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# OBSERVATIONS ON LITTLE STUDIED PROTISTS (CHYTRIDS AND AN AMOEBA), AFFECTING PHYTOPLANKTON POPULATIONS IN THE UPPER REACHES OF THE SCHELDE ESTUARY (BELGIUM)

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ABSTRACT. — In the summer of 1996, a detailed survey of natural phytoplankton samples from the freshwater part of the Schelde estuary was carried out to check for the occurrence of poorly studied heterotrophic protists, the herbivorous amoeba *Asterocaelum algophilum* and chytrid parasites. The plankton community during the summer bloom was dominated by centric diatoms (*Cyclotella scaldensis*, *C. atomus*). Fungal infestations of these bloom-forming species were not observed in fixed samples, although severe infections were frequently observed in net samples which were maintained in the laboratory for a few days. The chytrid infecting the diatom *Actinocyclus normanii* was identified as *Podochytrium cornutum.* Four morphologically distinct chytrids resembling the genus *Podochytrium* parasitized cells of *C. scaldensis.* In contrast to the populations of centric diatoms, infection of the riverine *Scenedesmus* population by the chytrid *Rhizophydium scenedesmi* occurred *in situ.* The infection prevalence was maximum ca. 24%, leading to a twofold reduction in the biomass of *Scenedesmus*. It is hypothesized that the condition of the host population is a discriminating factor since only the allochtonous phytoplankton populations get infected, when entering the estuary. On the other hand, *Asterocaelum algophilum* was shown to feed voraciously on centric diatom cells. *Cyclotella atomus*, *C. scaldensis* and *Stephanodiscus hantzschii* were its main prey; infrequently, some green algae (*Crucigenia*) were found ingested too. At times, the amoeba was capable of grazing away ca. 25% of the diatoms per day. On average, larger diatom cells were found relatively more frequently inside 'feeding cysts' of this unusual amoeba when compared to the undigested centric diatom populations, which suggests the occurrence of some size-selectivity in the feeding behaviour of *A. algophilum.* Since sometimes more than 70% of the *C. scaldensis* cells and more than 50% of the *S. hantzschii* cells were found ingested, the impact of grazing by *A. algophilum* on the populations of the larger diatom species in the estuary can be significant. As a result, more attention should be paid to the presence and role of these organisms in aquatic food web studies.

KEY WORDS. — *Asterocaelum*, chytrids, diatoms, herbivorous amoebae, phytoplankton, Schelde estuary.

## INTRODUCTION

In marine ecosystems, unicellular organisms like ciliates and heterotrophic flagellates are now considered to be equally important grazers of phytoplankton as metazoan zooplankton (CALBET & LANDRY 2004). Much less attention has been paid so far to other groups of protists, which, however, may interact significantly with the phytoplankton too.

Chytrids are among the protistan groups which still receive relatively little attention in aquatic studies. They constitute a group of primitive, microscopic fungi that belong to the order Chytridiomycota. Many chytrid species are parasites of phytoplankton. They infect their hosts via a zoospore that attaches itself to the cell wall. The attached zoospore encysts, penetrates the host cell with a germ tube, forms a rhizoidal system that digests the cytoplasm and eventually develops into a sporangium that produces new zoospores (see Fig. 1). Chytrids are common in marine and freshwater ecosystems and many different phytoplankton species are known to become infected with chytrid parasites. In freshwater lakes, fungal infections of phytoplankton can occur throughout the year (HOLFELD 1998) and maximal infection prevalence ranges from 80% (e.g. CANTER & LUND 1953, HOLFELD 1998) to almost 100% (ANDERSON *et al.* 1995), resulting in a strong numerical reduction of the host population. Chytrids thus can have a serious impact on the primary production and species succession in phytoplankton communities. The severity of infection depends on many different parameters. Epidemics in phytoplankton populations arise more easily when growth conditions for the algae are unfavourable (SPARROW 1943, REYNOLDS 1984). However, the infection of 'healthy' phytoplankton populations is also frequently reported (CANTER & LUND 1948, 1969, MASTERS 1971, DOGGETT & PORTER 1994, HOLFELD 1998, 2000). As the migratory capacities of zoospores are limited, the chance of an encounter between an infective zoospore and a vulnerable host cell increases with the number of

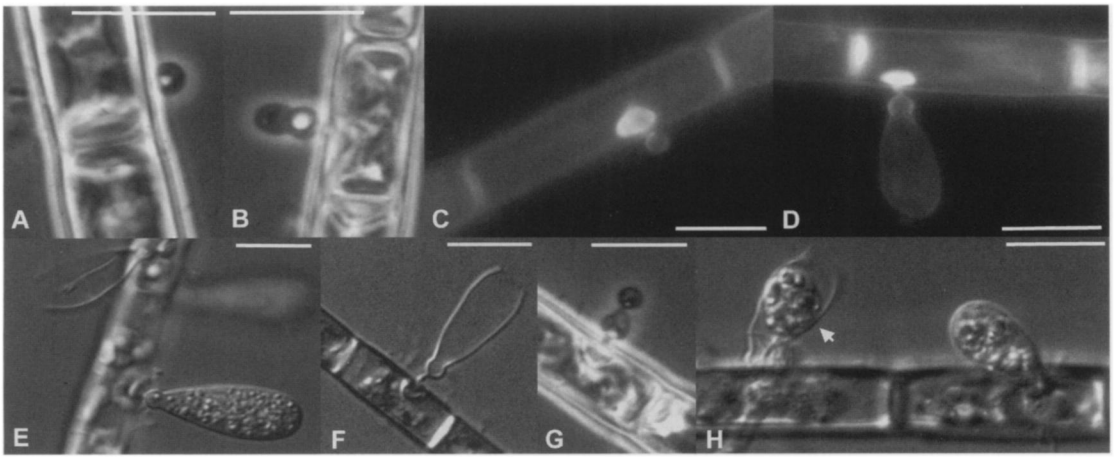


**Fig. 1.** Life cycle of *Diplochytridium sexuale* Karling, a parasitic chytrid infecting cells of the filamentous Xanthophyte *Tribonema vulgare* Pascher observed in a small eutrophicated, clear water pond (Maten 13, Genk, Belgium) in November 1998. A. Encysted zoospore with a large, refractive lipoidal globule. B. Sporangium development. Growth takes places only at the apex of the zoospore cyst leaving behind a basal, thick-walled appendage. C-D. The rhizoidal system (a more or less globular apophysis without rhizoids) inside the host cells, visualised by epi-fluorescence microscopy after staining with Calcofluor White (MÜLLER & SENGBUSCH 1983). The apophysis is already completely developed before sporangium growth takes place. E. Mature clavate zoosporangium with many lipoidal bodies. F. Empty sporangium with a large, apical exit pore. Also visible are the germ tube and the apophysis inside the host cell. G. Fusion of a 'female' encysted zoospore and a 'male' zoospore to eventually form a resting spore. H. Unknown structure, which could be interpreted as a resting spore. Scale bar = 10 µm.

host cells. For this reason, the size of the algal population is believed to be a significant parameter for the development of fungal infections (KUDOH & TAKAHASHI 1990). As a result, highest infections can be found in periods of maximal host cells densities (HOLFELD 1998) and more infections can be found in eutrophic waters, where the abundance of phytoplankton is generally higher (LUND 1957). Light is another discriminating factor. Chytrid zoospores were found to be positively phototactic (BEAKES *et al.* 1993): they have a lower mobility in the dark and lose their ability to recognise and infect host cells (BRUNING 1991b). Temperature (VAN DONK & RINGELBERG 1983, BRUNING *et al.* 1992) and the availability of nutrients (BRUNING 1991a, VAN DONK & BRUNING 1995) can also affect fungal epidemics.

Free-living amoebae constitute another group of protists that are often overlooked in the plankton. Naked amoebae are abundant in sediments and in the water column when the water is rich in suspended particulates (ANDERSON & ROGERSON 1995, MURZOV & CARON 1996, ROGERSON & GWALTNEY 2000). Some amoebae feed on phytoplankton and may have a significant impact on phytoplankton populations. *Asterocaelum*, for instance, is capable of feeding on several algal species. This amoeba secretes a firm wall after the ingestion of one to several prey items and transforms itself into a so-called digestion cyst (Fig. 5 Q-T). Eventually, one to several amoebae will emerge from this cyst leaving it empty except for the remains of its prey (CANTER 1973, CANTER-LUND & LUND 1995). In Loch Leven, *Asterocaelum algophilum* Canter was found to ingest up to 27% of the cells of centric diatoms (BAILEY-WATTS & LUND 1973).

Chytrids and *Asterocaelum* share a common feature, namely that their populations develop and disappear rapidly. Chytrids or *Asterocaelum* can terminate a phytoplankton bloom within one week or even less. This partly explains why they are rarely reported in monitoring studies since the frequency of sampling is often lower than once a week. Chytrids and *Asterocaelum* only affect one or a few species of the phytoplankton community, often the dominant ones. Therefore, they affect more the species composition of the phytoplankton community rather than the primary production. Thus they can end a phytoplankton bloom by decimating the population of the bloom-forming phytoplankton species (e.g. COOK & AHEARN 1976, BRUNING *et al.* 1992).

In 1996, a monitoring study was carried out in the upper Schelde estuary to evaluate the effect of short-term flood events on phytoplankton dynamics. During this 3-month study, the dynamics of individual algal species abundances was followed in detail and outbreaks of both chytrids and the algivore amoeba *Asterocaelum algophilum* were observed. Here, we estimate the potential impact of these outbreaks on some algal species and the phytoplankton dynamics in general.

## MATERIAL AND METHODS

### STUDY AREA

The Schelde estuary is a macrotidal coastal plain estuary situated in Western Europe (Fig. 2). In contrast to many other European estuaries, where locks were constructed at the freshwater seawater interface, the Schelde estuary still possesses an extensive freshwater tidal zone in its upper reaches. In these upper reaches, dense phytoplankton blooms occur during spring and summer. These phytoplankton blooms are typically dominated by diatoms but green algae can also be co-dominant in the tributary rivers and near the head of the estuary in summer (MUYLAERT *et al.* 2000b). The zooplankton community in the upper estuary is dominated by rotifers, which frequently attain ca. 1000 individuals $L^{-1}$ (SOETAERT & VAN RIJSWIJK 1993, MUYLAERT *et al.* 2000a, TACKX *et al.* 2004).

The Schelde estuary is strongly polluted with dissolved and particulate matter. During the sampling period, average concentrations of orthophosphate and total dissolved nitrogen were 340 µg $L^{-1}$ and 6040 µg $L^{-1}$, respectively. Therefore, it is very likely that the concentration of nutrients was not a limiting factor for photosynthesis (HEIP 1988). The sampling site (Hamme-Driegoten, see Fig. 2) is situated in the freshwater part (salinity range 0.1 – 0.7‰) of the estuary, close to the maximal turbidity zone, which is revealed by the low average Secchi-depth (23.5 cm). The average conductivity at the site was 1120 µS $cm^{-1}$ while the pH was more or less constant, ca. 7.5. Climatological conditions were typical for that time of the

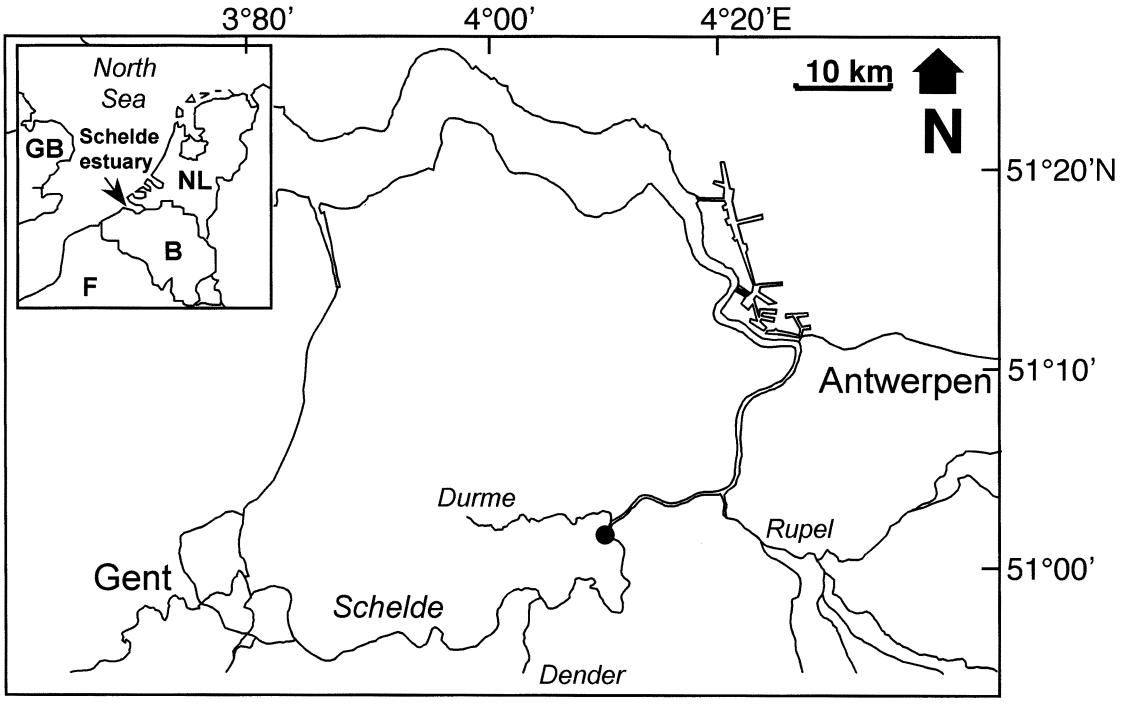


**Fig. 2.** Site map of the Schelde estuary, with indication of the sampling location (black dot) in the freshwater tidal reaches of the Schelde river.

year except for the occurrence of a marked summer storm on August 30th with heavy rainfall and high wind speeds.

### Sampling and analysis

Samples were taken every two days at low tide on a platform at 3-5 m distance from the bank, from July 31th to October 30th, 1996. For quantitative analysis, 40 mL of surface water were immediately fixed with 25 µL alkaline Lugol's iodine, 1 mL formaldehyde (35%) saturated with borax and 50 µL $NaS_2O_3$ (Sherr & Sherr 1993). Surface water (4 L) was concentrated with a plankton-net (mesh size 10 µm) to a volume of about 50 mL. Back in the laboratory, these qualitative samples were put on a desk in front of a window at room temperature and were frequently checked for the occurrence of parasitic fungi.

The quantitative samples were stored in a refrigerator in the laboratory until enumeration with a Wild M40 inverted microscope using the method of Utermöhl (1958). At least 50 individuals of each dominant phytoplankton taxon were counted. Linear dimensions of cells were measured and the biovolume was calculated assuming simple geometric cell shapes. Biovolumes were converted to C-biomass using a conversion factor of 0.22 pg C $\mu m^{-3}$ (Reynolds 1984). Chytrids were observed at high magnification (1000 X) with a Leitz Diaplan microscope and identified using the guide of Karling (1977). The infection prevalence was evaluated and dead algae on which the parasite had completed its life cycle (empty sporangia) were not included in this percentage (Bruning *et al.* 1992). Similarly, digestion cysts of *Asterocaelum algophilum* were counted and cysts with and without amoebae were distinguished. Free-living individuals of the amoeba were rarely encountered in the field samples. In order to detect any food preference, detailed observations of the content of digestion cysts of *A. algophilum* were carried out on 5 field samples from periods of high cyst abundance. Since diatom frustules could only be detected visually in digestion cysts without amoebae, the average number of ingested diatoms was based on the enumeration of the contents of at least 25 empty digestion cysts. This value was multiplied with the total number of digestion cysts at the same day to obtain the amount of ingested diatoms.

## RESULTS

### THE PHYTOPLANKTON COMMUNITY

The phytoplankton cell densities reached high levels up to ca. 76 000 cells $mL^{-1}$ (equivalent to 3 600 µg C $L^{-1}$) towards the end of August (Fig. 3). After this maximum, a sharp decline was observed due to a heavy storm, and the densities fluctuated around 11 000 cells $mL^{-1}$ (1 000 µg C $L^{-1}$) during September and October. In total, 75 algal taxa were identified, of which representatives of the Bacillariophyta and Chlorophyta were the most abundant (Fig. 3). Before the storm, diatoms contributed most to total biomass (ca. 80%). After the storm, their relative contribution had declined to ca. 61%. The biomass of green algae was more or less stable during the entire study but their mean contribution to the total phytoplankton biomass after the storm (31%) doubled when compared to the mean contribution before the storm (14.5%). On average, ca. 80% of total algal biomass was composed of only 6 taxa, i.e. centric diatoms: *Cyclotella atomus* Hust. (24%), *C. scaldensis* Muylaert & Sabbe (24%), *Actinocyclus normanii* (W. Greg.) Hust. (9 %), *Aulacoseira granulata* (Ehr.) Simons. (6%), *Thalassiosira* spp. (6%) and green algae: *Scenedesmus* spp. (12%). More details about the phytoplankton community and its relation with biotic and abiotic parameters can be found in MUYLAERT *et al.* (2001).

### FUNGAL PARASITES

In many field samples, the chytrid *Rhizophydium scenedesmi* (Fott) Karling was detected on *Scenedesmus* coenobia (Fig. 5 N-O). This chytrid is mainly known to infect mass cultures of *Scenedesmus* spp. (FOTT 1967, SOEDER & MAIWEG 1969, LUKAVSKÝ 1970) but has also been observed on other green algae (MASTERS 1971). They were first found in the samples collected in mid August and were abundant during the rest of the study (Fig. 4). This fungus showed maximal infection prevalence (24%) shortly after the maximal density peak of 4 000 *Scenedesmus* coenobia

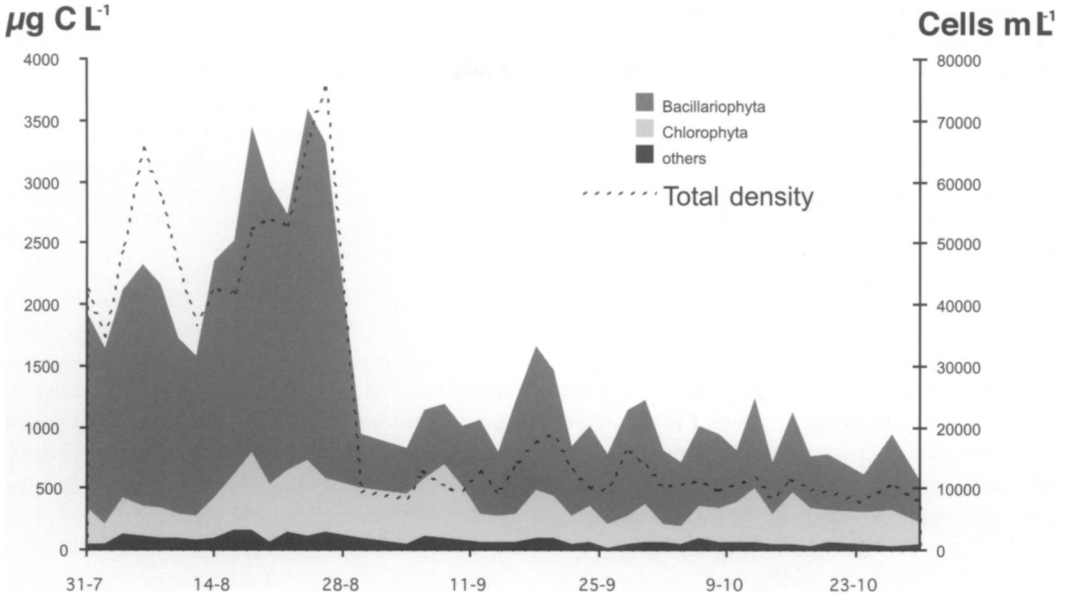


**Fig. 3.** Fluctuation in biomass (µg C $L^{-1}$) of the dominant phytoplankton taxa and total phytoplankton density (cells $mL^{-1}$) in the upper reaches of the Schelde estuary in the summer 1996.

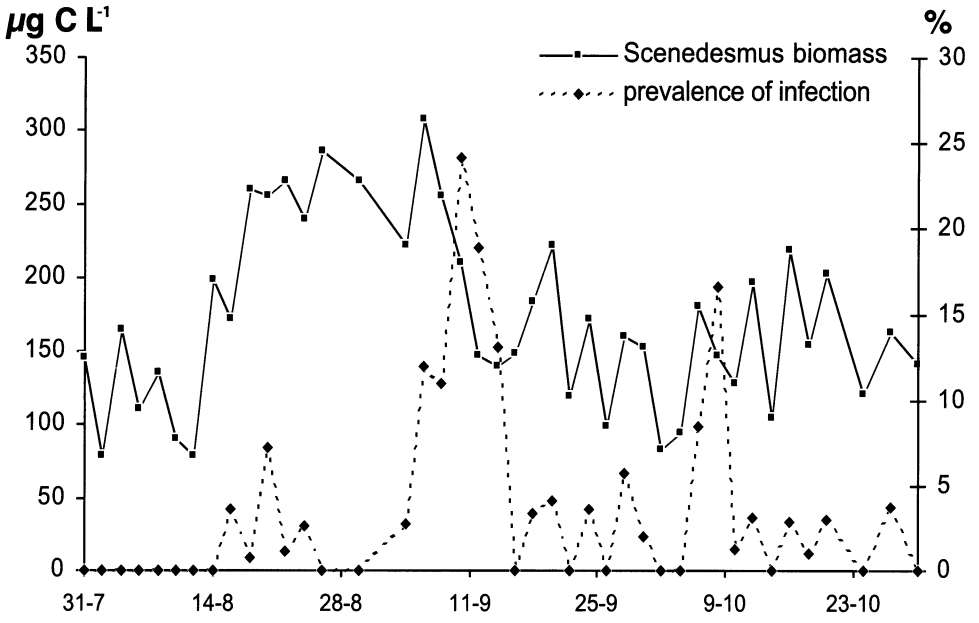


**Fig. 4.** Fluctuation in biomass (µg C $L^{-1}$) of the *Scenedesmus* population at the sampling location and the infection prevalence of *Rhizophydium scenedesmi* (Fott) Karling as expressed in % of total *Scenedesmus* coenobia. Coenobia on which the fungus had completed its lifecycle (empty sporangia) were not included in this parameter.

$mL^{-1}$ in September, coinciding with a reduction in *Scenedesmus* densities by more than half.

Infection of centric diatoms was not observed in the fixed samples but infected diatoms were frequently observed in the concentrated samples, which were maintained for a few days at room temperature in the laboratory. Four morphologically distinct chytrids were observed frequently parasiting *Cyclotella scaldensis* cells (Fig. 5 A-H). All observed sporangia, which were eucarpic, monocentric and epibiotic, had a more or less clearly visible basal cell but differed in the number and location of the dehiscence pores. The sporangia of the first morphotype were obovate with one apical dehiscence pore (Fig. 5 A-B); those of the second were transversally obovate, with a single lateral dehiscence pore (Fig. 5 C-D). The third morphotype had transversally elliptic sporangia, with two lateral pores (Fig. 5 E-F), and the fourth had a clearly visible basal cell and an elongated main body, with a single apical pore (Fig. 5 G-H). Whether these different morphotypes belong to one (morphologically variable) species or represent different species is not clear. All four share many characters with *Podochytrium chitinophilum* Willoughby, described as a saprophyte on chitin (Willoughby 1961), although other representatives of this genus have been reported as parasites on diatoms (Karling 1977). The morphotype with two lateral exit pores, strongly resembles an unidentified chytrid on *Cyclotella,* illustrated previously by Canter-Lund & Lund (1995: 321).

On several occasions, the chytrid *Podochytrium cornutum* Sparrow was observed on cells of *Actinocyclus normanii*. The chytrid penetrated the diatom cell via the openings of the labiate processes (Fig. 5 J-M). *P. cornutum* was originally described as a parasite of the centric diatom genus *Stephanodiscus* (Sparrow 1951, Karling 1977), but Schuchardt & Holfeld (1991) found this chytrid infecting the centric diatom *Actinocyclus normanii* in the Weser estuary. Although they have a larger sporangium size, *P. cornutum* from the Schelde estuary share the absence of a septum

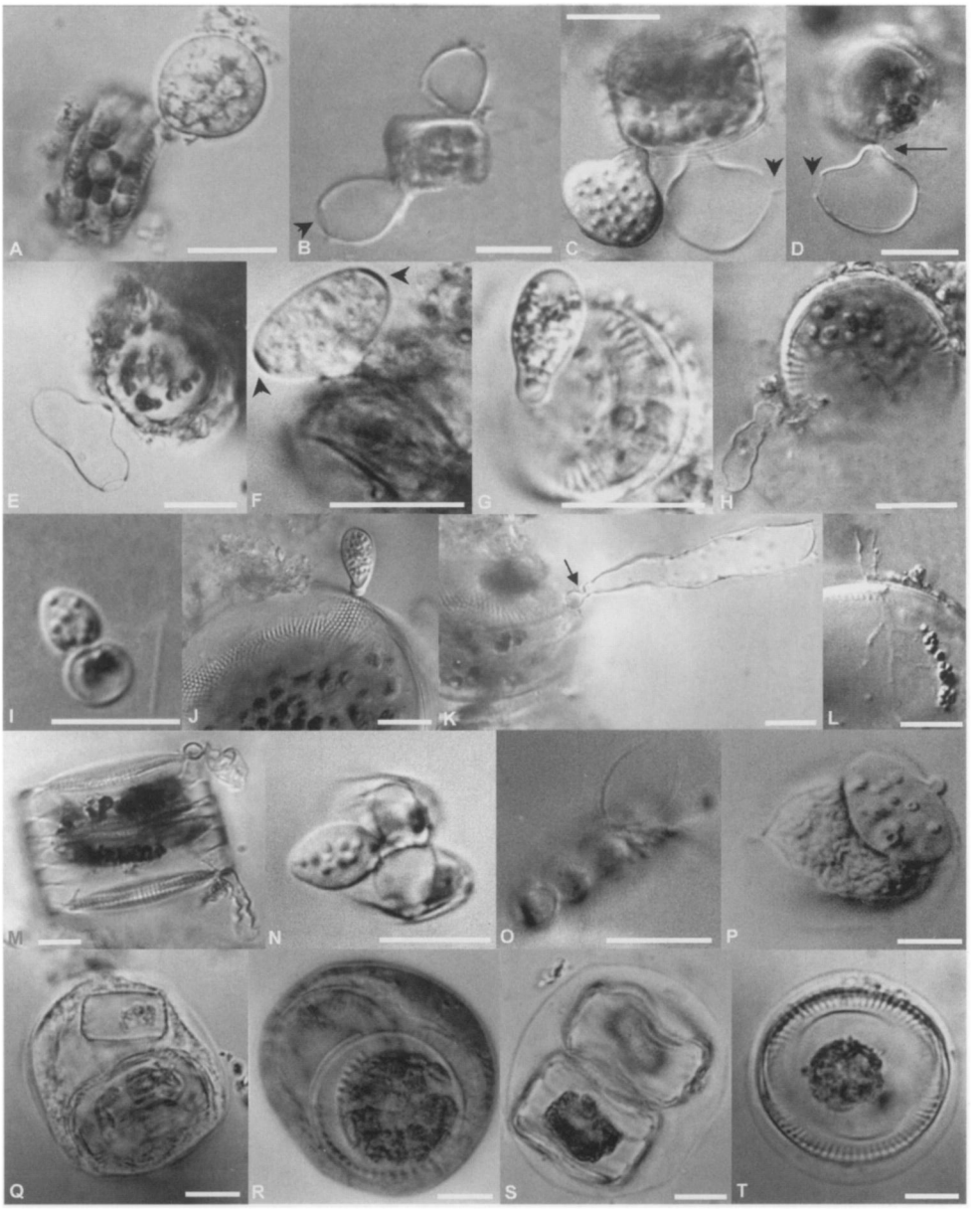


**Fig. 5.** Different protists, capable of affecting phytoplankton populations in the upper reaches of the Schelde estuary. A-H. *Podochytrium*-like chytrids, infecting the centric diatom *Cyclotella scaldensis*. Four different morphotypes were observed, all had a more or less clearly visible basal cell (long arrow), but they differed in the amount and location of the dehiscence pores (small arrows). I. Mature sporangium of an unknown chytrid on *Cyclotella atomus*. J-M. The chytrid *Podochytrium cornutum,* infecting cells of *Actinocyclus normanii*. These chytrids have a narrow but large sporangium and a clearly visible basal cell with an inclination just above (arrow). The exit pore is situated at the apex and sometimes the lid is still attached. The rhizoidal system consists of branched mycelium. N-O. Mature and empty sporangium of *Rhizophydium scenedesmi* (Fott) Karling, infecting a *Scenedesmus* coenobium. P. Sporangium of *Entophlyctis apiculata*, a parasitic biflagellate fungus inside a *Pteromonas* cel. Q-T. Digestion cyst from the herbivore amoeba *Asterocaelum algophilum* with and without amoebae. The food contents (centric diatoms) can be easily distinguished.

between the basal cell and the main body with specimens from the Weser estuary, which is in contrast with the description of this species by KARLING (1977: 82 and plate 33).

An unknown chytrid on *Cyclotella atomus* (Fig. 5 I) and *Entophlyctis apiculata* Fischer, an endobiotic chytrid parasite of autotrophic flagellates (KARLING 1977, SHIN *et al.* 2001), inside a *Pteromonas* cell (Figure 5 P), were only seen once.

### PREDATORY AMOEBAE

During the entire study, digesting cysts of *Asterocaelum algophilum* were observed frequently in the field samples (Fig. 5 Q-T). The centric diatoms *Cyclotella atomus*, *C. scaldensis* and *Stephanodiscus hantzschii* Grun., were its main prey in the estuary. Ingested chlorophytes (*Crucigenia* sp.) were observed only rarely. Maximal cyst abundances were observed soon after (mid August, September) or during (October) maximal concentrations of centric diatoms (Fig. 6A). The build-up of a second maximal abundance at the end of August was disrupted by the storm, which flushed the complete phytoplankton community to the river mouth. In general, between 5 and 25 % of the diatom population was found ingested. Before the storm, the feeding cysts contained either only frustules of *C. atomus* or a mixture of different centric diatom taxa (Fig. 6B). After the storm, frustules of *C. scaldensis* were mainly observed inside feeding cysts. In comparison with the population of undigested diatoms, the relative contribution of larger cells (*C. scaldensis* and *S. hantzschii*) was higher in the feeding cysts, especially after the storm (Fig. 6C). Small diatom cells (*C. atomus*) represented only ca. 30% of the diet of this amoeba at mid August, and this diatom contributed less than 2% in the period after the storm. Although more cells of *C. atomus* were captured (on average 4 per cyst) in comparison with *C. scaldensis* and *S. hantzschii* (both less than one per cyst), only about 20% of the *C. atomus* population was found ingested while at times more than 70% of the *C. scaldensis* cells and 50% of *S. hantszchii* cells were reported to be inside the feeding cysts, especially during mid August when there was a peak in the number of amoebae (Fig. 6D). Lower values were obtained just before the summer storm, but by then the amoebae population had probably not reached its maximum yet. In September and October, the average number of *C. atomus* cells decreased to less than one per cyst while the amount of *C. scaldensis* and *S. hantzschii* increased (from 0.64 to 1.66 and from 0.18 to 0.33 individuals per cyst, respectively). The impact on the population was still high for *C. scaldensis* and *S. hantzschii,* i.e. respectively maximum 35 and 55 % of the population inside feeding cysts, and remained low for *C. atomus* (maximum 5%).

## DISCUSSION

### THE SIGNIFICANCE OF FUNGAL INFECTIONS

The observed phytoplankton community, with a high contribution of centric diatoms to total phytoplankton biomass, is typical for the freshwater tidal part of estuaries (ANDERSON 1986, JACKSON *et al.* 1987, SCHUCHARDT & SCHIRMER 1991, MALLIN & PAERL 1992) and the co-dominance of *Cyclotella scaldensis* and *Cyclotella atomus* is frequently documented in such a habitat (SABATER & KLEE 1990, MURAKAMI *et al.* 1992, KLARER & MILLIE 1994, MURAKAMI *et al.* 1994, KISS 1996). Although chytrid parasites of centric diatoms were present in the estuary (indicated by the strong infestations in concentrated samples in the laboratory), significant infection of the diatoms in the estuary was never observed. On the other hand, chytrid infestation of the green alga *Scenedesmus* was evident in the field. It seems that the susceptibility to severe infection differed between the two algal populations. Since fungal infections are highest in periods of maximal host densities (HOLFELD 1998), the abundance of host cells is a significant factor (KUDOH & TAKAHASHI 1990). The success of infection depends on the capacity of zoospores to find new host cells. But as host cells with a bigger cell volume can become infected at lower densities (HOLFELD 1998), the use of cell volume and/or biomass is preferable. Maximal biomass values of the

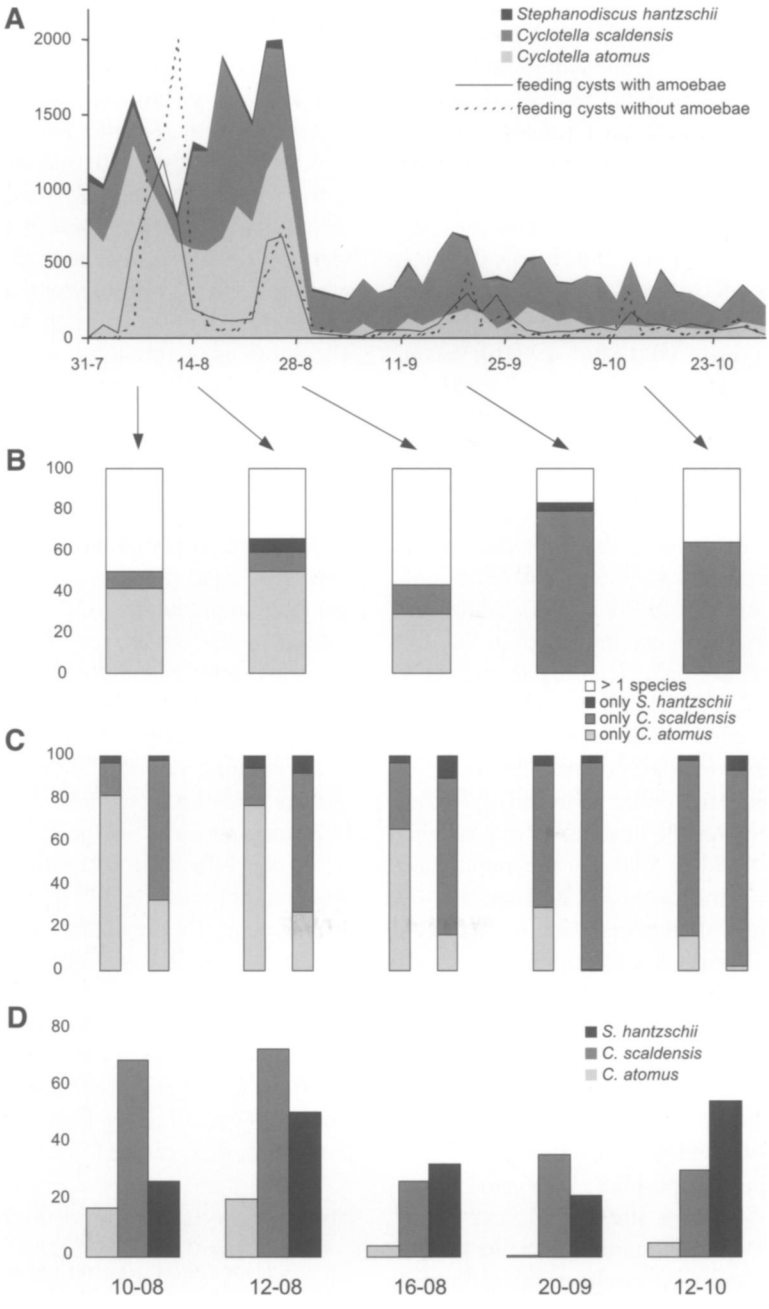


**Fig. 6.** A. Fluctuations in the density (cells $mL^{-1}$) of feeding cysts of *Asterocaelum algophilum* with and without naked amoeba and in the biomass (µg C $L^{-1}$) of different centric diatom species, its principal prey. B. Relative contribution (%) of feeding cysts with specific prey items to total number of cysts analysed in samples from 5 selected data of high amoebae abundance C. Relative biomass contribution (%) of each centric diatom species *in situ* (left column) and in the feeding cysts of *A. algophilum* (right column) to the total biomass of centric diatom species outside and inside digestion cysts respectively. D. The relative contribution (%) of different ingested diatom species by *A. algophilum* to the total (*in situ* + digested) biomass of the different diatom species.

diatom species in the samples were higher than those of *Scenedesmus*. Although the highest infection of the *Scenedesmus* population did occur when densities of the coenobia were maximal, the start of a severe infection at the study site was probably not triggered by high density or biomass of the host cells alone.

Fungal epidemics can be determined by physical (light, temperature) and chemical (nutrients) conditions. Zoospores have a lower mobility in the dark and lose their ability to recognize and infect host cells (BRUNING 1991b). Some ultrastructural organelles in the zoospores exhibit photoreceptoric characteristics and these zoospores seem to enter quiescence in very low light levels (BEAKES *et al.* 1993). The development time of the sporangia is dependent on temperature and nutrients. It increases with decreasing temperature (BRUNING *et al.* 1992) and at limiting phosphorus levels (BRUNING 1991a). Since the environmental variables are the same for the different chytrids at the study site, they are probably of less importance in explaining the fact that *Scenedesmus* seemed more vulnerable to infection in comparison with the centric diatoms.

Fungal epidemics arise more easily when growth conditions for the host cells are less optimal (REYNOLDS 1984). Even if the population looks 'healthy', intrusion of the host cell by the developing fungus often takes place through cell wall fissures and/or weakened regions of the cell wall (BEAKES *et al.* 1992, DOGGETT & PORTER 1994). The environmental conditions at the study site were ideal for the development of centric diatom populations. These organisms are especially adapted to strongly changing light levels (REYNOLDS 1994), which typify this part of the estuary. Their species optimum in the estuary was located at the sampling site (MUYLAERT *et al.* 2001) and their good condition is reflected by their high abundance. In contrast, *Scenedesmus* are not typical for the estuary, their population optimum is situated far more upstream in the river and their densities at the sampling site only increase by import from the river after periods of rainfall. In the estuary, *Scenedesmus* are subject to increasing levels of salinity and decreasing light intensities, which weaken the population and lead to their final senescence (MUYLAERT *et al.* 2001). Most likely, the conditions had become unfavourable in the static concentrated samples in the laboratory too, which made the centric diatoms more susceptible to infection.

In contrast with fungal infections by virulent parasitic chytrids as is more characteristic for phytoplankton populations in lakes, it seems that epidemics will occur in the estuary mainly when growth conditions worsen (e.g., when phytoplankton changes from an autochtonous to an allochtonous community or by changing seasonal conditions). This is so for the riverine green algae (*Scenedesmus* spp.) at the sampling spot and presumably for the observed population of centric diatoms more downstream in the estuary. Although conclusions on the possible role these chytrids could have in the removal of senescent algal blooms in the estuary are rather speculative on the basis of our data, other observations on the occurrence of chytrid infections in estuaries and rivers support our hypothesis. SCHUCHARDT & HOLFELD (1991) found an inverse relationship between the degree of infection of *Podochytrium cornutum* on *Actinocyclus normanii* and the densities of the host in the Weser estuary. Infection increased in the downstream direction where densities of the host cells decreased owing to severe light limitation. MUYLAERT (1994) observed an infection of a population of the centric diatom *Stephanodiscus hantzschii* by *Chytridium* sp. when it entered the Elbe estuary. In the Danube river, a severe chytrid infection of the centric diatom population was observed in Austria (STOYNEVA 1998), where diatoms entered a highly polluted (mainly by coal-mining industry) part of the river (STOYNEVA pers. comm.).

### THE IMPORTANCE OF PREDATORY AMOEBAE

*Asterocaelum algophilum* was first described in eutrophic lakes in the UK and later observed in English rivers as well (CANTER 1973, 1980). In the Schelde estuary, this amoeba can be found predominantly in the freshwater and oligohaline reaches during the summer when the diatoms bloom (MUYLAERT *et al.* 2000b). Although *A. algophilum* was shown to grow well in culture on

a diet of different chlorophytes (BAILEY-WATTS & LUND 1973), in nature it feeds predominantly on diatoms (CANTER 1973, 1980) as is also confirmed by our observations. Different diatom species were found ingested by this amoeba in the Schelde estuary, depending on what was available in the water column. Before the summer storm, about half of the feeding cysts contained exclusively *C. atomus*, which dominated the phytoplankton at that time, while the other half contained a mixture of *C. atomus* and *C. scaldensis*. After the storm, *C. scaldensis* became the most abundant diatom as was reflected in the feeding cysts of *A. algophilum*. It seems that *A. algophilum* needs a certain amount of prey biomass before developing into a feeding cyst. When it fed only on *C. atomus,* it needed on average 4 cells per specimen before encysting, when feeding only on *C. scaldensis,* it needed in general only one diatom cell. This is in agreement with the data by CANTER (1973), who found that encystment of *A. algophilum* took place after the ingestion of 3 cells of *Cyclotella pseudostelligera* Hust. or 1 cell of the larger species *Stephanodiscus rotula* (Kütz.) Hendey in the eutrophic shallow lake (Loch Leven) where this amoeba was first discovered.

However, the observation that relatively more larger cells were found inside the cysts in comparison with the live population of centric diatoms, suggests some size selectivity in the feeding behaviour of *A. algophilum*. Occasionally, more of these larger diatoms were found ingested than remaining in the water and as a result, the grazing impact from the amoebae on the larger, less abundant diatoms (*C. scaldensis*, *S. hantzschii*) was probably stronger than on the small, very abundant ones (*C. atomus*). This was especially obvious for *S. hantzschii*, whose biomass decreased six-fold at the maximal abundance of *A. algophilum* in mid August, in comparison with the biomass of *C. atomus* and *C. scaldensis* that decreased only two-fold.

How this amoeba carries out its selective feeding behaviour remains unclear. Culture experiments have indicated that *A. algophilum* needs a solid surface in order to graze on its prey (BAILEY-WATTS & LUND 1973). However, in the turbulent environment of an estuary it seems unlikely that grazing by *A. algophilum* takes place on sunken diatoms on the sediments and that feeding cysts of the amoebae could be found in the plankton due to resuspension. Since this amoeba is algivore, it is unlikely that it lives associated with suspended particulate matter in the estuary too. These estuarine flocks can function as important loci for protozooplankton activity, where amoebae can feed on attached bacteria (ROGERSON & LAYBOURN-PARRY 1992). For this reason, amoebae can sometimes reach very high abundances in the water column (ROGERSON & GWALTNEY 2000, ROGERSON *et al.* 2003). Most probably, *A. algophilum* has a real planktonic life style in the Schelde estuary. Its particular morphology probably explains how it can carry out its size-selective grazing behaviour in the turbulent water column of the Schelde estuary. The amoeboid stage of *A. algophylum* possesses long pseudopodia (up to 140 µm long), which it can use to capture its prey (CANTER 1973). These long extensions increase the volume where it can make contact with its prey. This increases also the chance to encounter the rarer, larger cells. Since *S. hantzschii* also possess some very long spines, the chance to get an encounter with *A. algophilum* is probably not as low as could be expected from the densities of this diatom. This possibly explains why this amoeba is so efficient in capturing and digesting this less abundant diatom species.

In this study, at most 25% of the population of centric diatoms were found inside feeding cysts of this amoeba. This is in agreement with BAILEY-WATTS & LUND (1973), who found a maximal proportion of ca. 27% inside feeding cyst in Loch Leven, resulting in a significant decline in the abundance of centric diatoms. The phytoplankton community in the freshwater part of the Schelde estuary is believed to be predominantly grazed by a community of filter feeding rotifers, which sometimes can exhibit relatively high grazing rates, between 33 and 84 % (LIONARD *et al.* 2005). In comparison, the observation that only one protozoan species is sometimes able to graze away a quarter of the phytoplankton biomass in this part of the estuary underlines the importance these small, often unnoticed organisms can have in aquatic food webs.

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