally ranging from 0.5 to 1.6 divisions d^{-1} and salinity had a significant effect on 5 of the 9 species (Fig. 6).

Germination and growth at different salinities with controlled pH

Culture pH dropped from 8.0 to between 7.9 and 7.7 during the first few days of the experiment, began to increase by Days 8 to 10, and reached maxima of 9.9 in uncontrolled cultures. Controlling pH, however, made no significant difference for germination time and growth of cultures (Fig. 7). A more significant result of the experiment in 2004 was that it confirmed germination times and growth rates found in 2001, and this suggests that the patterns are not specific to a particular year.

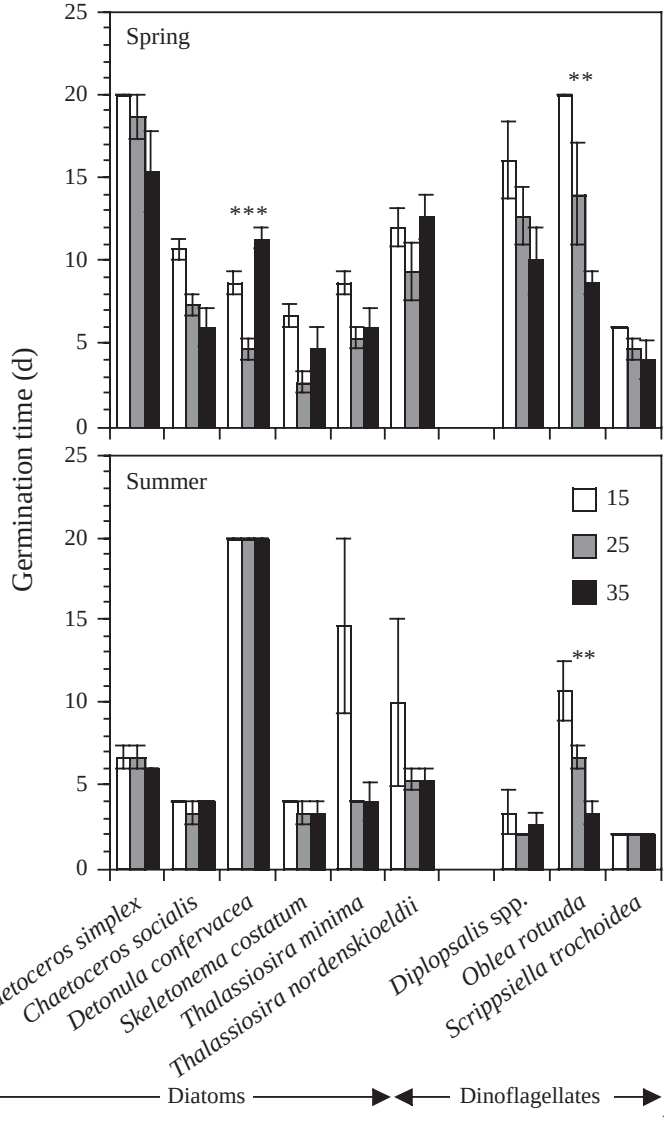

Fig. 5. Days between culture inoculation and germination of resting stages (average ± SE, n = 3) of 9 dominant taxa at salinities of 15, 25, and 35‰. Sediment used in cultures was collected from Gullmar Fjord in 2000. Significant effects of salinity are **p = 0.01, ***p = 0.001

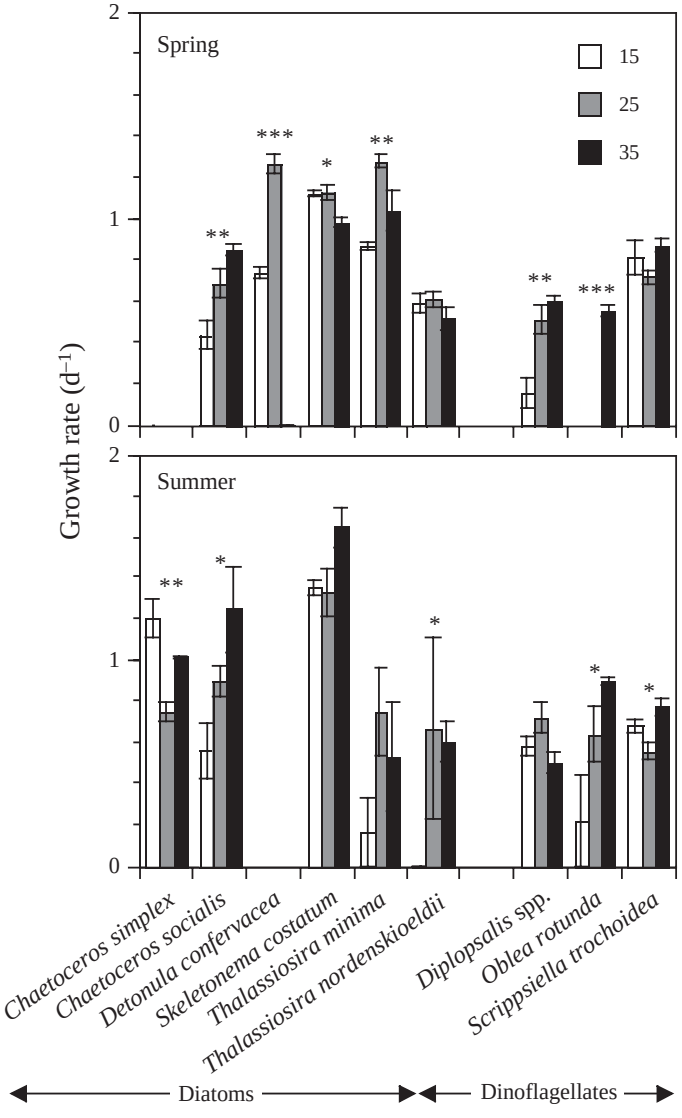

Fig. 6. Growth rates of vegetative cells (average ± SE, n = 3) of 9 dominant taxa at salinities of 15, 25, and 35‰. Sediment used in cultures was collected from Gullmar Fjord in 2000. Reliable estimates of growth rate could not be calculated for *Chaetoceros simplex* in the spring treatment. Significant effects of salinity are *p = 0.05, **p = 0.01, ***p = 0.001

Microplankton distributions on the Swedish west coast

The 9 dominant taxa in the laboratory experiments were also recorded in the plankton along the Swedish west coast (Fig. 8). *Chaetoceros simplex* and *Skeletonema costatum* were found throughout the growing season and at a wide range of salinities. *Scrippsiella trochoidea* and *C. socialis* were found throughout the year at salinities ranging from 21 to 32‰. *Thalassiosira nordenskioeldii* and *Detonula confervacea* occurred primarily in late winter and spring, when salinities ranged from 21 to 27‰, but *T. nordenskioeldii* was occasionally found at salinities outside this range. Due to its small size and similarity to other species, there is only 1 confirmed record of *T. minima* with corresponding salinity data. This data point shows that *T. minima* can reach high concentrations in spring at 23.7‰. *Diplopsalis* spp. were primarily present in autumn and winter, and at moderate salinities, 23.5 to 28.5‰. Although records of *Oblea rotunda* are few, data suggest that this species is most abundant in spring and autumn at salinities of 26 to 32‰.

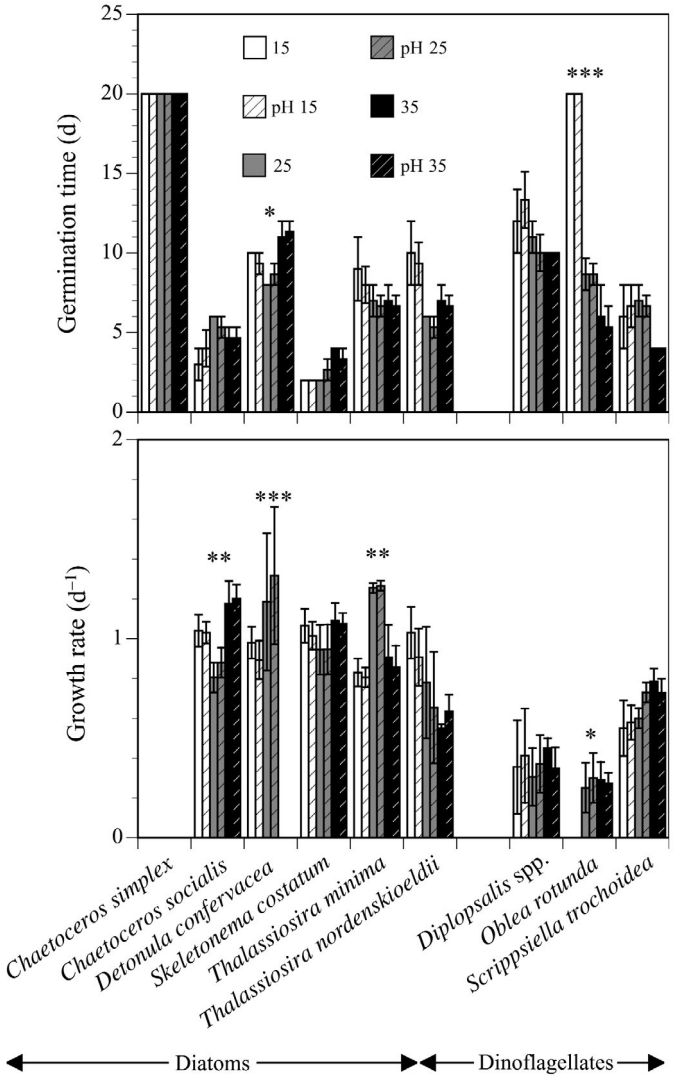

Fig. 7. Growth rates of vegetative cells and days between culture inoculation and germination of resting stages of 9 dominant taxa in spring conditions at salinities of 15, 25 and 35‰. Cultures regularly adjusted to pH 8 are plotted with striped bars; other bars show unadjusted pH (average ± SE, n = 2). Adjusting pH had no significant effects on germination time or growth. Sediment used in cultures was collected from Gullmar Fjord in 2003. Significant effects of salinity are *p = 0.05, **p = 0.01, ***p = 0.001

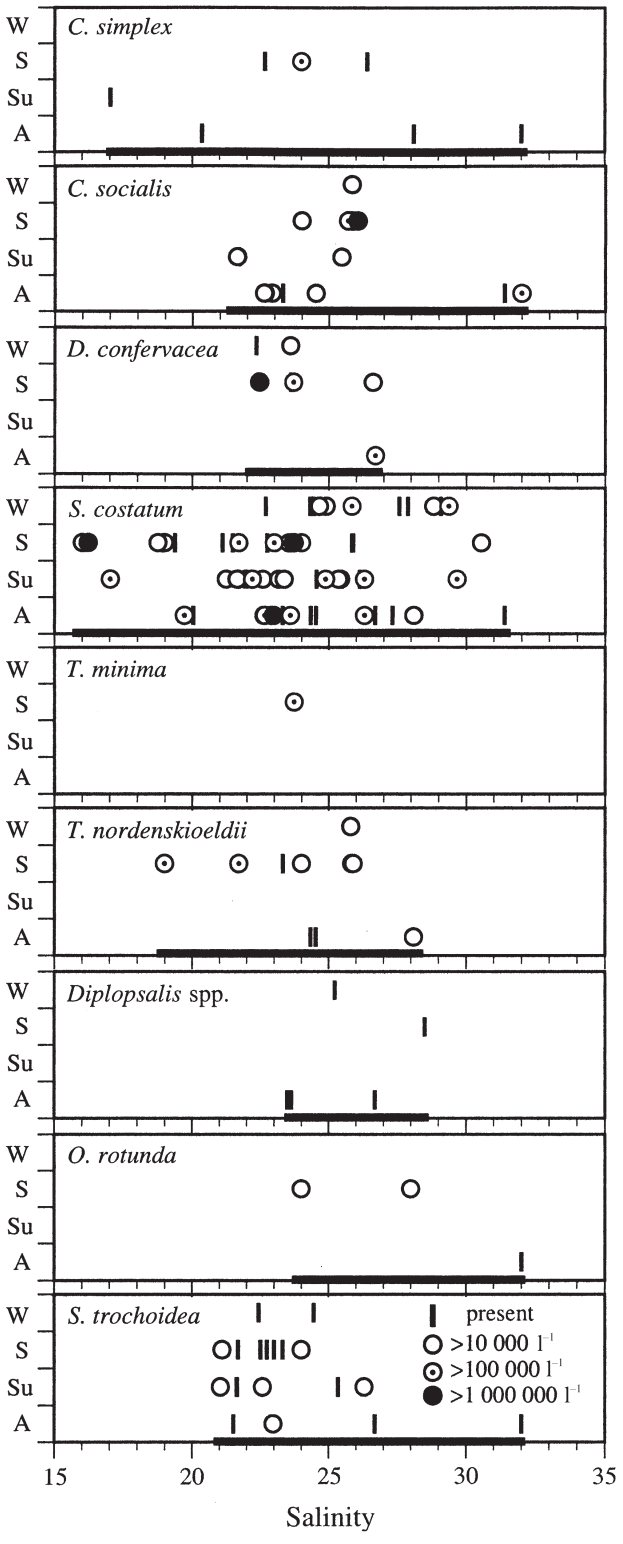

Fig. 8. Seasonal abundance of 9 microplankton taxa in fjords on the Swedish west coast versus sea surface salinity from 1993 to 2003. W: winter (December–February); S: spring (March–May); Su: summer (June–August); A: autumn (September–November). Bold lines indicate the natural salinity range for each species

DISCUSSION

For many coastal microplankton, seasonal variations in temperature and light are important factors governing the transition from dormancy to vegetative growth (e.g. Binder & Anderson 1987, Eilertsen et al. 1995). The present study shows that for some species, germination and subsequent vegetative growth may also be regulated by changes in salinity.

Resting stages in sediments on the Swedish west coast

Not all taxa observed in the plankton record are present in the sediments (McQuoid 2002, Godhe & McQuoid 2003). Differences in taxonomic composition between plankton and sediment reflect losses due to grazing and germination, and gains from lateral advection. Most importantly, the presence of a species in the sediment cultures requires that they survive 8 mo of storage in darkness. Most of the species observed in the sediment cultures are known for their ability to form resting stages. This study, however, provides new evidence of dormant life stages for *Thalassiosira minima* and *T. pseudonana*. Repeated incubations of the sediment collected in 2000 showed growth of *T. minima* and *T. pseudonana* after more than 2 yr of storage. To date, no resting spores have been found for these species, so it is likely that they have survived years of storage as physiologically resting cells. Diatoms such as *Skeletonema costatum* are also known to survive as resting cells in Scandinavian sediments (McQuoid 2002).

Germination at varying salinities

Diatoms require light for germination (Hollibaugh et al. 1981). Consequently, they must either germinate from shallow areas or be transported to near-surface waters. Salinity of surface waters in Gullmar Fjord can vary greatly (Fig. 2), especially in spring. It would, thus, be advantageous for species with a spring maximum to germinate at a wide range of salinities. Results of this study show that, for many diatoms, germination can occur at salinities between 15 and 35‰ (Figs. 5 & 7). Although able to germinate at all tested salinities, faster germination of *Detonula confervacea* at 25‰ suggests that this species may be sensitive to large salinity variations. Similarly, the absence of *Chaetoteros diadema*, *Leptocylindrus danicus*, and *Odontella aurita* at 15‰ (Table 1) suggests that low salinity may negatively influence the germination of these species. This would not likely affect the overall success of *L. danicus* and *O. aurita*, because they are usually abundant in summer and winter (Lange et al. 1992), respectively, when surface salinities tend to be greater than 20‰. It could, however, be a disadvantage for *D. confervacea* and *C. diadema*, because these species are common in spring (Tiselius & Kylenstierna 1996) when salinity is most variable.

In contrast to diatoms, many dinoflagellates can germinate in darkness (Bravo & Anderson 1994) and do not rely on resuspension of deep sediment. Although salinity of shallow areas may be variable, deep water in Gullmar Fjord usually has a narrow range of salinity (32 to 35‰) (Fig. 2). It is therefore not surprising that all the dinoflagellates in the sediment cultures could germinate at high salinity (Table 1), and that germination of *Oblea rotunda* was significantly faster at 35‰ (Figs. 5 & 7). Previous studies have found that germination of *Alexandrium tamarense* is lowest during summer monsoons, when sea surface salinity drops below 30‰ (Kim et al. 2002). Similar experiments with *A. minutum*, however, show adaptation to lower salinities (14 to 26‰) (Cannon 1993). Although some dinoflagellates germinate only at a particular season, *Scrippsiella trochoidea* is known to excyst readily and shows no strong annual germination rhythm (Binder & Anderson 1987, Nuzzo & Montresor 1999). In Onagawa Bay, Japan and Yongil Bay, Korea, *S. trochoidea* germinates throughout the year, even when vegetative growth is severely limited by temperature (Ishikawa & Taniguchi 1997, Kim & Han 2000). Consequently, the development of planktonic *S. trochoidea* populations may depend more on factors influencing growth than on factors regulating excystment. Once germling cells reach the upper water column, they can be exposed to a much wider range of salinity, so a species' success will also depend on the effects of salinity on vegetative growth.

Influence of salinity on vegetative growth and seasonal microplankton patterns on the Swedish west coast

The seasonal distributions of microplankton species observed in this study agree with previous reports from the Swedish west coast (Lindahl & Hernroth 1983, Tiselius & Kuylenstierna 1996) and also reveal species-specific salinity ranges (Fig. 8). The experimental results show that a species' natural salinity range may be due, in part, to the influence of salinity on the growth of new vegetative cells. All 9 of the species analyzed were significantly influenced by salinity in 1 or both seasonal treatments (Figs. 6 & 7) and several species showed strong seasonal patterns.

Along the Swedish west coast, *Detonula confervacea*, *Thalassiosira nordenskioeldii*, and *T. minima* bloom in the early spring, and all 3 species showed higher growth rates and maximum numbers in the spring experiment. *D. confervacea* occurred in the fjords over a narrow salinity range and did not grow in the experiments at 35‰. Although these results support previous laboratory experiments that show an optimal salinity range of 15 to 30‰ for *D. confervacea* (Smayda 1969), this species is sometimes associated with sea ice (Robineau et al. 1997) which may contain brine-filled channels. Thus, poor growth of *D. confer-*

vacea at 35‰ may be specific to some clones only. *T. nordenskioeldii* was present at a wider range of salinities in the fjords than *D. confervacea*, but did not show consistent trends among the 3 experiments, so no specific salinity 'preferences' could be identified for *T. nordenskioeldii*. *T. minima* grew fastest and reached highest concentrations at 25 or 35‰. Although there is a poor plankton record for this species, resting stages of *T. minima* are well documented on the Swedish west coast and are most abundant at stations on the outer coast where sea surface salinities are generally higher (McQuoid 2002).

Following the spring bloom, microplankton on the Swedish west coast are usually dominated by flagellates and a variety of diatoms. In the fjords, *Chaetoceros simplex* was common in spring and autumn; however, in the experiments it was most abundant in summer conditions. Because *C. simplex* is a small, unicellular species, it is not easy to identify. It may be that the cells recorded in the fjords include other unicellular *Chaetoceros* spp. that are more common in spring, or perhaps the late summer form of *C. simplex* produces spores and the spring form does not. This diversity may also account for the wide salinity range observed for *C. simplex* in the fjords. A similarly wide salinity range was estimated for a clone of *C. simplex* from the Sargasso Sea (Brand 1984). The growth of *C. simplex* in these experiments supports the idea that this species is not specifically adapted to a particular salinity.

Chaetoceros socialis was present at all tested salinities, but had its fastest growth at the highest salinity. This species can be present at any time of year and is most abundant in spring and autumn diatom blooms in Swedish fjords, where surface salinities are usually between 20 and 25‰. But outside the fjords in the Skagerrak, *C. socialis* is common during late summer and autumn, a time when salinities are generally greater than 29‰ (Lange et al. 1992, Gustafson & Stigebrandt 1996).

The heterotrophic dinoflagellate *Oblea rotunda* is rarely mentioned in microplankton surveys, probably owing to its small size and relatively low concentrations (Lewis 1990). The few observations available suggest that *O. rotunda* has highest concentrations in the fjords during spring. In the experiment, however, it grew slightly better in the summer treatment. *O. rotunda* can use a wide variety of prey but is often associated with diatom blooms (Strom & Buskey 1993), in particular, *Ditylum brightwellii*, which was abundant in the cultures at all salinities. *O. rotunda* was present at moderate to high salinities in the fjords and was clearly favored at 35‰ in the experiments. Given that unidentified members of the genus *Oblea* have been found in the Salton Sea, which can exceed 40‰ (Hurlbert et al. 2001), the upper limit of salinity for *O. rotunda* may be even higher than 35‰. Whereas *O. rotunda* appears to be favored by high salinity in many locations, a variant of this species has been discovered at very low salinity (<7‰) (Chomérat et al. 2004), a finding that highlights the importance of clonal differences. The success of some dinoflagellates at high salinities may account for their abundance in offshore waters (e.g. Kim et al. 2004), and *O. rotunda* is known to occur in the Skagerrak as well as in Swedish fjords.

Diplopsalis spp. are present in the fjords year round but are most common in autumn. In the experiments, *Diplopsalis* spp. grew fastest and reached highest concentrations at 25 or 35‰; however, this species is not recorded in the fjords at salinities >29‰. These heterotrophs have a generalist feeding behavior, and little is known about their association with particular environmental conditions. *Diplopsalis* spp. that grew from sediments on the Swedish west coast appear to be most successful at salinities between 23 and 35‰ (Figs. 6–8). In addition to the effects of salinity on the heterotrophic species themselves, there may also be an indirect effect of salinity though selection of prey. A generalist feeding behavior would, therefore, be advantageous in regions with high salinity variability.

In contrast to species with a distinct seasonal distribution, *Skeletonema costatum* and *Scrippsiella trochoidea* were present in the fjords most of the year. They have previously been characterized as occurring throughout the growing season (Lange et al. 1992, Ishikawa & Taniguchi 1997) and at a wide range of salinities (e.g. Brand 1984). *S. costatum* is abundant in the North Sea, can form large blooms (up to 15 million cells l^{-1}) on the Swedish west coast, and is also present in all areas of the Baltic Sea (Snoeijs et al. 1993–1998). Laboratory experiments have shown that *S. costatum* cultures are well adapted to salinity changes, although growth at salinities <5‰ is low (Rijstenbil et al. 1989). Furthermore, numerical models suggest that *S. costatum* is less sensitive to salinity changes than the dinoflagellate *Alexandrium tamarense* (Yamamoto & Seike 2003). Thus, *S. costatum* can succeed under a wide range of salinities. *S. trochoidea* is well adapted to grow at 21 to 35‰. This species is successful in coastal environments and can be a common member of both the plankton and sediment communities on the Swedish west coast (Godhe & McQuoid 2003). A wide range of salinity tolerance, coupled with a quick germination response and a short dormancy period (Binder & Anderson 1987), allow this species to adapt quickly to changes in the environment.

The observations of species at particular salinities, and their germination and growth in the 3 experimental salinity treatments can be summarized to show species-specific salinity 'preferences' (Fig. 9). Many species are euryhaline and *Skeletonema costatum* is particularly well adapted to changes in salinity.

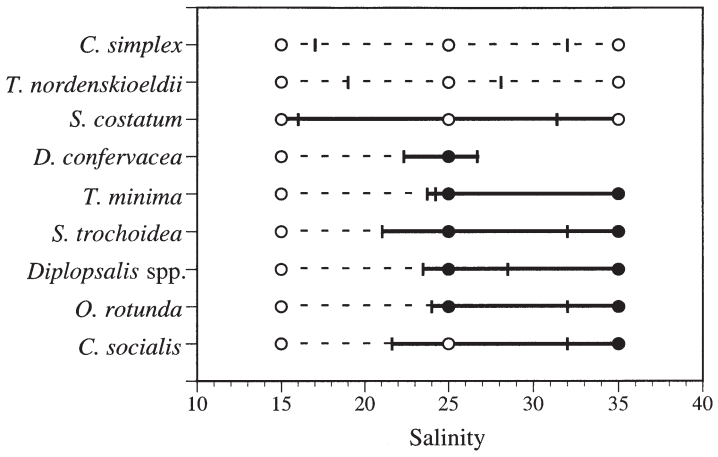

Fig. 9. Summary of salinity effects on germination and growth of 9 microplankton taxa. Vertical bars indicate the natural observed salinity range. ○: growth in experimental cultures. ●: significantly better growth in experimental cultures. For each species that shows a clear pattern, bold lines propose the optimal salinity range

Detonula confervacea is sensitive to high salinities and could be at a disadvantage in spring months with high salinity compared to other taxa. *Chaetoceros socialis* and *Thalassiosira minima*, in contrast, would be good competitors at high salinity. The 3 dinoflagellates examined are all well adapted to salinities between 22 and 35‰, and *Oblea rotunda* is favored at the high end of this range. These differences in salinity tolerance may contribute to variations in microplankton composition if altered climate and circulation change salinity along the Swedish west coast. In these fjords, abundance of species favored by high salinity (i.e. *C. socialis*, *O. rotunda*, *T. minima*) may reflect intrusion of high-salinity surface water, rather than local seeding by resting spores. Saline surface water from the Skagerrak can be transported into Gullmar Fjord by winds from the southwest (Lindahl et al. 1998), and these are usually enhanced during positive NAO. In contrast, periods with negative values of NAO should favor species that tolerate moderate to low salinities. Historical records of *D. confervacea* from Koljö Fjord sediments confirm that this species is more abundant when NAO is negative (McQuoid & Nordberg 2003).

Indirect and direct effects of salinity on microplankton populations

Variations in microplankton associated with changes in salinity may result from a combination of biological and physical processes, and these effects may be direct or indirect. Changes in salinity can alter the buffering capacity of seawater. In less saline seawater with a lower buffering capacity, pH can reach high values during blooms, and may result in the dominance of taxa that are tolerant of high pH (Hinga 2002). In the present experiment, pH did not reach high values (>8.5) until after Day 12, by which time most species had germinated and were in exponential growth. Although high pH may temporarily reduce species richness during the late successional stages of a bloom, it appears that salinity-related changes in pH did not significantly influence germination and early exponential growth of the microplankton examined in this study.

The influence of changing salinity on diatom bloom development and species composition is evident in areas where reductions in salinity have contributed to increased water column stability, and thus decreased nutrient replenishment of surface waters (e.g. Goffart et al. 2002). In such situations, diatom growth is indirectly reduced by salinity. Similar effects have been described for diatom blooms in Scandinavian fjords during increased river discharge (Kristiansen 1998). Because cultures in the present study were given high nutrient medium, results indicate that growth of some diatom species may be reduced as a direct consequence of changes in salinity. In contrast to diatoms, dinoflagellate growth is sometimes stimulated by high river runoff or heavy rainfall (Blanco et al. 1985, Weise et al. 2002). Freshwater inputs can indirectly influence microplankton composition through changes in water column stability and by concentration of cells at frontal zones. However, runoff can also bring humic substances, which are known to have a positive effect on growth for some dinoflagellates (e.g. Carlsson et al. 1995). In this study, humic substances did not accompany decreases in salinity, and dinoflagellate growth was not enhanced in low-salinity treatments.

Models have shown that, during high NAO periods, increased westerly winds lead to increased salt content in the North Sea, but decreased salinity in the Baltic Sea (Schrum 2001). Surface water salinities along the Swedish west coast depend on local rivers, circulation patterns in the Skagerrak, and changing outflow from the Baltic Sea (Gustafson & Stigebrandt 1996), therefore, fjords on the Swedish west coast can also be expected to show salinity variations related to climate change. Although seasonal factors, such as temperature and light, may strongly regulate the germination of microplankton resting stages, the results of this study indicate that salinity may also be an important controlling factor for some species. In addition to effects through water column stability, buffering capacity, and nutrient supply, salinity changes may alter microplankton communities on the Swedish west coast through direct effects on resting-stage germination and vegetative growth.

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