Hydrobiologia (2005) 540:105–115
P. Meire & S. van Damme (eds), Ecological Structures and Functions in the Scheldt Estuary: from Past to Future
DOI 10.1007/s10750-004-7123-x

Primary Research Paper

Influence of changes in salinity and light intensity on growth of phytoplankton communities from the Schelde river and estuary (Belgium/The Netherlands)

Marie Lionard[1,*], Koenraad Muylaert[1], Dirk Van Gansbeke[2] & Wim Vyverman[1]
[1]*Laboratory for Protistology and Aquatic Ecology, Department of Biology, University of Ghent, Krijgslaan 281 – S8, B-9000 Gent, Belgium*
[2]*Marine Biology Section, Department of Biology, University of Ghent, Krijgslaan 281-S8, B-9000 Gent, Belgium*
*(*Author for correspondence: Tel.: +32-09-264-8511; Fax: +32-09-264-8599; E-mail: marie.lionard@UGent.be)*

Key words: phytoplankton, HPLC, pigments, estuary, river, irradiance, salinity

Abstract

In the Schelde continuum, a succession in the phytoplankton community is observed along the transition from the river to the freshwater tidal reaches of the estuary and from the freshwater to brackish reaches of the estuary. The goal of this study was to experimentally evaluate the contribution of changes in salinity and light climate to this succession. In summer 2000 and in spring 2001, phytoplankton communities from the river, the freshwater tidal reaches and the brackish reaches of the estuary were incubated under high or low light intensities and exposed to a change in salinity. HPLC analysis was used to evaluate the response of different algal groups to changes in light intensity and salinity. When incubated at a light intensity corresponding to the mean underwater light intensity of the freshwater tidal reaches, growth of phytoplankton from the river as well as from freshwater tidal reaches was significantly lower than when incubated at a light intensity corresponding to the mean underwater light intensity of the river. The phytoplankton community from the freshwater tidal reaches did not appear to be better adapted to low light intensities than the phytoplankton community from the river. Although diatoms were expected to be less sensitive to a reduction in light intensity than green algae, the opposite response was observed. Freshwater and brackish water phytoplankton were negatively affected by respectively an increase or decrease in salinity. However, the effect of salinity was not strong enough to explain the disappearance of freshwater and brackish water phytoplankton between a salinity of 0.5 and 10 psu, suggesting that other factors also play a role. In the freshwater phytoplankton communities from the river and the freshwater tidal reaches, green algae and diatoms responded in a similar way to an increase in salinity. In the brackish water phytoplankton community, fucoxanthin displayed a different response to salinity than lutein and chlorophyll *a*.

Introduction

In estuaries, freshwater supplied by rivers is mixed with seawater brought in by the tides. This mixing creates the well-known estuarine salinity gradient, with seawater near the mouth of the estuary to pure freshwater near the head of the estuary. Between a salinity of 0.5 and 10 psu, freshwater phytoplankton species are usually replaced by marine species (e.g. Remane & Schlieper, 1952; Snoeijs, 1995). In the Schelde estuary, a succession can be observed from typical freshwater diatoms like *Cyclotella* and *Stephanodiscus* below 0.5 psu to marine diatoms like *Thalassiosira* species, *Skeletonema costatum* and *Ditylum brightwellii* above 10 psu (Rijstenbil et al., 1993; Muylaert & Sabbe, 1999; Muylaert et al., 2000a). The phytoplankton succession along the salinity gradient has generally been ascribed to the fact that most phytoplankton species are stenohaline and suffer osmotic stress upon exposure to salinity changes (Remane & Schlieper, 1958; Admiraal, 1977; Miller & Kamykowski, 1986; Rijstenbil, 1989; Kirst, 1990; Flameling & Kromkamp 1994; Bisson & Kirst, 1995).

In most estuaries, the influence of the tides extends further upstream than the influence of seawater. As a result, the upper reaches of estuaries are freshwater systems subjected to a strong tidal regime: the freshwater tidal reaches. Salinity in these freshwater tidal reaches is identical to salinity in the rivers (<0.5 psu) and the freshwater tidal reaches of estuaries only differ from rivers in the presence of tidal activity. This tidal activity is responsible for the resuspension of bottom sediments and, as a result, the transition from river to freshwater tidal estuary is characterized by an increase in turbidity (Muylaert et al., 1997). As the capacity of the freshwater tidal reaches should be sufficient to contain water supplied by the river as well as water brought in by the tides, the riverine–estuarine transition is also characterized by an increase in water depth. The increases in turbidity and water depth along the transition from river to freshwater tidal estuary cause a strong increase in the mixing depth to euphotic depth ratio (Z_m/Z_{eu}) and imply a large reduction in the light available to phytoplankton. In two studies from the Schelde estuary, clear differences in phytoplankton species composition were observed between the tributary rivers and the freshwater tidal side-basins of the estuary they discharge into (Muylaert et al., 1997; Muylaert et al., 2000a). Diatoms were found to be the dominant phytoplankton group in the freshwater tidal reaches while chlorophytes were found to be more successful in the tributary rivers, especially in summer. Changes in phytoplankton community composition along the riverine–estuarine transition were ascribed to different adaptations of the phytoplankton community to the light climate. While diatoms are generally known to be adapted to low light levels and are therefore capable of surviving in the turbid estuary, green algae are known to depend on relatively high light intensities and would therefore be expected to survive only in the river (Richardson et al., 1983).

The goal of this study was to experimentally test the hypotheses that the succession along the riverine–estuarine transition is regulated by light limitation and that the succession along the salinity gradient is regulated by osmotic stress. Experiments were carried out with natural phytoplankton communities that were exposed to changes in salinity and light climate.

Materials and methods

Study site

The Schelde estuary is a macrotidal estuary situated in Western Europe (Belgium and The Netherlands) (Fig. 1). High population densities and intensive industrial activities in the catchment of the estuary result in high loadings of organic matter and inorganic nutrients. In Figure 2, changes in salinity, SPM concentration, water column depth and the Z_m/Z_{eu} ratio along the riverine–estuarine continuum of the Schelde are presented. Salinity and SPM concentrations shown in Figure 2 are averages and standard deviation of monthly samples collected between 1995 and 2001 (unpublished data provided by Stefan Van Damme). Depth data are based on Soetaert & Herman (1995) between the mouth of the estuary and km 80 and on Muylaert et al. (this volume) between km 80 and Gent. As the estuary is never strongly stratified, Z_m was considered to equal water column depth. Z_{eu} was calculated from SPM concentrations as $4.61/K_d$ (Kirk 1994) while K_d was estimated from SPM data according to the conversion factor of 0.06 m^{-1} (mg l^{-1})$^{-1}$ for turbid estuaries published in Cloern (1987). In contrast to many other European estuaries, the Schelde estuary still possesses extensive freshwater tidal reaches that comprise the upper 60 km of the estuary. As freshwater discharge is low compared to the total volume of the estuary, the salinity gradient is gradual and salinity increases only slowly from 0.5 to 20 psu over a distance of 60 km. Due to the presence of tidal activity in the freshwater tidal reaches, the riverine–estuarine transition in the Schelde estuary is characterized by a doubling of SPM concentrations. Together with an increase in mean water column depth, this causes a strong increase in the Z_m/Z_{eu} ratio. Between a salinity of 0.5 and 10 psu, suspended matter concentrations are similar to those of the freshwater tidal reaches but water depth increases strongly, resulting in an increase in the Z_m/Z_{eu} ratio when compared to the freshwater tidal reaches. At salinities above 10 psu, suspended

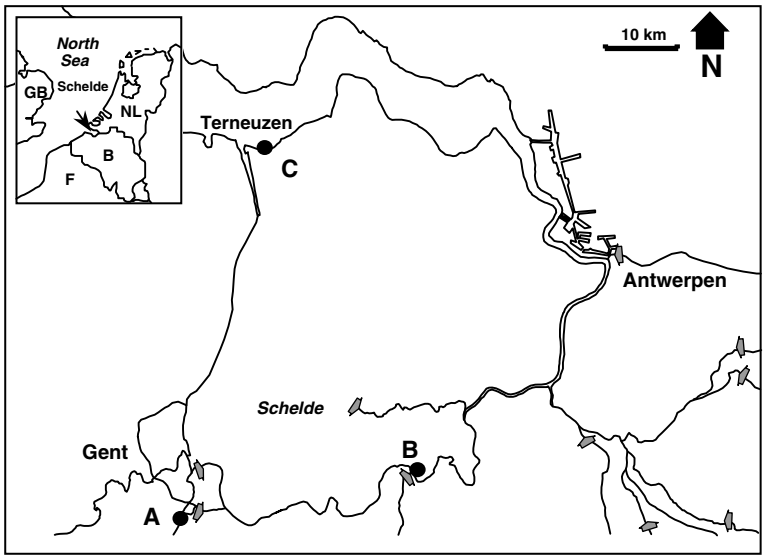

Figure 1. Map of the Schelde estuary showing the location of the sampling sites: the river Schelde in Gent (a), the freshwater tidal reaches of the Schelde estuary in Dendermonde (b) and the brackish reaches of the Schelde estuary in Terneuzen (c). The grey arrows indicate the upstream limit of tidal influence in the Schelde estuary and its side-basins.

matter concentrations decrease again while water depth remains constant, resulting in a Z_m/Z_{eu} ratio that is comparable or slightly lower than in the freshwater tidal reaches.

For the experiments, three sites situated along the riverine–estuarine continuum were sampled: the river Schelde near Gent (site A) where Z_m/Z_{eu} and salinity are low, the freshwater tidal reaches near Dendermonde where Z_m/Z_{eu} is high and salinity is low (site B) and the brackish reaches of the estuary near Terneuzen where Z_m/Z_{eu} as well as salinity are high (site C). Water was collected from a jetty at the three sites in summer (August 2000) and spring (April 2001). To eliminate as much zooplankton as possible, the water was filtered over a 50 μm nylon mesh immediately upon sampling. Salinity and temperature were measured at the sites using a handheld WTW meter.

Experimental setup

The goal of our experiments was to evaluate the influence of changes in salinity and light climate on growth of phytoplankton communities during transport along the riverine–estuarine continuum. Across the riverine–estuarine transition, phytoplankton experiences a strong reduction in the available light. At the freshwater sea water interface in the estuary, freshwater phytoplankton communities from the freshwater tidal reaches are exposed to brackish water. Similarly, due to tidal mixing of seawater with freshwater, brackish water phytoplankton communities experience a decrease in salinity at the freshwater seawater interface.

To simulate the mixing of freshwater and seawater in the estuary and evaluate the impact of corresponding changes in salinity on the phytoplankton communities, freshwater containing phytoplankton collected at sites A and B was mixed with GF/F filtered, phytoplankton-free brackish water from site C (treatments A c L and B c L). Similarly, brackish water containing phytoplankton from site C was mixed with GF/F filtered fresh water from site B (treatment C b L). As control treatments, 50 μm filtrate from each site was mixed with GF/F filtrate from the same site (treatments A a L, B b L and C c L). The resulting dilution of water aimed not only at simulating the mixing of water in the estuary but also reduced the grazing impact by grazers <50 μm in the experimental incubations through dilution (cf. Landry & Hassett, 1982). To be able to study the effect of salinity independently from that of light, all treatments were incubated at the same light intensity, which corresponded to the average light intensity experienced by phytoplankton in the river.

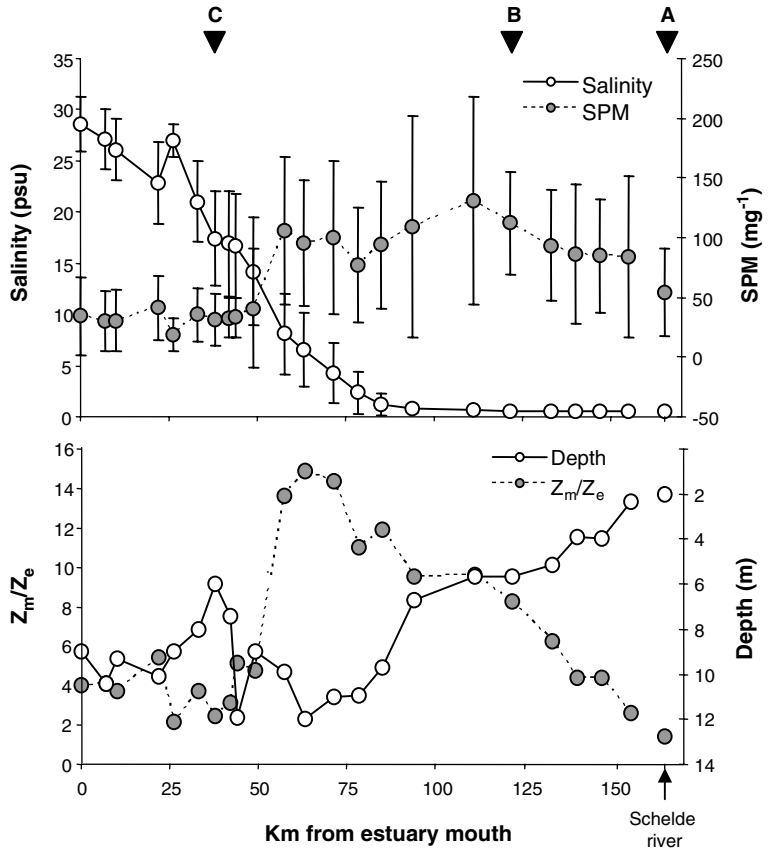

Figure 2. Mean salinity and suspended particulate matter (SPM) concentration (above) and water column depth and mixing depth to euphotic depth ratio (Z_m/Z_{eu}) (below) along the riverine–estuarine continuum of the Schelde. The location of the sampling sites is indicated above the graphs.

To evaluate whether the succession in the phytoplankton community along the riverine–estuarine transition was caused by different adaptations of the phytoplankton communities to the underwater light climate, phytoplankton communities from sites A and B were incubated at high or low light intensities. The high and low light intensities corresponded to the mean underwater light intensity of respectively the river and the freshwater tidal reaches. To allow for comparison with the other experimental treatments, water containing phytoplankton was mixed with phytoplankton-free GF/F filtrate from the same location. As a result, the high light intensity treatments (A a L and B b L) corresponded to the control treatments of the salinity experiments. Although salinity did not differ between the river and the freshwater tidal reaches, we nevertheless tested whether changes in the chemical composition of the water along the riverine–estuarine transition could affect riverine phytoplankton that is transported into the freshwater tidal estuary. Therefore, 50 μm filtrate from site A was mixed with GF/F filtrate from site B (treatment A b L).

The mean underwater light intensities in the river and freshwater tidal reaches were estimated from water column depth and the light attenuation K_d. Assuming a mean water column depth of 2 m and a K_d of 3.25 m^{-1} in the river, the mean underwater light intensity in the river was estimated to be about 15% of the surface irradiance. Average daily irradiance at the water surface was assumed to be 2000 μEinst m^{-2} s^{-1} in summer and 1250 μEinst m^{-2} s^{-1} in spring. Irradiances used in the high light intensity treatments were therefore set at 190 μEinst m^{-2} s^{-1} in spring and

300 μEinst m^{-2} s^{-1} in summer. Because mean water column depth as well as K_d are about twice as high in the freshwater tidal reaches than in the river, the mean underwater light intensity level in the freshwater tidal reaches was estimated to be about 4 times lower than that in the river. Irradiance in the low light intensity incubations was reduced to 25% of that in the high light intensity incubations using grey neutral density filters. Irradiance was supplied by daylight fluorescent tubes at a 12/12 h light dark cycle in spring and a 15/9 h light dark cycle in summer. Incubations were carried out in a temperature controlled room at 20 °C in summer and 10 °C in spring, which was within 1 °C of the *in situ* temperature. For each treatment, 6 replicates were incubated in 250 ml tissue culture flasks. Three replicates were collected after 1 day while the remaining 3 replicates were collected after 3 days. In spring, when temperature was low, only a weak response was observed after 1 day and therefore only the data from the replicates collected after 3 days are presented. In summer, the response of the phytoplankton community was much faster and only data from the replica's that were collected after 1 day are presented. From each bottle, a 100 ml subsample was filtered onto a GF/F filter. The filter was dried between blotting paper to remove excess water. The dried filter was then immediately wrapped in aluminium foil and stored at −80 °C. Pigments were extracted in a 2% ammonium acetate solution in methanol by means of sonnication using a tip sonnicator. Pigment extracts were analysed by means of HPLC according to the method of Wright et al. (1991).

Results

Temperature at the sampling sites ranged between 20 and 21 °C in summer and between 9.5 and 10.4 °C in spring. Salinity at site C was 22.7 psu in summer and 18 psu in spring. At sites A and B, salinity was always below 0.5 psu, the detection limit of the sensor used.

Total phytoplankton biomass, as estimated by chlorophyll *a* concentration, was substantially higher in summer (Fig. 3) when compared to spring (Fig. 4). In spring, phytoplankton biomass was maximal at site A (15 μg l^{-1}), decreased slightly at site B (13 μg l^{-1}) and was lowest at site C (4.3 μg l^{-1}). In summer, phytoplankton biomass was maximal at site B (135 μg l^{-1}), was about half as high at site A (79 μg l^{-1}) and only 10 μg l^{-1} at site C. The observed change in the location of the chlorophyll *a* maximum from the river in spring to the freshwater tidal reaches in summer and the decline in chlorophyll *a* concentration towards the brackish reaches of the estuary are in agreement with previous studies (Van Spaendonk et al., 1993; Van Damme, 1999; Muylaert et al., 2000a and 2001).

During HPLC analysis, the major accessory pigments observed were indicator pigments for diatoms (chlorophyll c, fucoxanthin, diatoxanthin and diadinoxanthin) or green algae (chlorophyll b, lutein, violaxanthin and neoxanthin). Indicator pigments typical of cyanobacteria (zeaxanthin), cryptophytes (alloxanthin), dinoflagellates (peridinin) and prymnesiophytes (19′-hexanoyloxyfucoxanthin) were not observed or only occurred in trace quantities (zeaxanthin and alloxanthin). This is in agreement with previous studies, which only reported diatoms and chlorophytes as important components of the phytoplankton community in the Schelde estuary (Rijstenbil et al., 1993; Muylaert & Sabbe, 1999; Muylaert et al., 2000a). Therefore we will focus only on indicator pigments of diatoms and chlorophytes. Fucoxanthin will be used as an indicator for diatoms while lutein will be used as an indicator for chlorophytes.

Given a fucoxanthin to chlorophyll *a* ratio varying between 0.5 and 1.5 for diatoms and a lutein to chlorophyll *a* ratio varying between 0.1 and 0.2 for chlorophytes (Tester et al., 1995; Roy et al., 1996; Descy et al., 2000), we estimated that diatoms dominated phytoplankton biomass at all sites in spring and at all sites but site A in summer. At site A during summer, green algae were the dominant algal group. This is in agreement with previous studies, who found that diatoms dominate the phytoplankton throughout the year in the estuary and in the river in spring while green algae are dominant only in the river in summer (Muylaert et al, 1997; 2000a and unpublished results). As lutein to chlorophyll *a* ratio's suggest that chlorophytes were important only in summer, the results for lutein are only presented for the summer experiment.

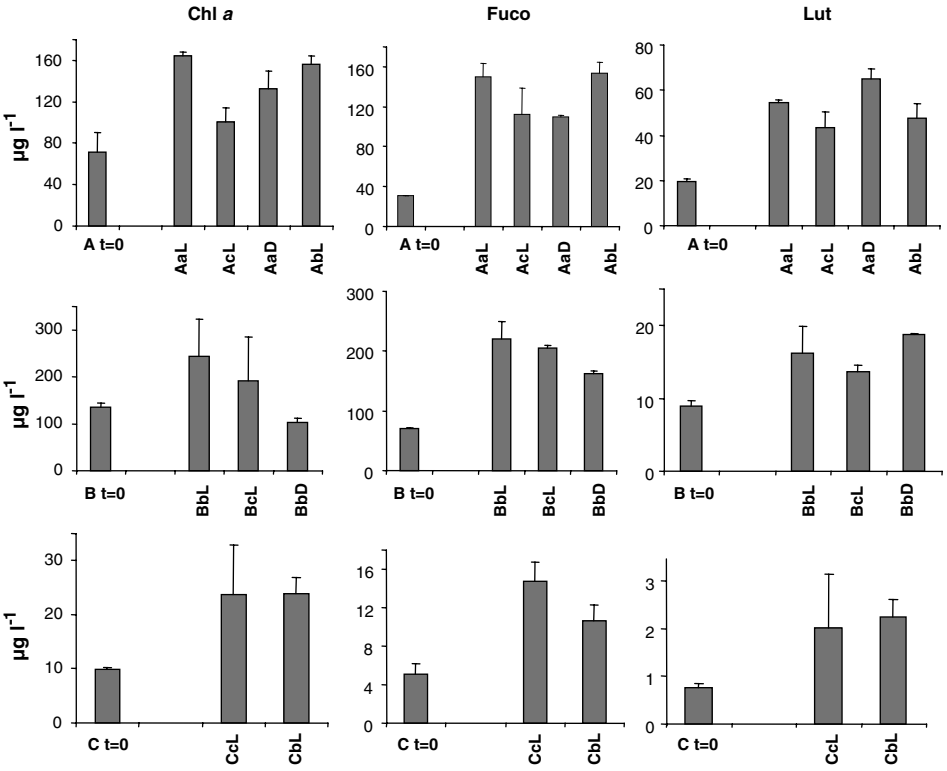

Figure 3. Results of the summer experiment: concentrations of chlorophyll *a* (Chl a), fucoxanthin (Fuco) and lutein (Lut) at the beginning of the experiment ($t = 0$) and after 1 day of incubation in the different treatments. Data shown are averages while error bars correspond to the standard deviation. The abbreviations of the treatments are explained in the materials & methods section.

Mean pigment concentrations in the different treatments are shown in Figure 3 for the summer experiment and Figure 4 for the spring experiment. Pigment concentrations in the different treatments were compared using simple *t*-tests (Table 1). Probably due to slight variations in irradiance levels in the incubator room, differences among replicas were sometimes relatively large. Therefore, a *p*-level of 0.1 was considered to be significant.

When the phytoplankton communities from sites A or B were exposed to high or low light intensities, a significantly higher chlorophyll *a* concentration was always observed in the high (A a L and B b L) compared to the low light intensity treatments (A a D and B b D). Fucoxanthin concentration displayed the same response to a change in light levels as chlorophyll *a*. As opposed to chlorophyll *a* and fucoxanthin, lutein concentration (monitored only in the summer experiments) remained constant (site B) or even increased (site A) in the low compared to the high light intensity treatments. When the freshwater phytoplankton community from site A was mixed with fresh water from site B (A b L), no significant change in salinity occurred and no significant difference was found with the control treatment (A a L) for any of the three pigments investigated.

Mixing of water from sites A or B with an equal volume of filtered water from site C resulted in an increase from 0.5 to 11.6 psu in summer and from 0.5 to 9.3 psu in spring. When the freshwater phytoplankton communities from sites A or B were mixed with brackish water from site C (treatments A c L and B c L), in the spring experiments, a significantly lower chlorophyll *a* concentration was observed relative to the control treatments (A a L and B b L). In the summer experiments, the same results were obtained at site A while at site B, chlorophyll *a* concentration did not differ significantly between the mixture with brackish water and the control treatment. In

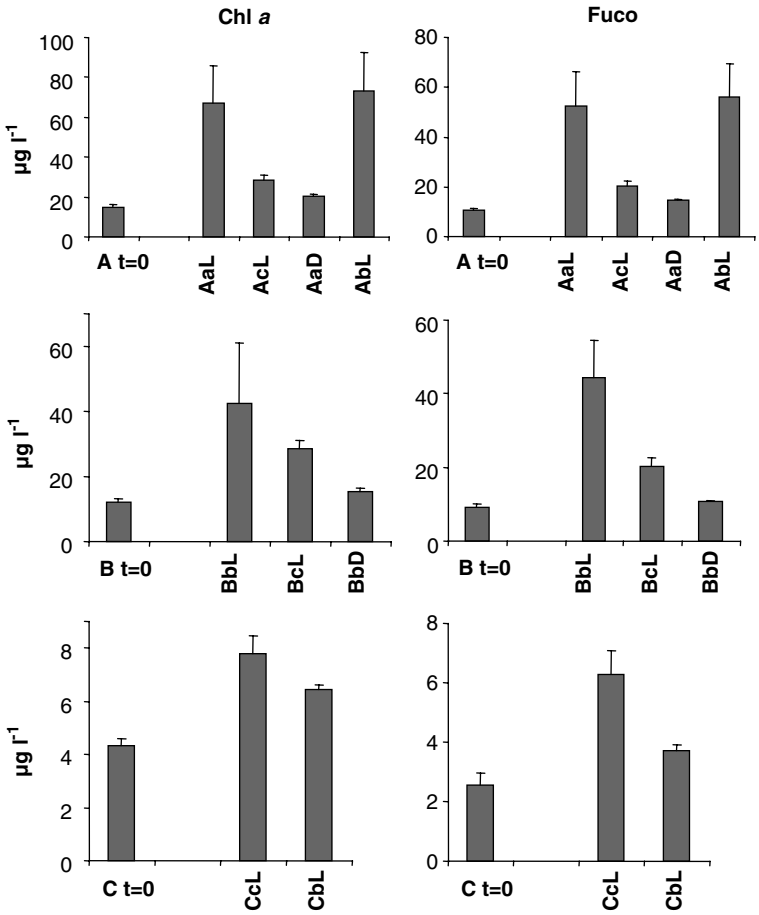

Figure 4. Results of the spring experiment: concentrations of chlorophyll *a* (Chl a) and fucoxanthin (Fuco) at the beginning of the experiment ($t = 0$) and after 3 days of incubation in the different treatments. Data shown are averages while error bars correspond to the standard deviation. The abbreviations of the treatments are explained in the materials & methods section.

the spring as well as the summer experiments, fucoxanthin displayed the same response as chlorophyll *a* to the mixing of the freshwater phytoplankton communities from sites A and B with brackish water. Lutein was only monitored in the summer experiments and also displayed the same response as chlorophyll *a*. Mixing of water from site C with an equal volume of filtered water from site B resulted in a decrease from 22.7 to 11.6 psu in summer and from 18 to 9.3 psu in spring. When the phytoplankton community from site C was mixed with freshwater from site B (treatment C b L), a significant decrease in chlorophyll *a* concentration relative to the control treatment (C c L) was observed in spring but not in summer. Fucoxanthin displayed a significant response to mixing with freshwater in both experiments. Like chlorophyll *a*, lutein did not respond significantly to mixing of the phytoplankton from site C with fresh water from site B in the summer experiments.

Discussion

Influence of light intensity

Light intensity had a strong and significant effect on growth of phytoplankton communities from the river as well as the freshwater tidal reaches. Chlorophyll *a* concentration was always lower in the low light intensity treatments (corresponding to the mean underwater light intensity of the freshwater tidal reaches) when compared to the

Table 1. Significance levels for *t*-tests comparing pigment concentrations between treatments after 3 days of incubation (in the spring experiment) or 1 day of incubation (in the summer experiment)

	Chlorophyll *a*		Fucoxanthin		Lutein
	Spring	Summer	Spring	Summer	Summer
A a L vs. A a D	0.012	0.035	0.009	0.008	0.017
A a L vs. A b L	0.709	0.217	0.762	0.713	0.154
A a L vs. A c L	0.023	0.001	0.016	0.093	0.065
A a D vs. A c L	0.006	0.060	0.005	0.837	0.023
B b L vs. B b D	0.008	0.039	0.005	0.025	0.413
B b L vs. B c L	0.084	0.503	0.017	0.423	0.319
B b D vs. B c L	0.008	0.178	0.003	0.000	0.005
C c L vs. C b L	0.032	0.993	0.005	0.049	0.756

high light intensity treatments (corresponding to the mean underwater light intensity of the river).

The phytoplankton community from the freshwater tidal reaches responded in a similar way to a reduction in light intensity than the phytoplankton community from the river in spring and was even more strongly inhibited by a reduction in light intensity than the phytoplankton from the river in summer. This suggests that the phytoplankton community from the freshwater tidal reaches is not better adapted to survival in the low light environment of the estuary than the phytoplankton community from the river. In spring, this was expected, as the phytoplankton communities in both the river and the freshwater tidal reaches were dominated by diatoms. In summer, green algae dominated the phytoplankton community in the river while diatoms were dominant in the freshwater tidal reaches. As chlorophytes require higher light levels for growth while diatoms are better adapted to survive under low light conditions (Richardson et al., 1983), the phytoplankton community from the river was expected to be more sensitive to a reduction in light intensity than the phytoplankton community from the freshwater tidal reaches.

Contrary to our expectations, diatoms were negatively affected by a reduction in light intensity while green algae displayed an equal (green algae from the freshwater tidal reaches) or even higher (green algae from the river) growth in the low light intensity compared to the high light intensity treatments. Therefore, other factors than changes in the light climate probably regulated the succession from green algae to diatoms along the riverine–estuarine transition. Changes in the chemical composition of the water probably did not play a role: when the riverine phytoplankton community was mixed with water from the freshwater tidal reaches no significant effect on chlorophyll *a* nor accessory pigments was observed. Grazing by rotifers may be important. Like in other freshwater tidal estuaries (e.g. Holst et al., 1998), rotifers are the dominant zooplankton in the freshwater tidal reaches of the Schelde (Muylaert et al., 2000b). Rotifers graze selectively on small phytoplankton like the coccoid chlorophytes that are dominant in the Schelde river and may have difficulties ingesting the relatively large diatoms dominating in the freshwater tidal reaches. In our experiments, the influence of rotifers was eliminated by filtration over a 50 μm mesh and dilution with filtered water.

Influence of salinity

When phytoplankton communities from the river or the freshwater tidal reaches were mixed with brackish water, a significant reduction in phytoplankton growth was observed in 3 out of 4 experiments. Only in the summer experiment with the phytoplankton community from the freshwater tidal reaches no effect of salinity could be statistically demonstrated. At the time of the summer experiments, initial phytoplankton biomass in the freshwater tidal reaches was already very high and increased further to more than 250 μg chlorophyll $a\,l^{-1}$ during the incubation in the control treatments

as well as in the high salinity treatments. It is plausible that at such high biomass level phytoplankton growth became controlled by an unknown factor. This is supported by the observation that in replicate experimental bottles that were incubated for two days longer biomass decreased again to a level below initial biomass (data not presented). Possibly, parasites of phytoplankton like chytrid fungi or the amoeba *Asterocaelum*, which often become abundant in the freshwater tidal reaches of the Schelde estuary when phytoplankton biomass is high (Muylaert et al., 2001, 2000b and unpublished observations), prevented growth of phytoplankton. Therefore, we are reluctant to conclude that the phytoplankton community from the freshwater tidal reaches was better adapted to survive an increase in salinity than the community from the river.

In the experiments with freshwater phytoplankton from the river or the freshwater tidal reaches the response of fucoxanthin and lutein was the same as the response of chlorophyll *a*, suggesting that diatoms and chlorophytes were affected in a similar way by an increase in salinity. This is in agreement with field observations in the Schelde estuary, which show that between a salinity of 0.5 and 10 psu all freshwater species, diatoms and chlorophytes alike, disappear and are replaced by marine species (Muylaert & Sabbe, 1999; Muylaert et al., 2000a).

When phytoplankton communities from the brackish water station were exposed to freshwater, a significant negative effect on both chlorophyll and fucoxanthin was observed in spring. In summer, however, only fucoxanthin displayed a significant negative response to a reduction in salinity while chlorophyll *a* and lutein were not affected. These results indicate that a reduction in salinity has a negative effect on the growth of brackish water phytoplankton but that different algal groups may be affected in a different way by a salinity reduction. Within the brackish reaches of the estuary, different salinity zones containing specific phytoplankton communities can often be discerned (Rijstenbil et al., 1993; Muylaert & Sabbe, 1999). This indicates that different brackish water phytoplankton species have different salinity tolerances. Therefore, we can expect some algal groups to be more sensitive to a salinity reduction than others.

The negative effect of changes in salinity on growth of freshwater and brackish water phytoplankton communities supports the hypothesis that the observed succession in phytoplankton community composition between a salinity of 0.5 and 10 psu from freshwater species to marine species is caused by salinity stress (Muylaert & Sabbe, 1999; Muylaert et al., 2000a). In our experiments, however, an increase or decrease in salinity did not cause mortality of freshwater or brackish water phytoplankton species but merely resulted in a reduction of growth rates. This is in agreement with results from culture experiments which demonstrated that, when phytoplankton is exposed to a change in salinity, osmoregulatory processes cause respiration to increase while growth often continues, albeit at a lower rate (Miller & Kamykowski, 1986; Flameling & Kromkamp, 1994). This suggests that salinity stress is not the only factor responsible for the decline of freshwater and brackish water phytoplankton between a salinity of 0.5 and 10 psu. In the Schelde estuary, like in many estuaries, the freshwater seawater interface is not only characterized by a strong change in salinity but it is also location of a peak in the Z_m/Z_{eu} ratio (Fig. 2). Therefore, extreme light limitation of phytoplankton primary production can be expected in this zone. In our experiments, we did not simultaneously test the effect of a reduction in light intensity and a change in salinity on phytoplankton growth. If the effects of light and salinity are complementary or enforce each other, the decline of freshwater and marine phytoplankton populations at the salinity gradient can probably be ascribed to a combination of salinity stress and light limitation.

Conclusions

When incubated at a light intensity corresponding to the mean underwater light intensity of the freshwater tidal reaches, growth of phytoplankton was significantly lower than when incubated at a light intensity corresponding to the mean underwater light intensity of the river. In spring as well as in summer, however, the phytoplankton community from the freshwater tidal reaches was not better adapted to low light intensities than the

phytoplankton community from the river. Moreover, contrary to our expectations, green algae appeared to perform better than diatoms under low light conditions. Therefore, a change in the light climate is probably not the primary cause of the succession from green algae to diatoms along the riverine–estuarine transition.

Salinity had a negative effect on growth of freshwater phytoplankton from the river and the freshwater tidal reaches as well as on brackish water phytoplankton. This effect, however, was not strong enough to explain the disappearance of freshwater and brackish water phytoplankton between a salinity of 0.5 and 10 psu. Probably, light limitation plays an equally important role in preventing growth of phytoplankton in this estuarine zone. In experiments with freshwater phytoplankton communities, all pigments responded in a similar way to an increase in salinity, suggesting that all freshwater algal groups occurring in the Schelde continuum are equally sensitive to changes in salinity. In the summer experiment with the brackish water phytoplankton community, fucoxanthin displayed a different response to a salinity increase than lutein and chlorophyll *a*, suggesting that different brackish water algal groups are affected in a different way by changes in salinity.

Acknowledgements

The research presented in this paper was carried out in the framework of the Flemish OMES project ('Onderzoek naar de Milieu-Effecten van het Sigmaplan') coordinated by Prof. Patrick Meire. Stefan Van Damme provided unpublished monitoring data on SPM and salinity. Koenraad Muylaert is a post-doctoral fellow of the Fund for Scientific Research (Flanders). The Fund for Scientific Research is thanked for funding the Scientific Community 'Ecological characterization of European estuaries, with emphasis on the Schelde estuary' (project nr. W 10/5 – CVW.D 13.816).

References

Admiraal, W., 1977. Salinity tolerance of benthic estuarine diatoms as tested with a rapid polarographic measurement of photosynthesis. Marine Biology 39: 11–18.

Bisson, M. A. & G. O. Kirst, 1995. Osmotic acclimation and turgor pressure regulation in algae. Naturwissenschaften 82: 461–471.

Cloern, J. E., 1987. Turbidity as a control on phytoplankton biomass and productivity in estuaries. Continental Shelf Research 5: 1367–1381.

Descy, J. P., H. W. Higgins, D. J. Mackey, J. P. Hurley & T. M. Frost, 2000. Pigment ratios and phytoplankton assessment in northern Wisconsin lakes. Journal of Phycology 36: 274–286.

Flameling, I. A. & J. Kromkamp, 1994. Responses of respiration and photosynthesis of Scenedesmus protuberans Fritsch to gradual and steep salinity increases. Journal of Plankton Research 16: 1781–1791.

Holst, H., H. Zimmermann, H. Kausch & W. Koste, 1998. Temporal and spatial dynamics of planktonic rotifers in the Elbe Estuary during spring. Estuarine Coastal and Shelf Science 47: 261–273.

Kirk, T. O., 1994. Ligh & Photosynthesis in Aquatic Ecosystems. Cambridge University Press. Cambridge. 507 pp.

Kirst, G. O., 1990. Salinity tolerance of eukaryotic marine algae. Annual Review of Plant Physiology and Plant Molecular Biology 40: 21–53.

Landry, M. R. & R. P. Hassett, 1982. Estimating the grazing of marine micro-zooplankton. Marine Biology 67: 283–288.

Miller, R. L. & D. L. Kamykowski, 1986. Effects of temperature and, salinity, irradiance and diurnal periodicity on growth and photosynthesis in the diatom Nitzschia americana: light-saturated growth. Journal of Phycology 22: 339–348.

Muylaert, K., A. Van Kerkvoorde, W. Vyverman & K. Sabbe, 1997. Structural characteristics of phytoplankton assemblages in tidal and non-tidal freshwater systems: a case-study from the Schelde basin. Freshwater Biology 38: 263–276.

Muylaert, K. & K. Sabbe, 1999. Spring phytoplankton assemblages in and around the maximum turbidity zone of the estuaries of the Elbe (Germany), the Schelde (Belgium/The Netherlands) and the Gironde (France). Journal of Marine Systems 22: 133–149.

Muylaert, K., Sabbe K. & W. Vyverman, 2000a. Spatial and temporal dynamics of phytoplankton communities in a freshwater tidal estuary (Schelde, Belgium). Estuarine, Coastal and Shelf Science 50: 673–687.

Muylaert, K., R. Van Mieghem, K. Sabbe, M. Tackx & W. Vyverman, 2000b. Dynamics and trophic roles of heterotrophic protists in the plankton of a freshwater tidal estuary. Hydrobiologia 432: 25–36.

Muylaert, K., J. Van Wichelen, K. Sabbe & W. Vyverman, 2001. Effects of freshets on phytoplankton dynamics in a freshwater tidal estuary (Schelde, Belgium). Archiv für Hydrobiologie 150: 269–288.

Muylaert, K., M. Tackx & W. Vyverman, 2005. Phytoplankton growth rates in the freshwater tidal reaches of the Schelde estuary (Belgium) estimated using a simple light-limited primary model. Hydrobiologia 540: 127–140.

Remane, A. & C. Schlieper, 1958. Die Biologie des Brackwassers. E. Schwiezerbart'sche Verlagsbuchhändlung. Stuttgart. 348 pp.

Richardson, K., J. Beardall & J. A. Raven, 1983. Adaptation of unicellular algae to irradiance- an analysis of strategies. New Phytologist 93: 157–191.

Rijstenbil, J. W., L. R. Mur, J. A.Wijnholds & J. J. Sinke, 1989. Impact of a temporal salinity decrease on growth and nitrogen metabolism of the marine diatom Skeletonema costatum in continuous cultures. Marine Biology 101: 121–129.

Rijstenbil, J. W., C. Bakker, R. H. Jackson, A. G. A. Merks & P. R. M. Devisscher, 1993. Spatial and temporal variation in community composition and photosynthetic characteristics of phytoplankton in the upper Westerschelde estuary (Belgium, SW Netherlands). Hydrobiologia 269: 263–273.

Roy, S., J.-P. Chanut, M. Gosselin & T. Sime-Ngando, 1996. Characterization of phytoplankton communities in the lower St. Lawrence Estuary using HPLC-detected pigments and cell microscopy. Marine Ecology Progress Series 142: 55–73.

Snoeijs, P., 1995. Effects of salinity on epiphytic diatom communities on Pilayella littoralis (Phaeophyceae) in the Baltic Sea. Ecoscience. 2: 382–394.

Soetaert, K. & P. M. J. Herman, 1995. Estimating estuarine residence times in the Westerschelde (The Netherlands) using a box model with fixed dispersion coefficients. Hydrobiologia 311: 215–224.

Tester P. A., M. E. Geesey, C. Z. Guo, H. W. Paerl, D. F. Millie, 1995. Evaluating phytoplankton dynamics in the Newport River estuary (North-Carolina, USA) by HPLC-derived pigment profiles. Marine Ecology Progress Series 124: 237–245.

Van Damme, S., T. Ysebaert , P. Meire & E. Van den Bergh, 1999. Habitatstructuren, waterkwaliteit en leefgemeenschappen in het Schelde-estuarium. Rapport van het Instituut voor Natuurbehoud 99.24. Instituut voor Natuurbehoud. Brussel.

Van Spaendonk, J. C. M., J. C. Kromkamp & P. R. M. Devisscher, 1993. Primary production of phytoplankton in a turbid coastal-plain estuary, the Westerschelde (The Netherlands). Netherlands Journal of Sea Research 31: 267–279.

Wright, S. W., S. W. Jeffrey, R. F. C. Mantoura, C. A. Llewellyn, T. Bjornland, D. Repeat & N. Welshmeyer, 1991. Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. Marine Ecology Progress Series 77: 183–196.