Studies on the dynamics of heterotrophic flagellates on biofilm communities

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Introduction

In natural stream systems, the organisms and processes are directly influenced by flow velocities. These are the major physical factors structuring the ecosystem (Schönborn 1992). The River Continuum Concept describes stream systems as a continuos series of physical gradients and resulting biota underlying the constant unidirectional flow (Vannote et al. 1980). Consequently, the biotic diversity may be low in systems without fluctuations in abiotic conditions (Connell 1978). The spatio-temporal dynamics of organic carbon and nutrients are described in the spiraling concept (Webster & Patten 1979, Tank & Webster 1998).

Throughout the world, many riverine systems are interrupted by weirs (Giller 2005, Nilsson et al. 2005). Common reasons to build weirs are the use of water force for energy supply or the regulation of the discharge of riverine systems. Weirs alter or even disturb the continuous flow conditions in rivers. As a result of altering the flow characteristics, a new concept - the serial discontinuity concept - had to be developed with respect to the anthropogenic impacts (Ward & Stanford 1983). It has been shown that the biggest problem caused by weirs is the barrier effect for biota and nutrients influencing the dynamics and composition of communities (Watters 1996).

The productivity, diversity and composition of the benthic community are extremely important for the degradation processes of stream ecosystems, especially in headwater regions and small streams (Ward 1976). Most studies about benthic communities consider larger organisms like invertebrates. Those studies describe the community composition and function of invertebrates in riverine biofilms (e.g. Arle 2005). The effects of weirs on the productivity and diversity on invertebrates are more studied and better understood than the effects of weirs on microorganisms on riverine biofilms (e.g. Stanley et al. 2002, Santucci et al. 2005).

Besides the benthos, also the habitat “biofilm” is very important for the self-purification and transfer of energy through trophic levels in riverine systems (Sorokin 1990, Sabater et al. 2002). The term biofilm is used to define the discrete aggregation of organisms (bacteria, algae, fungi, protozoa and micrometazoa) and their metabolic products at an interface (Hamilton 1987).

After the notification of the significance of the microbial loop in the pelagial (Azam et al. 1983) the interest on interactions in riverine biofilms increased due to the finding that up to 99% of microbial activity in rivers takes place on riverine biofilms (Arndt et al. 2003, Parry 2004). But still very little is known about the development, structure, function and dynamic of riverine biofilm communities including all groups of organisms.

Until now, studies focused manly on benthic ciliate communities (e.g. Gong et al. 2005, Norf et al. 2007), although it is recognised that heterotrophic flagellates are as important as ciliates in the microbial loop. The grazing by protozoans make dissolved organic carbon finally available for higher organisms by grazing on protozoa.

One reason for the fact that the composition of heterotrophic flagellate communities is not well studied is that there are problems in the taxonomic resolution. The abundances of heterotrophic flagellates are mainly counted by the use of the DAPI-technique (Porter & Feig 1980). The live-counting technique is crucial in determining the taxonomic structure of the heterotrophic flagellate community because the movement is one of the main determination features in heterotrophic flagellates (Arndt et al. 2000). Only a few studies considered the colonisation and structure of heterotrophic flagellate communities on biofilms (e.g. Esser 2006). There are several important gaps in our understanding of the functioning of biofilm communities, which should be addressed in the course of the present study: The temporal changes in the abundance and community structure of heterotrophic flagellate communities, the influence of important abiotic factors such as flow velocity and the population dynamics of benthic heterotrophic flagellates.

There are two ways of studying dynamics of heterotrophic flagellates on biofilms: 1) field experiments and 2) laboratory experiments. Field experiments have the advantage that they are run under natural conditions. The disadvantage is that generally no single influencing factor can be investigated. Laboratory experiments allow the reduction of influencing factors.

The present study on the dynamics of heterotrophic flagellates on biofilm communities included field and laboratory experiments. The field experiments were done as a part of a Research Training Group, GRK 266/3, (Chapter 1 – 4)

to investigate riverine biofilms in a degraded stream ecosystem. The results for the heterotrophic flagellates are presented in this thesis considering the influence of different flow velocities on the temporal colonisation, abundance and diversity of heterotrophic flagellates on riverine biofilms. The laboratory experiments were carried out to study the impact of flow velocities on different benthic flagellate species and to analyse the parameters of population dynamics of a benthic flagellate.

Chapter 1 is intended to review the present knowledge regarding the impact of weirs on physical, chemical and biological conditions in streams and rivers. The seasonal dynamics of heterotrophic flagellate communities affected by small low-head weirs are presented in Chapter 2. In Chapter 3 the effect of weirs on the short-term (between 1 and 14 days) colonisation of heterotrophic flagellates on riverine biofilms in spring and summer is described. Chapter 4 deals with the short-term colonisation of heterotrophic flagellates under normal and high water level conditions on riverine biofilms. Results from chapter 3 were included and extended by field experiments in autumn and under high water level conditions. The investigations were done to determine the colonisation, abundance and diversity of benthic flagellate communities on riverine biofilms at different flow velocities. The single chapters are manuscripts which are published (Chapter 1 and 3) or will be submitted (Chapter 2 and 4). The studies of the riverine biofilms were done in the same stream with the same sampling method, so that parts of the text are redundant, especially in the material and method sections.

Chapter 5 and 6 are dealing with dynamics of heterotrophic flagellates under laboratory conditions. The water-current in the micro-layer above the substrate and impacts on the colonisation of selected heterotrophic flagellates were investigated in Chapter 5. There are no investigations available on population dynamics of biofilm-dwelling heterotrophic flagellates. For the first time it was possible to determine all population dynamic parameters (growth rate, division rate and also mortality) for a selected euglenids species. The selected organism (*Entosiphon sulcatum*) is not bursting at the moment of dying like other protozoans. To understand the changes of population dynamic

parameters of heterotrophic flagellates, laboratory experiments were conducted under different temperature conditions.

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CHAPTER I:

QUERYING THE OBVIOUS: LESSONS FROM A DEGRADED STREAM

ABSTRACT: A detailed assessment of degradation issues is essential for the development of reasonable restoration strategies. The assessment may be a difficult task when fluxes of organic matter and energy are concerned which are primarily mediated by microorganisms. In small streams biofilms are hot spots of trophic interactions. Small weirs cause small scale changes of flow velocity, which affects the formation, structure, and function of biofilms. Weirs are superficially considered as disturbing cross-barriers that should immediately be removed for the restoration of riparian systems. However, our empirical studies of weirs in the stream Ilm/Germany and conceptual modeling approaches revealed a rather beneficial effect, because weirs compensate the loss of natural retention structures in straightened rivers. Longer processing time of particular organic matter (POM) in the weir reservoirs may have a positive effect on biofilm productivity and nutrient cycling in aquatic ecosystems. This is a striking example of thorough investigations that resulted in a complete and surprising reassessment of a degradation situation, and for a case in which uninformed gut-feeling decisions about management plans would have had detrimental effects.

Key Words: stream ecosystems, disturbance, microorganisms, microbial activity, weirs, conceptual model

INTRODUCTION

The first step before any restoration management plan could be developed is indeed to determine the state of degradation. Apparently this statement is trivial, because degraded systems are supposed to be easy to identify. But in fact, revealing the very reasons for degradation is a rather demanding task. Degradation may be due to hidden pathways that are not immediately obvious, but that need to be carefully scrutinized for reasonable decisions about promising restoration strategies. Even more so, reliance on superficial characteristics of a degraded system could be dangerously misleading and may result in not only unsuccessful, but even detrimental management actions.

In particular this caution applies to the fluxes of energy and matter through ecosystems that are mainly mediated by microorganisms. The scientific knowledge about the microbiology in aquatic ecosystems is still rather incomplete, the methods for quantitative investigations are demanding, and even the systematic classification of organisms is challenging. The essential turnover processes in streams take place in the sediment or at the water-sediment interface, i.e. in a changing environment that is anything but easily assessable. Here we present our experiences from studies in the Ilm, a 3rd order hard water stream in Thuringia, Germany, which resulted in a surprising reassessment of an ”obvious” degradation situation.

Degradation of running water ecosystems

All major rivers worldwide are largely influenced by human impacts (Giller 2005), which change the lotic character, affect the ecosystem structure and function, alter the habitat heterogeneity, and fragment riparian zones (Jansson et al. 2000; Giller 2005). More than 50% of the rivers in the northern hemisphere are affected by dams (Nilsson et al. 2005), which modify flow regimes, interrupt sediment transport, deteriorate water quality, and break biological continuity (Ward & Stanford 1979; Petts 1984). Large dams alter the river continuum (Vannote et al. 1980) by disturbing the spiraling of resources (nutrients and organic matter) and disconnecting upstream and downstream reaches.

The vast majority of dam structures in Central European stream systems, however, are weirs with a hydraulic head not greater than 5 m (Poff & Hart 2002), which in contrast to large dams have only small or moderate effects (Hart et al. 2002). Weirs do not substantially alter the natural discharge regime, but rather affect the local flow velocity patterns, sediment composition (Magilligan & Nislow 2001; Stanley et al. 2002), and particulate organic matter budgets (Wagner 2003). Barrier effects of weirs on movement and population dynamics of migratory fish species are evident (Mills 1989; Lewis 1991; Morita & Yamamoto 2002), but the consequences for microbial and macroinvertebrate communities are less well documented (Pringle 2003; Arle 2005).

Rivers cause a permanent discontinuous transition between transport and storage of organic matter, largely in the form of dissolved organic matter (Wetzel 1992). According to the microbial loop concept (Azam et al. 1983) the transfer of energy and matter to higher trophic levels in aquatic ecosystems is largely mediated by microorganisms which convert dissolved organic matter (DOM) into particulate organic matter (POM; Kerner et al. 2003). In small streams the retention of DOM mainly occurs in biofilms (Schwoerbel 1994; Fischer et al. 2002). Biofilms are complex assemblages of bacteria, fungi, algae, micro- and meiofauna within a polysaccharide matrix (Lock 1981; Lock et al. 1984) and are formed at any submerged surfaces such as stones, plants, and roots (Zubkov & Sleigh 1999). Biofilms are hot spots for the turnover of organic matter in small streams, since the majority of bacteria lives attached to the streambed (Geesey et al. 1978). Biofilm bacteria display higher sugar assimilation rates (Fletcher 1986) and higher enzyme activities (Romaní & Sabater 1999*b*) compared to planktonic bacteria. Biofilms provide an important food resource for higher grazing organisms like aquatic snails (Sheldon & Walker 1997; Lawrence 2002).

Effects of weirs on aquatic communities

Weir reservoirs create distinct physical conditions which differ considerably from free-flowing natural reaches (Baekken et al. 1981; Stanley et al. 2002), but chemical and thermal differences often occur only locally (Santucci et al. 2005). Our studies of weirs in the Ilm confirmed that although flow velocity was reduced in the reservoirs (0.10 ± 0.02 m/second) compared to

the outlet and natural sites (0.35 ± 0.10 m/second), pH, oxygen content, turbidity, conductivity, and temperature in the water column were not affected. However, because the Ilm is heavily fragmented by more than 50 weirs on an entire length of only 137 km, cumulative effects may occur. So in a series of studies we tried to disentangle the complex interaction between altered flow velocity, the formation and function of biofilms, and the benthic invertebrate communities near weirs.

As a general pattern, the abundance of microorganisms and the accumulation of biomass in biofilms are negatively correlated with increasing flow velocity (up to 0.30 m/second; Lau & Liu 1993; Battin et al. 2003*a*; Battin et al. 2003*b*). At even higher flow velocity and turbulence, biofilm erosion or sloughing of the adhered biomass occurs (Characklis 1990; Costerton et al. 1995). On the other hand, uptake of DOM is primarily limited by diffusion through the laminar sublayer or by processes within the biofilm (Gantzer et al. 1989). Thus, the higher DOM content and nutrient availability in stream water during high discharge periods might cause higher extracellular enzyme activities of biofilms (Romaní & Sabater 1999*a*). Consequently, bacterial productivity may increase during high discharge periods. Bacterial turnover depends both on external and internal carbon supply, because algae colonizing biofilms release extracellular organic carbon that can be rapidly utilized by bacteria (Sundh & Bell 1992).

The next higher trophic level in biofilms are protists such as heterotrophic flagellates and ciliates, which feed on bacteria and algae (Azam et al. 1983). Increasing flow velocity results in higher contact rates between planktonic protists that pass the biofilm (Hunt & Parry 1998). Peritrich ciliates on surfaces benefit from the enhanced advection of prey at increasing flow velocity (Shimeta et al. 2001), and thus the clearance rate of some benthic bacterivorous ciliates may improve. In general, grazing pressure from ciliates and flagellates on bacteria in rivers is low (0.02 – 1.67%; Gücker & Fischer 2003). Higher organisms such as ostracodes predominantly consume algae and extrapolymeric substances, while unspecific feeders like snails and mayflies can efficiently reduce the biofilm thickness (Lawrence 2002).

In our study, the biofilm abundances of bacteria, heterotrophic flagellates, and ciliates in weir reservoirs were similar to those in the respective outlets, but slightly higher than at natural sites. Extracellular enzyme activities

were highest at outlet sites, but biofilm thickness and chlorophyll *a* content were enhanced at reservoirs, indicating a tight mutualistic interaction between bacterial production and the photosynthetic activity of algae and/or cyanobacteria. Thus, at low flow velocities the high potential release of extracellular organic carbon by algae and the limited diffusion of nutrients from the water column into the biofilm might restrict the efficiency of allochthonous DOM turnover in the reservoir. However, enhanced enzymatic and microbial activity in the water might compensate for organic matter processing.

Only few invertebrates feeding on biofilms were present in the reservoirs, and so carbon and energy flow from biofilms to invertebrate communities might be small. Biomass of invertebrates was similar in the reservoirs and natural sites, but slightly higher in the outlet. In general the aquatic community appeared to be not significantly affected by the weirs. However, invertebrate species diversity was reduced ahead of the weirs, and detritivorous collector–gatherers dominated (88% of all invertebrates; Arle 2005), as is confirmed by other studies (Stanley et al. 2002; Santucci et al. 2005). Within the reservoirs, the benthos normally undergoes a succession towards lentic life forms, but these changes are locally restricted and appear to have no effect on downstream reaches.

Cross-barriers can reduce the longitudinal connectivity by preventing or impeding the migration of organisms throughout the stream system (Pringle 2003) that lead to fragmentation of the habitat and isolated populations (Pechlaner 1986; Winston et al. 1991; Drinkwater & Frank 1994; Marchant & Hehir 2002). Depending on size and operational type, small low head weirs might also act as barriers to some invertebrate species (Watters 1996; Cortes et al. 1998; Benstead et al. 1999; Conception & Nelson 1999; Stanley et al. 2002). We observed a slightly modified invertebrate downstream drift within the weir reservoirs of the Ilm, but the barrier effect was unimportant for the maintenance of diverse invertebrate communities upstream and downstream of the weir (Arle 2005). The strong spatial restriction of impacts from each single weir probably explains the absence of any notable cumulative effect of multiple weirs on invertebrate communities, even in the heavily fragmented Ilm.

Another effect of weirs is the retention of particulate organic matter (POM). Many headwater streams like the Ilm are energetically dependent on allochthonous organic material (Fisher & Likens 1973; Cummins 1974). Large

amounts of POM are stored in the reservoir and detritivorous collector–gatherers dominate during low discharges. Only major floods can reset the system (Fjellheim et al. 1993). Trapping of POM in reservoirs of large dams leads to a local increase of respiratory activity of heterotrophic organisms (Ward & Stanford 1983). A similar increase might occur in the reservoir of weirs. The reservoirs in the Ilm in fact contained higher POM standing stocks than outlet and natural sites (Arle 2005). Therefore, we hypothesized that in straightened, homogeneously structured streams, i.e. with reduced size of riparian corridors and in absence of natural retention zones, multiple weirs may compensate the loss of natural retention structures, because POM as the energy base for stream biota will be longer retained in the system.

Modeling disturbed streams

The interactions between flow velocity and nutrient cycling among the different trophic levels of aquatic systems are hard to unravel, and so modeling is an appropriate tool to reveal the causal relationships and the expected outcome from different scenarios. DOM spiraling modeling suggests that uptake lengths for DOM increase with decreasing flow velocity (Kaplan & Newbold 2003). Rapid uptake of labile DOM from the stream water will result in a greater concentration difference between water column and biofilm and, thus, will be more strongly influenced by turbulent mixing than the uptake of less labile DOM (Kaplan & Newbold 2003). Classic approaches like the river continuum concept (Vannote et al. 1980) and the nutrient spiraling concept (Elwood et al. 1983) consider riverine ecosystems as continuous ecosystems. However, due to structural alteration many stream systems worldwide are far away from their natural character.

To describe DOM turnover efficiency in regulated streams we developed a conceptual model that regards the effect of physical (e.g. temperature, flow velocity) and biotic (e.g. growth rates, trophic interactions) parameters, as well as their synergistic interactions (e.g. growth rates depend on temperature). In the presence of weirs, flow velocity and POM storage are altered. Under normal discharge, the decrease of flow velocity will lead to a decrease of DOM uptake (Kaplan & Newbold 2003) and, thus, the DOM turnover efficiency of the stream system also decreases. The retention of POM

in the reservoir, however, may stimulate bacterial transformation, providing an additional DOM source for the biofilm. Under high discharge conditions, sediment particles will be mobilized in unregulated stream reaches, leading to a detachment of benthic biofilms and a temporary reduction of DOM turnover. Since weirs mediate the discharge regimes and lower upstream flow velocities, biofilm detachment might be prevented in the reservoirs, creating spots with continuing DOM turnover. Major floods, however, will lead to a depletion of POM and to a reset of the aquatic community also in the reservoirs.

From implementing different flow regimes into our model we found support for the hypothesis that decreased DOM turnover efficiency in weir reservoirs might be counteracted by increased residence time of organic matter. In addition, the high abundance of detritivorous collector–gatherers provides a direct trophic link from organic material to invertebrates. The increased residence time and the channeling of organic matter directly to the invertebrate community are important for straightened streams with severely reduced natural retention capability.

Consequences for restoration ecology

The complexity of running water ecosystems requires sufficient analyses of the functional and structural parameters before restoration management plans are set up (SER 2002). To evaluate the impact of human alterations on the ecological “health” of rivers and streams, biomonitoring approaches are used (Bunn & Davies 2000). Large amounts of money have been spent on restoration projects (Roni et al. 2002), but their success often remains unclear. Therefore, a standard program for restoration approaches is required (Giller 2005), in which the optimal conditions for the targeted ecosystem have to be evaluated and necessary measurements have to be defined (Palmer et al. 2005). Additionally, conceptual hypotheses and models should be employed to set up the aims of the proposed activities (Jansson et al. 2005).

Weirs were widely used in river regulation to control floods and to provide power e.g. for generating electricity. In river restoration, dam removal is commonly seen as an obvious and relatively inexpensive option when the ecological health of the system is of prime consideration (Santucci et al. 2005).

However, artificial weirs provide an efficient tool to reestablish the riffle-pool character in regulated rivers (Gordon et al. 1996). Straightening increases flow velocities, resulting in shorter turnover lengths, but also in a reduced stream length. Therefore, the increased turnover capacity in straightened zones has to be balanced with the loss of turnover area and the faster downstream transport of nutrients. Multiple weirs might increase the POM-retention capacity and improve nutrient cycling, which are indicated from studies of their natural analogs, i.e. debris dams and beaver dams. Reservoirs of small weirs can be key zones of transient storage for organic matter processing, and as artificial pool sites they might be valuable “refuge zones” especially under flooding conditions.

We are still far away from a complete understanding of the effects of weirs on aquatic communities and organic matter turnover in streams, which is a prerequisite for efficient management of these structures. The recent development of molecular techniques and other sophisticated methods have enabled aquatic ecologists to get a first glimpse into the microbial “black box”. However, to define the optimal conditions for the targeted ecosystem, much more research is needed to thoroughly understand the structure-function relationships of microbial biofilms, the hot spot of organic matter turnover in small streams. Our case studies in the stream Ilm indicated that the “obvious degradation issue” of fragmentation by weirs should rather be considered beneficial, because it compensates the loss of natural retention structures due to straightening. If a complete removal of weirs is nonetheless considered, it has to be necessarily combined with (1) the restoration of the entire riparian zone, and (2) the recreation of a heterogeneous channel structure with a variety of natural retention structures. Uninformed gut-feeling decisions for weir-removal without considering the retention function of the structures would have had detrimental effects.

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CHAPTER II:

SEASONAL DYNAMICS OF A HETEROTROPHIC FLAGELLATE COMMUNITY ON BIOFILMS OF THE RIVER ILM: EFFECTS OF SMALL WEIRS

INTROCUTION

Stream regulation by damming is known to alter physical, chemical and biological conditions in streams (Ward & Stanford 1979, 1983). There is a good documentation of the profound effects of large storage weirs. Such weirs are essential for power supply, flood control, and water storage. Regulation by these weirs has substantial economic benefits, but there are costs of fundamental alterations of stream systems. Small weirs with a hydraulic head < 5 meter (low-head) and impounded areas of < 20 hectare are much more numerous than large storage weirs in Central European streams and rivers. Most weirs which are removed or reconstructed (fish passes & -ladders) by the regional stream managers in Europe at present are small low-head weirs. Small low-head weirs alter the discharge regime by weakening flood peaks and altering local flow velocity patterns and sediment composition (Magilligan & Nislow 2001, Stanley et al. 2002). The ecological consequences of small low-head weirs are poorly understood (Benstead et al. 1999, Hart et al. 2002, Poff & Hart 2002) and comparably less scientific interest regards this kind of human impact to stream ecosystems.

The effect of fragmentation on movement and dynamics of fish has been extensively studied (e.g. Mills 1989, Lewis 1991, Morita & Yamamoto 2002), but effects on macroinvertebrates and microbial communities are less documented (Pringle 2003, Arle 2005). Microbial communities forming biofilms are hot spots for the turnover of organic matter in small streams, since the majority of bacteria lives attached to the streambed (Geesey et al. 1978). Therefore, the interest in biofilms has increased over the last thirty years. Interactions between biofilm bacteria and protozoans, especially heterotrophic flagellates, are very important since it is known that these are main consumers of bacteria in planktonic situations (Azam et al. 1983). One important factor for the colonisation of microorganisms on biofilms in stream ecosystems is the flow velocity (Hunt & Parry 1998, Battin et al. 2003b).

The knowledge on the colonisation of microorganisms, especially of heterotrophic flagellates, is still limited. In the present study, alteration of the flow velocity caused by a small low-head weir and the effect on the abundance and diversity of the heterotrophic flagellate community was investigated.

MATERIALS AND METHODS

Study area. The study was conducted in a 3rd order river (River Ilm, Thuringia, Germany, Strahler 1957). The stream has a catchment area of approximately 1035 km² (Krey 1995). Over the whole length of the river (130 km), 57 small low-head weirs are continuously distributed at an average distance of 2.3 km. All these weirs are low-head, run-off-weirs with a hydraulic head < 4 meters (min. 0.7 – max. 3.1 meters). The reservoirs are characterised by small impounded areas of < 3 hectare due to a steep slope of 3.16%. The reference site of this study was located in the unaffected, epirhithral region (km 127) and the sampling sites of the three weirs (reservoir with corresponding outlet) were in the metarhithral region (km 103) of the River Ilm (Fig. 1).

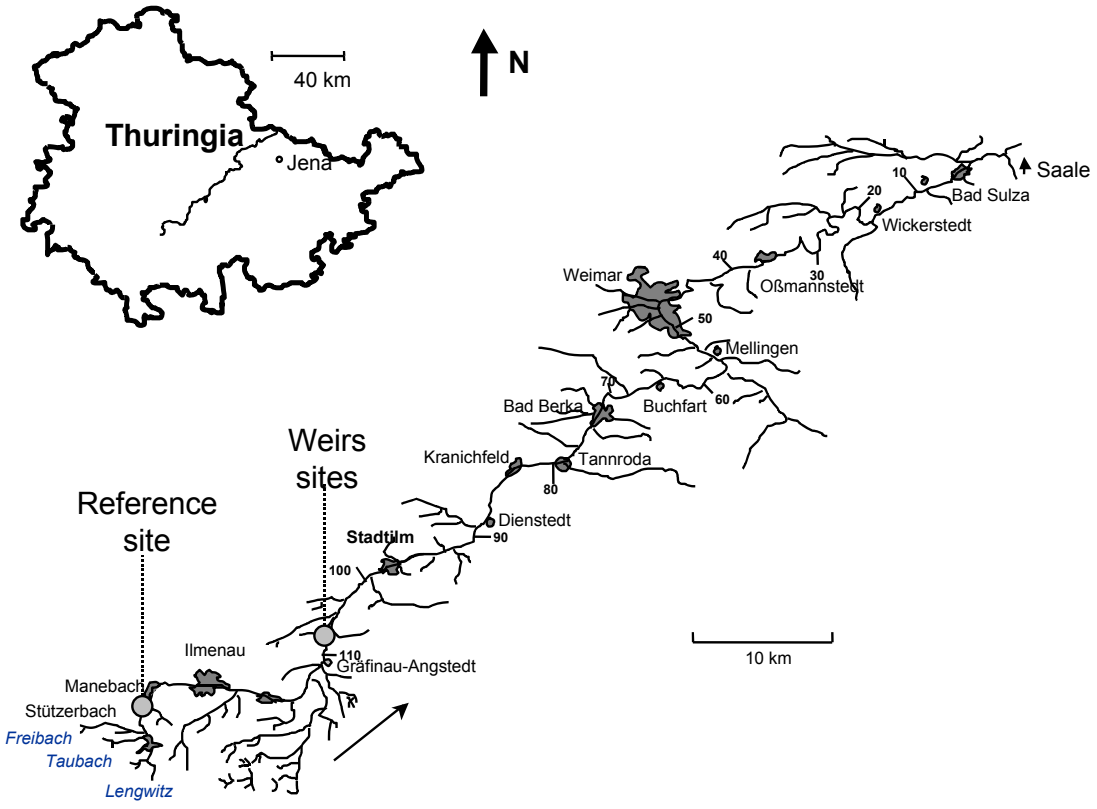

Fig. 1. Map of the 3rd order stream Ilm and its location in Thuringia (Germany) with the positions of the sampling sites.

Sampling design. Between April 2003 until April 2004, the abundance and structure of the heterotrophic flagellate community was examined every two months (excepted February 2004). Artificial substrate was used for the colonisation experiments. The colonisation development of microorganisms on glass slides is similar to that on natural substratum e.g. stones (Naumann 1919, Bamforth 1982). Glass slides (76 x 26 mm) were exposed horizontally and close to the stream bed in sample collection vessels (stainless steel cylinders, pore diameter 8 mm, modified after Alfreider et al. (1997, Fig. 2) for 14 days.

Fig. 2. Pictures of biofilm grown on a glass slide (above) and the sample collection vessel modified after Alfreider et al. (1997).

Cylinders were used to avoid destruction of the glass slides by floating stones. Sample collection vessels were used to avoid destruction of the glass slides by floating stones. Glass slides were placed in Greiner-Tubes (50 ml), which were filled with filtered, autoclaved river water (filter size 0.2 µm) and stored cool (6°C) until further processing. Within 6 - 7h the abundance and diversity of heterotrophic flagellates was estimated by the direct live counting technique (Arndt et al. 2000) under a phase contrast microscope (Zeiss Axiovert 25, 400 x magnification) and with the help of video recording (excepted April 2003). For each sample (three replicates were taken at each sampling site), at least 60 individuals or 30 visual fields were scanned by placing the slide directly under the microscope. Simultaneously all other organisms, e.g. ciliates, diatoms, were recorded, too.

Environmental parameters. Abiotic factors were measured at every sampling date. The flow velocity was measured with a Flow-Mate (Model 2000,

Mash-McBirney Inc., USA) at the exposition depth of the glass slides. For temperature, oxygen, conductivity, turbidity, and pH a Water Quality Checker (U-10, Horiba, Kyoto, Japan) and probes from WTW (Weilheim, Germany) were used. The discharge was obtained from the nearest permanent hydrographic station (Gräfinau-Angstedt, 117 km) and data were provided by the Staatliches Umweltamt Erfurt.

Statistical analyses. The influence of flow velocity on the colonisation of the heterotrophic flagellates was estimated using a One-way-ANOVA followed by a post-hoc Bonferroni test (SPSS 11.0, Chicago, USA). The alpha level of significance for all statistical tests was 0.05. To test the influence of other environmental parameters a principle component analysis (PCA, Canoco 4.5) was used. Similarity among the species pools for each sampling site was compared using Sørensen's similarity index (Pielou 1984):

$$S = ((2*a)/(b+c))*100,$$

where a = the number of species present in both species pools being compared, b = the number of species present in the first species pool but not in the second, and c = the number of species present in the second but not in the first.

RESULTS

The discharge ranged between 0.5 $m^3 s^{-1}$ and 11 $m^3 s^{-1}$. At this station, the averaged annual discharge (long-term mean from 1923 to 2003) approximates 2.44 $m^3 s^{-1}$ (Staatliches Umweltamt Erfurt, unpubl. data). During the study period discharge peaks occurred during winter and spring and lowest values occurred during the summer months. Highest flow velocity values were measured at the reference and the outflow sites (ranged from 0.15 to 0.5 m s^{-1}). The reservoir sites were characterised by a low flow velocity of < 0.1 m s^{-1} with the lowest flow velocity measured in August (0.023 m s^{-1}, Fig. 3).

Environmental factors were not significantly different between the three reservoir sites as well as outflow sites of the weirs, so that in the following the mean value of each site was used. The different sampling sites showed no significant difference in the oxygen content, temperature, and pH (Fig. 3). A high turbidity was found at both weir sites. At the reference the turbidity was

mainly zero. The conductivity was between two and five times higher at the weir sites in contrast to the reference.

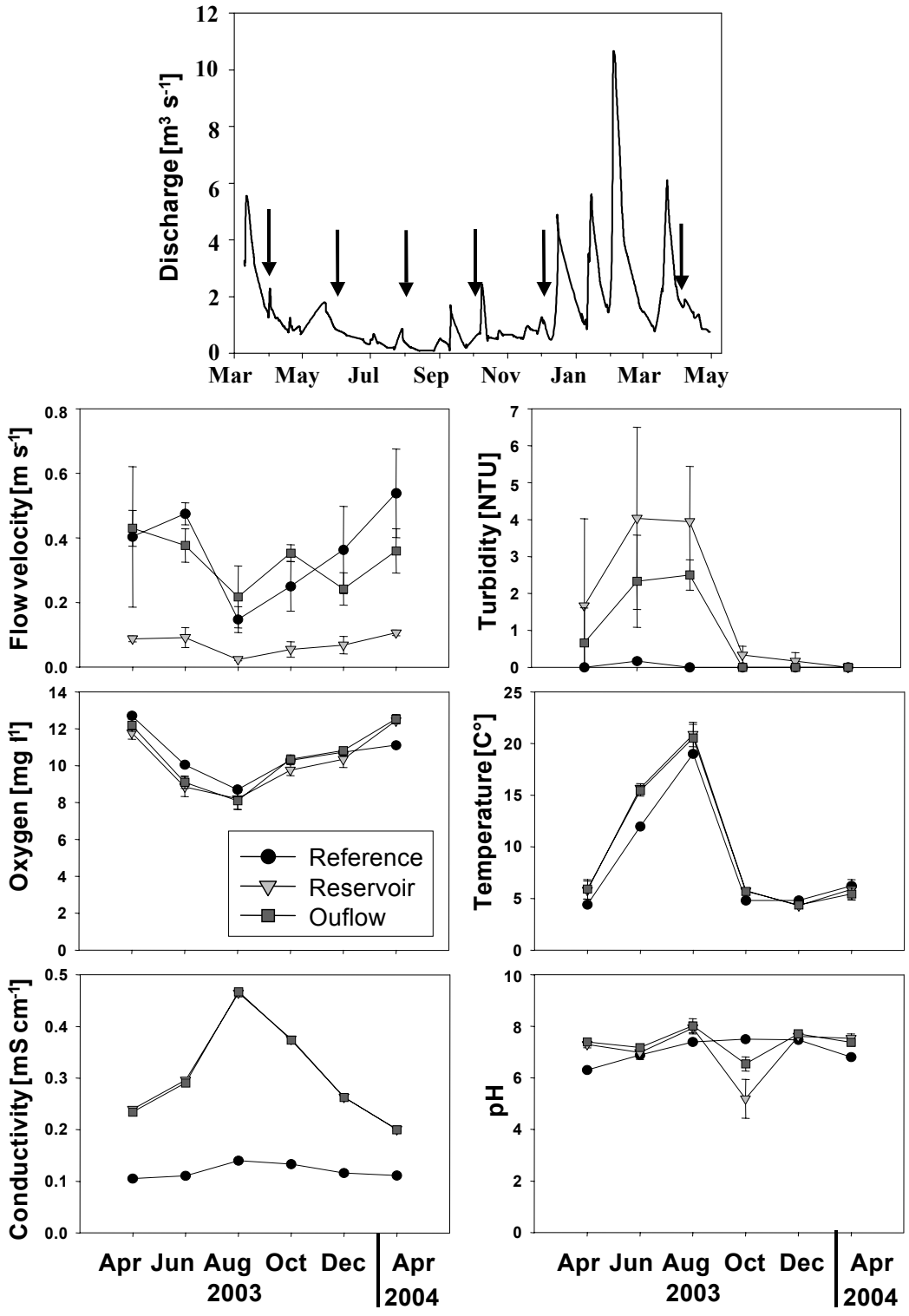

Fig. 3. Abiotic factors (discharge (measured at the water level gauge Gräfinau-Angstedt (117 km), data provided by the Staatliches Umweltamt Erfurt), flow velocity, turbidity, oxygen, temperature, conductivity, and pH) of all sampling sites (reference, reservoir, and outflow) over time. Reservoir and outflow are given as mean values of three examined weirs. Error bars indicate standard deviation (n = 3) and arrows indicate the dates of sampling.

The abundance of the heterotrophic flagellates was highest at the reference site at every sampling date (Fig. 4, for reservoir and outflow the mean values of three weirs were used). This trend was significant in April, June and August ($p < 0.05$). Lowest abundance was found in August at all sampling sites and highest in spring and early summer. The high standard deviations in the numbers of flagellates originated from the heterogeneity of the biofilms. Nevertheless, the abundance of heterotrophic flagellates was similar or even higher at outflow sites (50 and 900 Ind. cm^{-2}) in contrast to the reservoirs examined (Fig. 4). The flow velocity had a significant positive effect on the abundance of heterotrophic flagellates ($p < 0.001$, $R^2 = 0.21$, Fig. 5). PCA did not show any correlation to other abiotic factors.

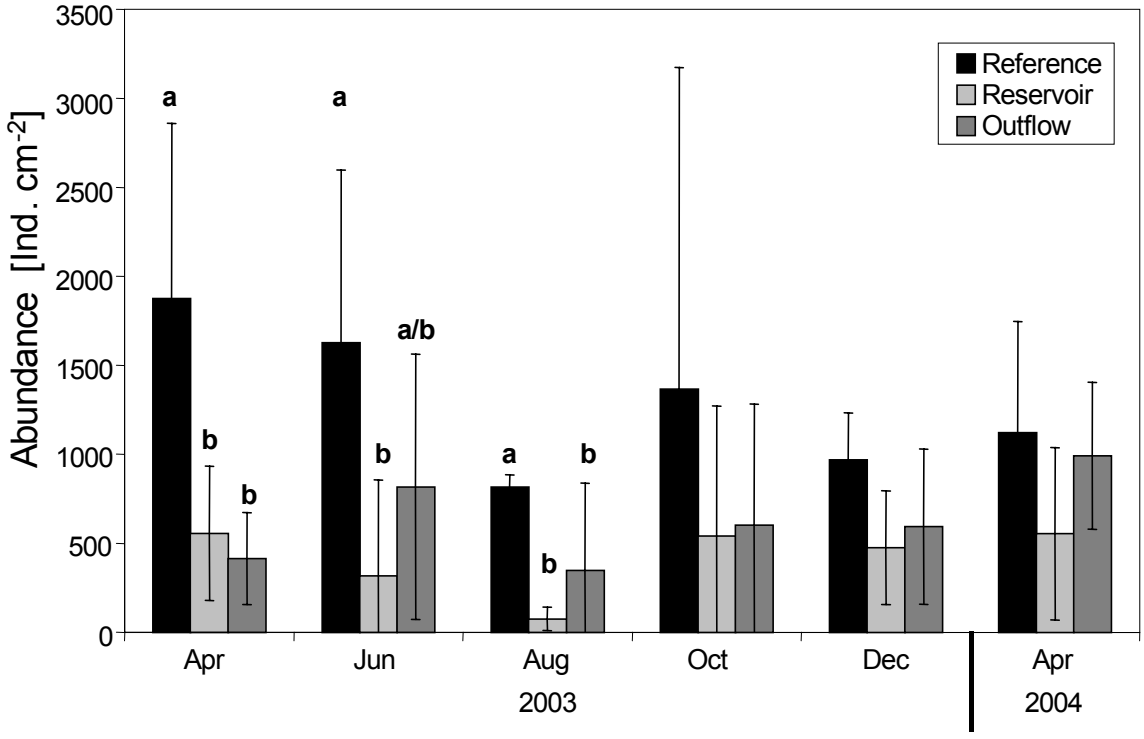

Fig. 4. Abundance of heterotrophic flagellates [Ind. cm^{-2}] on biofilms at all sampling sites (reference, reservoir, and outflow) in the River Ilm. Reservoir and outflow are given as mean values of three examined weirs. Error bars indicate standard deviation (n = 3). Different letters indicate significant differences ($p < 0.05$).

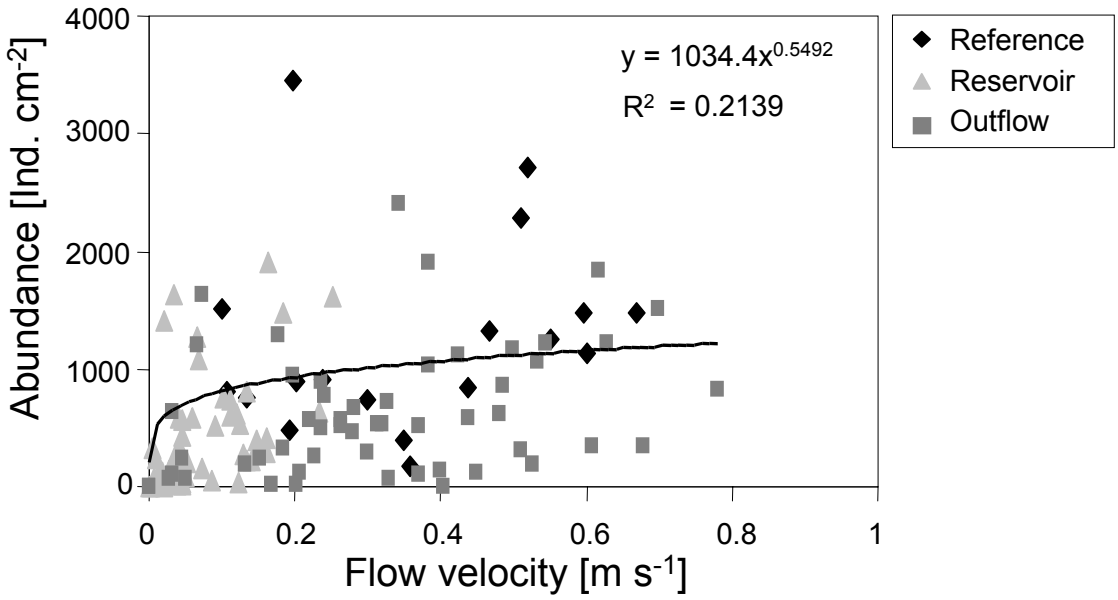

Fig. 5. The relationship between abundance of heterotrophic flagellates and corresponding flow velocity for all sampling sites and dates on biofilms in the River Ilm ($p < 0.001$, $R^2 = 0.21$).

The taxonomic structure of the heterotrophic flagellate community was not affected by small weirs (Fig. 6 and 8). In both Figures, the term “Other” refers to all individuals which could not be identified and three groups of heterotrophic flagellates (Apusomonadida, Ciliophryida and Cryptomonadida) which made up less than 10 % of the overall flagellate abundance at the respective sampling site. Kinetoplastida were the dominant group in summer while Cercomonadida were more important in October. In April 2004, euglenids dominated the community (about 50 %) at the outflow sites of the weirs. Ancyromonadida contributed between 10 and 25 % to the total abundance at all sampling sites and dates. The values of the Sørensen’s quotient of similarity were between 70 and 97 % and showed high similarity between communities of different sites (Fig. 7).

Abundance of bacteria, amoebae, and heliozoans were similar at all sampling sites (Table 1). Diatoms had a higher abundance at the weir sites. The lowest abundance of metazoans was found at the reservoir site of the weirs. The colonisation pattern of ciliates was like that of the heterotrophic flagellates.

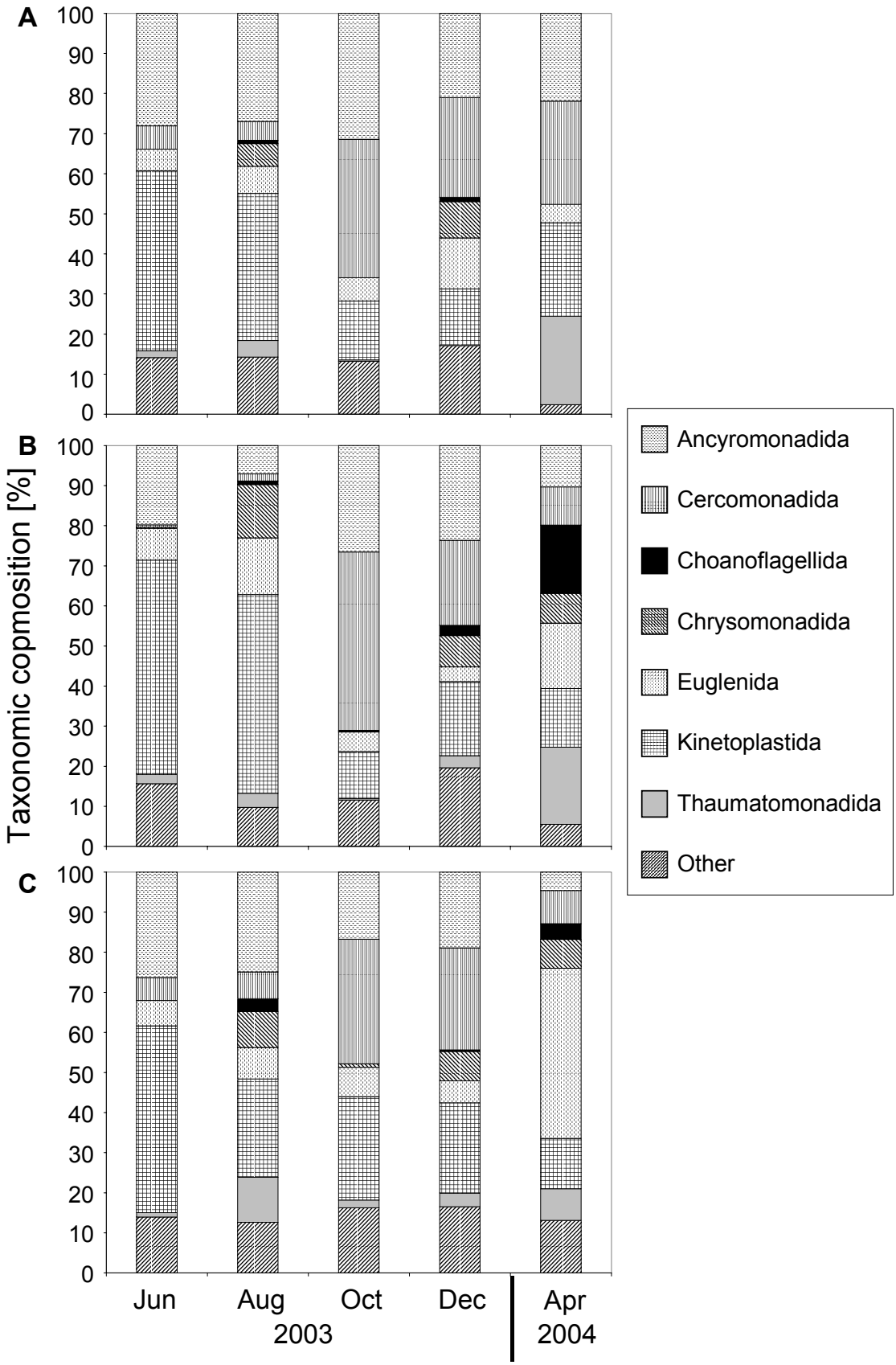

Fig. 6. Taxonomic composition (contribution of taxonomic groups to total abundance) of the heterotrophic flagellate community [%] on biofilms of the River Ilm (A = Reference site, B = Reservoir site, and C = Outflow site).

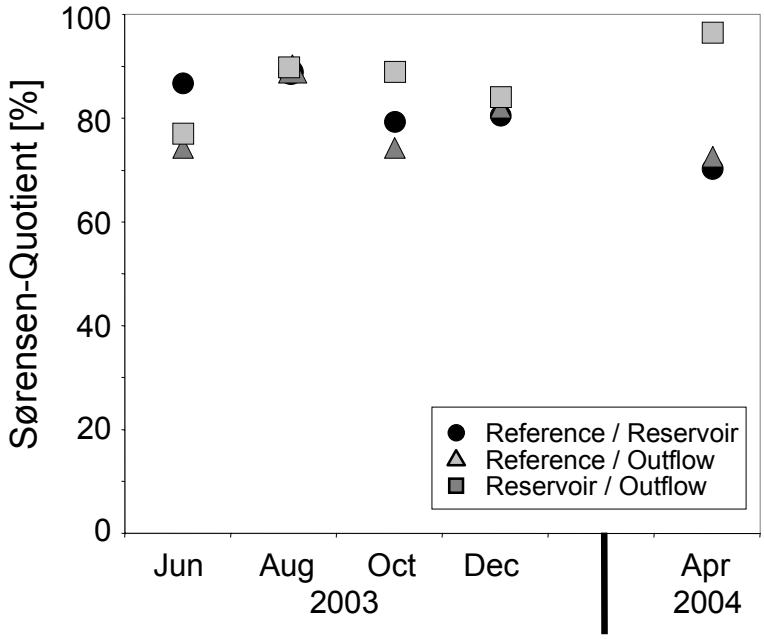

Fig. 7. Sørensen`s – Quotient of similarity [%] of the heterotrophic flagellate communities on biofilms in the River Ilm for the three sampling sites (reference, reservoir, and outflow).

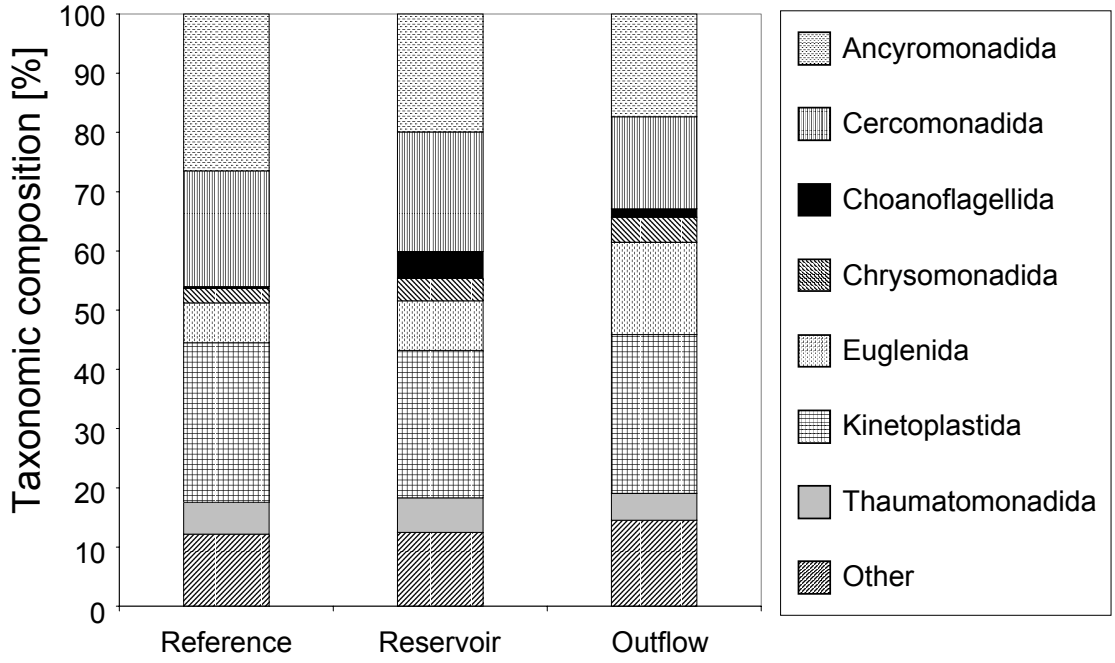

Fig. 8. Average taxonomic composition (contribution of taxonomic groups to total abundance) of the heterotrophic flagellate community [%] on biofilms in the River Ilm at the sampling sites (reference, reservoir, and outflow) for the whole sampling period.

Table 1: The average abundance of microorganisms [Ind. cm^{-2}] on biofilms at all sampling sites in the River Ilm.

Ind. cm^{-2}	Reference	Reservoir	Outflow
Bacteria (*10^6)	5.61 ± 3.59	11.05 ± 1.10	7.94 ± 2.05
Diatoms (*10^3)	1.81 ± 0.58	3.06 ± 2.63	4.09 ± 2.84
Flagellates (*10^3)	1.30 ± 0.40	0.44 ± 0.27	0.65 ± 0.26
Ciliates (*10^2)	0.94 ± 0.41	0.30 ± 0.15	0.61 ± 0.21
Amoebae (*10^2)	0.12 ± 0.14	0.11 ± 0.09	0.09 ± 0.05
Metazoans (*10^2)	0.12 ± 0.13	0.02 ± 0.02	0.08 ± 0.04
Heliozoans (*10^2)	0.03 ± 0.04	0.01 ± 0.01	0.01 ± 0.01

DISCUSSION

The flow velocity of the River Ilm was reduced in the reservoirs compared to outflow and reference sites. However, pH, oxygen content, turbidity, conductivity, and temperature in the water column were not affected by the examined low-head weirs. Also Baekken et al. (1981) and Stanley et al. (2002) reported no unusual changes of physical and chemical parameters at low-head weirs which are in accordance to the present investigation.

Results of the present investigation indicated that mainly the abundance and not the structure of the heterotrophic flagellate community was positively affected by the flow velocity (R^2 = 0.21). Hart (1992) found a positive correlation between *Cladophora* and the flow velocity, too. Schmitz (1985) showed that the optimal growth conditions for ciliates were at a flow velocity of 0.3 m s^{-1} in the River Rhine. The present investigation showed optimal growth conditions for heterotrophic flagellates around 0.4 m s^{-1}. So, it seems that ciliates and heterotrophic flagellates have a similar preference for relatively high flow velocities. Also, Hunt and Parry (1998) found a higher abundance of heterotrophic flagellates at a riffle site than at a pool site in a river. An increased flow resulted in an increased probability of attachment on recently colonised surfaces (Hunt & Parry 1998). Similar patterns were documented by Korte and Blinn (1983) in a study of epilithic diatom colonisation, where, after 14 days the number of diatoms were some 3 - 4 times higher on substratum in riffle

compared to pool sites. The structure of the diatom communities was similar at both sites. Furthermore, they found no difference in the physical and chemical parameters.

All other measured environmental parameters in this study did not have a significant effect on the abundance of heterotrophic flagellates. Lamb and Lowe (1987) stated that the flow velocity is likely the most important factor for the structure of the biofilm. High flow velocities are often associated with a monolayer biofilm and low flow velocities with more complex structures (Battin et al. 2003a). The abundance and structure of the heterotrophic flagellate community in the River Ilm showed no differences by comparing a 14 days old biofilm with a three month old biofilm (unpublished data).

The knowledge of the taxonomic structure and dynamic of heterotrophic flagellates on biofilms of rivers is scarce. In the present study 10 groups of heterotrophic flagellates were found. These discoveries agree with the results of investigations in the River Rhine (e.g. Arndt et al. 2003, Esser 2006).

Studies considering the effect of small weirs on biofilm communities and river conditions are still missing. This is the first investigation which considered the effect of small weirs on the abundance and diversity of heterotrophic flagellates on riverine biofilms. Small weirs are a common tool for structural restoration, especially where full restoration of the stream channel is not possible (Hey 1994) and they can have a positive effect on the biota (Mellquist 1985). But the size and the spacing of the weirs are critical factors (Hey 1994). The present study showed that the structure of the flagellate community was not altered by small low-head weirs, but significant differences were found in their abundance.

To verify these results detail investigations should be done on the colonisation pattern with the help of shorter incubation times of biofilms (for discussion see Chapter 4). Also, it might be that the micro-topography on a surface influences the hydrodynamic conditions above biofilms. Recent studies showed that in the sediment surface topography has an effect on the material and gas exchange (Huettel et al. 2003, Røy et al. 2005). For detail discussion on the effect of flow velocities and surface topography on the behaviour of different morphotypes of heterotrophic flagellates see Chapter 5.

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CHAPTER III:

EFFECT OF WEIRS ON THE FLAGELLTE COMMUNITY IN BIOFILMS

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Effect of weirs on the flagellate community in biofilms

Marlene Willkomm

Introduction

Many rivers are interrupted in their continual flow of material and water (River Continuum Concept, VANNOTE et al. 1980) by weirs and hydroelectric power plants. Along the River Ilm (Thuringia, Germany), with a length of 130 river kilometres, 57 weirs were built. These transverse structures influence not only the continuous transport of suspended and dissolved materials but also several other environmental factors, such as velocity and sedimentation rate.

The highest microbial activity takes place in the biofilm (surface-associated organisms) in small streams or in the rhithral region of streams (SCHWOERBEL 1994). Knowledge of the role of substrate-associated protozoans is modest. Heterotrophic flagellates are the major consumers of bacteria and transform dissolved organic matter into particulate organic matter, which in turn is available for consumers in the classic food chain (AZAM et al. 1983). The present study investigated the influence of weirs on the colonisation of biofilms, especially on the associated heterotrophic flagellate communities.

Key words: protozoan, heterotrophic flagellates, biofilm, river, weir

Material and methods

The River Ilm is a third-order stream that rises in the northeastern Thuringian wood and flows into the River Saale, which belongs to the catchment area of the River Elbe. It has a long rhithral and a short epipotmal region (SCHÖNBORN 1996). The reference site of this study was located in the unaffected, epirhithral region, and the sampling sites at the weir were in the metarhithral region of the river. To investigate the heterotrophic flagellate community in biofilms, glass slides (7.6 x 2.6 cm) were exposed in a horizontal position close to the riverbed. In spring and summer samples were taken after 1, 3, 5, 7 and 14 days. Abundance of heterotrophic flagellates was estimated with the direct live counting technique (ARNDT et al. 2000) under a phase contrast microscope (Zeiss Axiovert 25, 400x magnification) with the help of video recording. Abiotic factors (flow velocity, temperature, oxygen, conductivity, turbidity and pH) were measured at every sampling.

Results

At the reference site the colonisation by heterotrophic flagellates showed an exponential growth in spring (Fig. 1). A similar pattern was found in summer, but the highest abundance (1620 Ind. cm^{-2}) was reached after only 7 days. The abundance was similar the first day in spring and summer (24 Ind. cm^{-2}), but the increase by day 14 was higher in spring (spring: 3100 Ind. cm^{-2}; summer: 1620 Ind. cm^{-2}). In spring a small increase in the abundance until day 7 was observed in the reservoir (1063 Ind. cm^{-2}) as well as in the outflow (1041 Ind. cm^{-2}). A similar pattern was found in the outflow, but not in the reservoir during the summer sampling (Fig. 1). At all three sampling sites and times the abiotic factors were similar; only the velocity (Fig. 2) and the conductivity (reference: ~0.11 mS cm^{-1}, reservoir and outflow: ~0.3 mS cm^{-1}) were different. The flow velocity was similar at the reference site (~0.3 m s^{-1}) and in the outflow (~0.3 m s^{-1}) of the weir at both sampling times. In the reservoir the flow velocity was lower (~0.09 m s^{-1}).

Discussion

Biofilms can be rapidly colonised by protozoans (FENCHEL & BLACKBURN 1999), a finding in accordance to the present investigation. After one day the first heterotrophic flagellates were found. Investigations in the River Rhine showed that the first flagellates settled down on glass slides after ten minutes (ARNDT et al. 2003). They found the highest abundance after one day and then a decrease until day 8. In the River Ilm the heterotrophic flagellate abundance increased over the time at the reference site.

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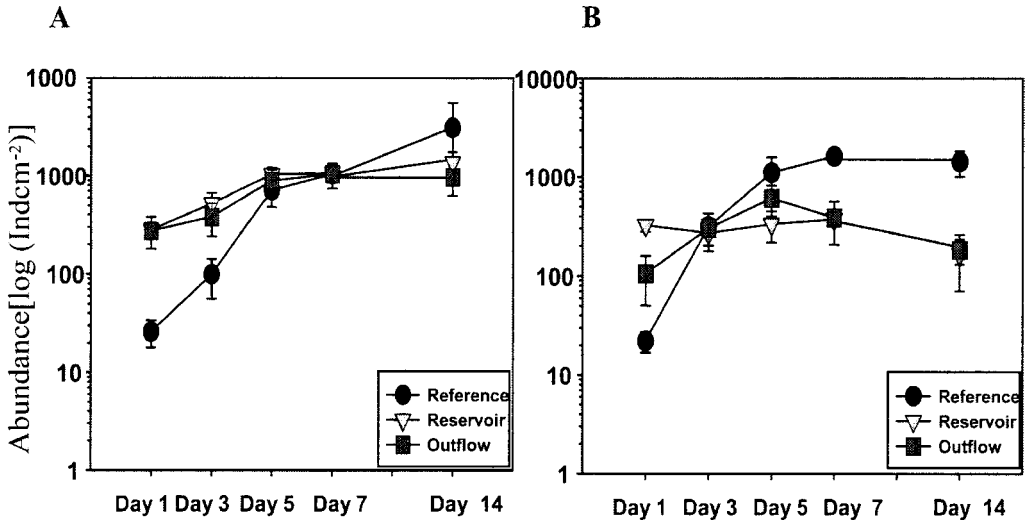

Fig. 1. Changes of heterotrophic flagellate abundance [Ind. cm^{-2}] on biofilms after day 1, 3, 5, 7 and 14 at the reference site, reservoir and outflow area at the weir (A) in spring and (B) in summer. Error bars indicate the standard deviation (n=3).

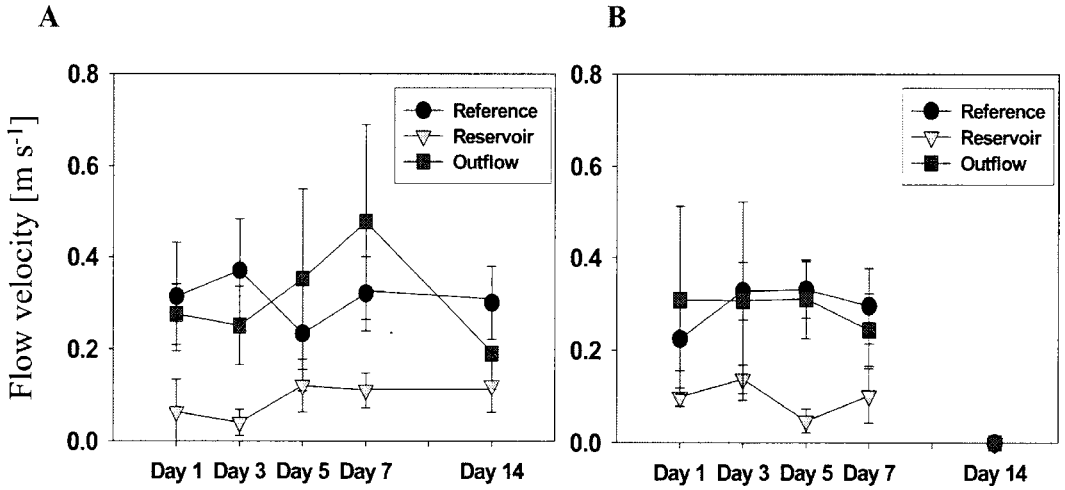

Fig. 2. Flow velocity [m s^{-1}], measured where the glass slides were placed, after day 1, 3, 5, 7 and 14 at the reference site, reservoir and outflow area at the weir (A) in spring and (B) in summer, no measurement on day 14. Error bars indicate the standard deviation (n=3).

This pattern was not found in the reservoir of the weir. It seems that low flow velocity allows rapid colonisation but no increase in abundance, in contrast to the undisturbed reference site. At the reference site with a higher flow velocity, a higher abundance of flagellates and an exponential growth was examined.

For the River Rhine, SCHMITZ (1985) describes the highest abundance of ciliates were found at velocities of 0.2 m s^{-1}. Above 0.8 m s^{-1} no ciliates were found. The optimum for colonisation of heterotrophic flagellates seems to be around 0.3 m s^{-1}, because the sampling sites with this velocity showed a higher

abundance. In addition to a high flow velocity, other environmental factors should be important, because more heterotrophic flagellates were always found at the reference site than at any site at the weir. It is important to investigate the bottom-up and top-down interactions on biofilms, might explain why the abundance of heterotrophic flagellates is highest at the reference site. The siltation on glass slides did not play an important role because the pattern of the distribution of heterotrophic flagellates was equal to the vertical position of glass slides at all sampling sites. Further investigations will reveal more about the role and pattern of heterotrophic flagellates on biofilms in affected and unaffected habitats.

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CHAPTER IV:

SHORT-TERM COLONISATION OF THE HETEROTROPHIC FLAGELLATE COMMUNITY ON BIOFILMS OF THE RIVER ILM (THURINGIA, GERMANY): EFFECT OF DIFFERENT WATER LEVELS

INTRODUCTION

Biofilms are complex communities of microorganisms (bacteria, algae, fungi, protozoa, and micrometazoa) which coexist within an extracellular polymeric substances matrix associated with water surrounded surfaces, like any substrate in rivers (Lock et al. 1984). In small rivers attached microbial communities play a major role in the energy flux of carbon (Bryers 1982). Heterotrophic flagellates as the main grazer of bacteria are an important link within the food chain (Azam et al. 1983). Furthermore, microorganisms in biofilms are a key element in the self-purification process in rivers (Pusch et al. 1998, Sabater et al. 2002). There are several advantages for heterotrophic flagellates to colonise on riverine biofilms, e.g. high food concentrations and refuge from predation (Arndt et al. 2003). The colonisation rate depends on age and taxonomic composition of biofilm communities as well as on flow velocity (Lau & Liu 1993, Railkin 1998). It has been shown that microorganism communities recover their numerical and species compositions through succession usually after 3 – 9 to 14 days (Peterson et al. 1994, Hamilton 1987). Flow velocity influences the colonisation of surfaces by increasing the contact rate of suspended cells with a surface (Hunt & Parry 1998). Rivers are hydrodynamic systems with pool (slow flow velocity) and riffle (high flow velocity) sections. Constructions like weirs can alter the natural flow regime of a river system. They enlarge the pool sections and strengthen the riffle sections just after a weir. Additionally, a weir is a barrier for organisms and nutrients.

In this study the short-term colonisation (abundance and diversity) of heterotrophic flagellates on riverine biofilms was investigated under normal and high water level at different season.

MATERIALS AND METHODS

Study area. The River Ilm has its source in the northern part of the Thuringian Forest (Germany) and runs into the River Saale (a affluent of the River Elbe) after 130 km. Additionally, 57 small low-head weirs with a hydraulic head < 4 m and an impounded area of < 3 ha were built approximately every 2.3 km. The capacity of the reservoirs are small caused by the high slope

of the stream (3.16 %) (Schönborn 1992). A small weir (reservoir and outflow site) (km 103) was chosen as sampling sites to study the effect of flow velocity on colonisation dynamics of biofilm-dwelling heterotrophic flagellates. Additionally, one unaffected reference site (km 127) was studied (a map of these sampling sites is shown in Chapter 2).

Sampling design. 1. Glass slides (artificial substratum) were exposed in spring (April), summer (July) and autumn (November) at a reference site (= riffle section) and one weir (reservoir (= pool section) and outflow (= riffle section)) for the investigation of a short-term colonisation of heterotrophic flagellates on biofilms under normal environmental river flow conditions. Samples were taken after 1, 3, 5, 7, and 14 days of biofilm development.

2. For the comparison of colonisation under normal and high water level glass slides were exposed using the same sampling sites for 1, 3, and 5 days in spring (April). The exposure started always with new glass slides under both water levels.

Glass slides (76 x 26 mm) were placed in sample collection vessels (modified after Alfreider et al. (1997)) made of perforated stainless steel (pore diameter 8 mm) and horizontally exposed close to the stream bed. These sample collection vessels were used to avoid destruction of the glass slides by floating stones. Glass slides were placed in Greiner-Tubes (50 ml), which were filled with filtered, autoclaved river water (filter size 0.2 µm) and stored cool (6°C) until further processing. Within 6 - 7 h the abundance and diversity of heterotrophic flagellates was estimated by the direct live counting technique (Arndt et al. 2000) under a phase contrast microscope (Zeiss Axiovert 25, 400x magnification) and by the help of video recording (exception: diversity from the first sampling in spring). For each sample (three replicates were taken at each sampling site), at least 60 individuals or 30 visual fields were scanned by placing the slide directly under the microscope. Simultaneously all other organisms, e.g. ciliates, diatoms, were recorded.

Environmental parameters. Abiotic factors were measured at all sampling sites and dates. The flow velocity was measured with a Flow-Mate (Model 2000, Mash-McBirney Inc., USA) where the glass slides were exposed. For temperature, oxygen, conductivity, turbidity and pH, a Water Quality Checker (U-10, Horiba, Kyoto, Japan) and probes from WTW (Weilheim,

Germany) were used. Three measurements were conduct as replicates at each sampling site. The discharge, provided by the Staatliches Umweltamt Erfurt, was obtained from the nearest permanent hydrographic station (Gräfinau-Angstedt, km 117).

Statistical analyses. The influence of flow velocity and the other abiotic parameters on the abundance and diversity of flagellates was estimated using a general linear model (univariate variance analysis) followed by a post-hoc Ryan-Einot-Gabriel-Welsch test (SPSS 11.0, Chicago, USA). The alpha level of significance for all statistical tests was 0.05. Similarities among the species pools for each sampling site were compared using Sørensen's similarity index (Pielou 1984):

$$S = ((2*a)/(b+c))*100,$$

where a = the number of species present in both species pools being compared, b = the number of species present in the first species pool but not in the second and c = the number of species present in the second but not in the first.

RESULTS

Environmental parameters. The discharge varied from 0.1 to 2.4 $m^3 s^{-1}$ at the different seasons during the first investigation (Fig. 1). The flow velocity was low in the reservoir during the sampling period (mean 0.10 ± 0.02 m s^{-1}). In comparison the velocities were higher at the outflow and reference sites (mean 0.35 ± 0.10 m s^{-1}). The other environmental factors, like pH, turbidity, and oxygen concentration were similar at the three sampling sites. Conductivity and temperature were lower at the reference site compared to both weir sites (Fig. 1). There was only seasonal variation for temperature and corresponding oxygen concentration during the sampling period.

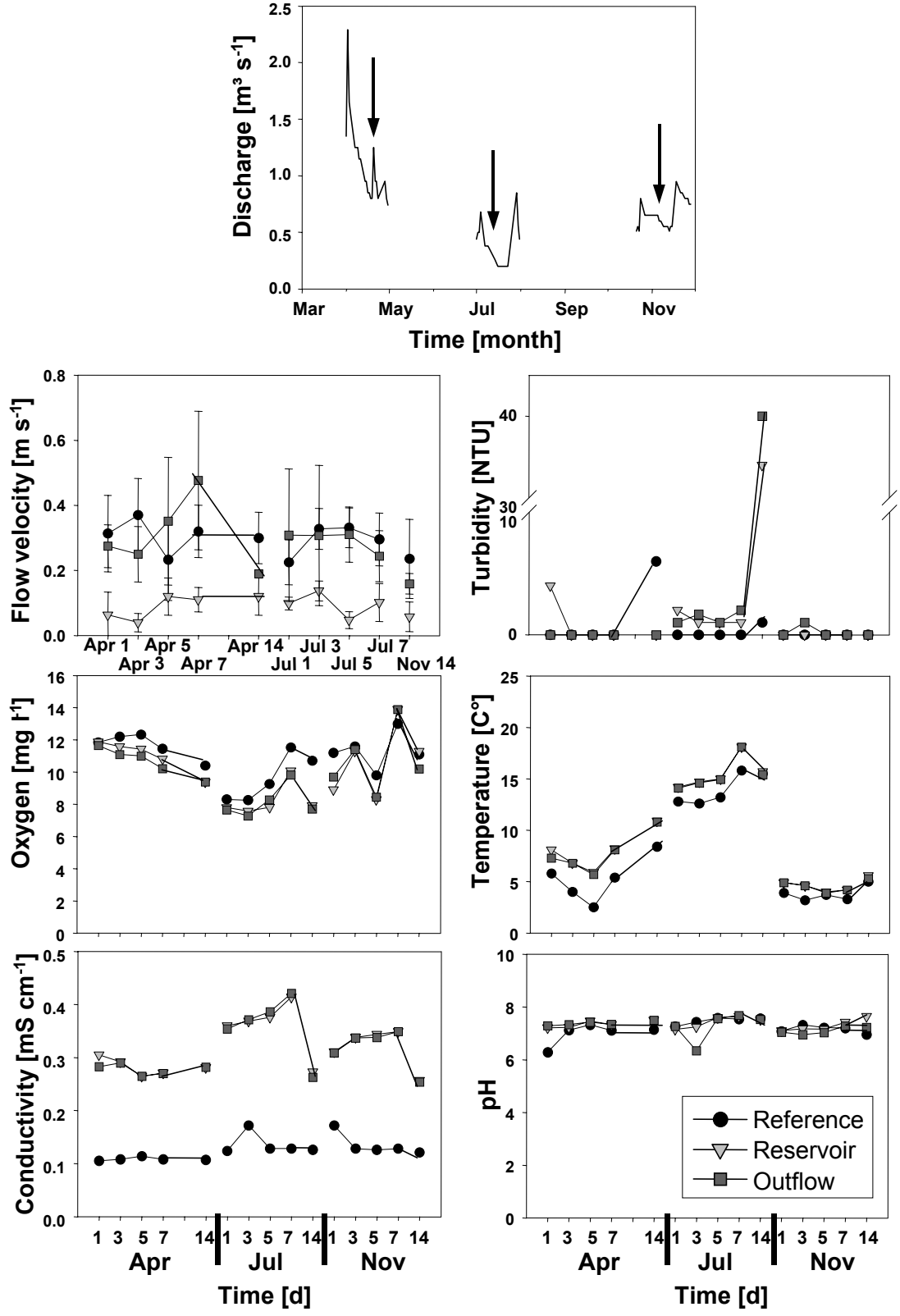

Fig. 1. Abiotic factors (discharge (measured at the water level gauge Gräfinau-Angstedt (117 km), data provided by the Staatliches Umweltamt Erfurt), flow velocity (after Willkomm 2006, for spring and summer), turbidity, oxygen, temperature, conductivity, and pH) of all sampling sites (reference, reservoir, and outflow) over time in the River Ilm. Error bars indicate standard deviation (n = 3) and arrows indicate the dates of sampling.

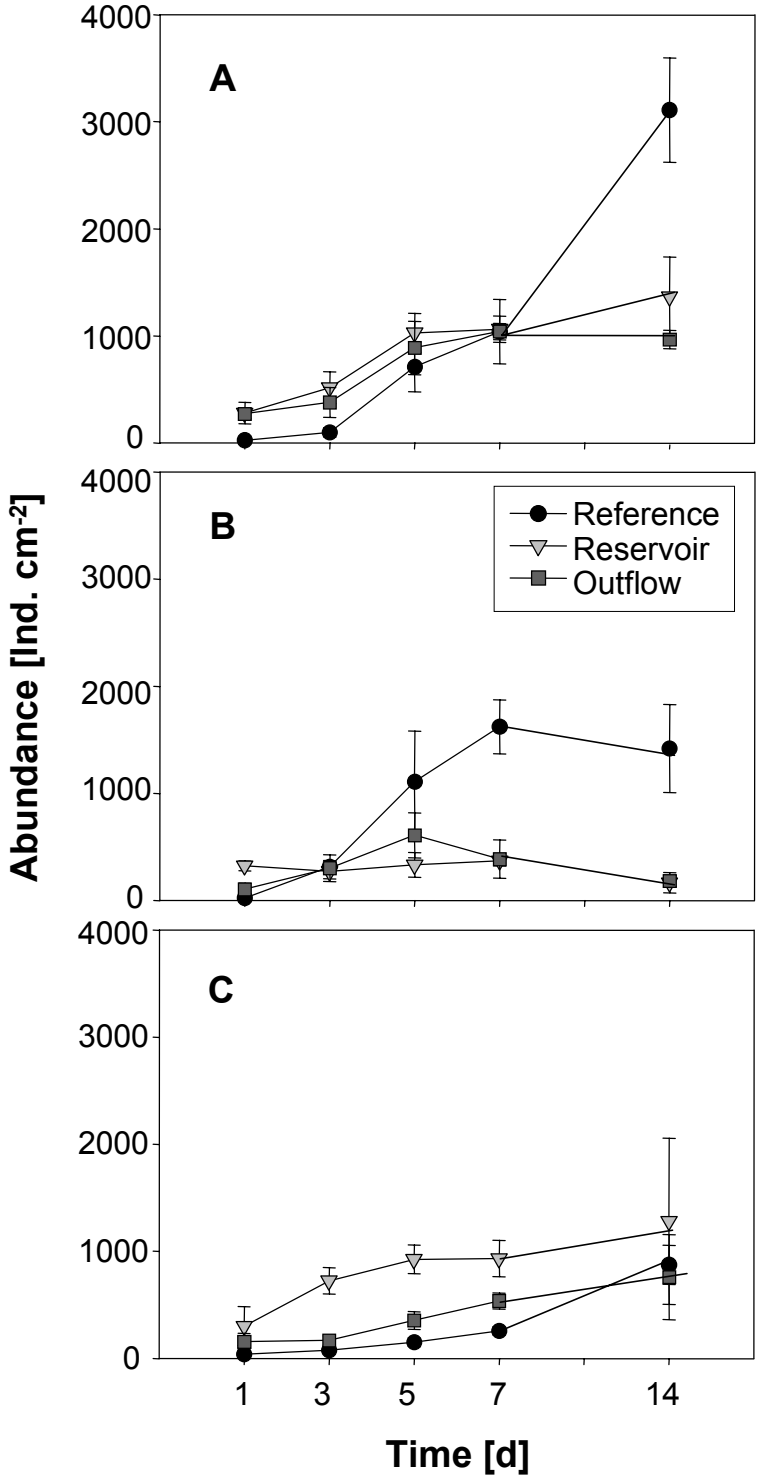

Fig. 2. Abundance of heterotrophic flagellates [Ind. cm^{-2}] at all sampling sites (A = spring, B = summer (after Willkomm 2006), and C = autumn) at day 1, 3, 5, 7, and 14 of biofilm development in the River Ilm (error bars indicate standard deviation, n = 3).

Species abundance. The highest abundance of heterotrophic flagellates (~3000 Ind. cm^{-2}) was found at the reference site in spring (Fig. 2 A). The

increase of abundance showed an exponential growth. A significant difference was determined between the abundance of heterotrophic flagellates at the reference site and both weir sites ($p < 0.05$) but not between the both weir sites. Similar results were determined for the sampling sites in summer (Fig. 2 B). However, the abundance of the heterotrophic flagellates reached a plateau at the 7th day (~1600 Ind. cm^{-2}) at the reference site. In autumn, the highest abundance was found at the reservoir (Fig. 2 C) but there was no significant difference between the sampling sites ($p > 0.05$).

Species diversity. The heterotrophic flagellate communities were dominated by Kinetoplastida and Ancyromonadida at both weir sites for all sampling dates in summer (Fig. 3 B and C). At the reference site, Ancyromonadida and Cercomonadida were most abundant (Fig. 3 A). In autumn Cercomonadida, Euglenida, and Kinetoplastida had a high contribution to the heterotrophic flagellate abundance at all sampling sites. Furthermore, Choanoflagellida were more abundant in autumn than in summer (Fig. 4). The Sørensen's similarity index showed the high similarity between both weir sites, too (Fig. 5 A). In autumn, the similarity was slightly higher than in summer (Fig. 5 B). The relationship between heterotrophic flagellates and bacteria on biofilms is shown in Figure 6. Bacterial abundance stayed constant after 7 days of exposure at both weir sites.

Influence of high water level. During the second part of the investigation, heavy rainfall started after the 3rd day leading to a discharge of max. 14 $m^3 s^{-1}$ which was much higher than the annual mean discharge of 2.45 $m^3 s^{-1}$ (Fig. 7). The flow velocities were 2 - times higher at the reference site and 2 to 3 - times higher at the weir sites during the high water situation. The turbidity was increased at both weir sites, too.

The abundance of heterotrophic flagellates increased until the 3rd day of the first part of this field experiment (= normal water level). At 3rd day, the abundance was significantly higher at both weir sites compared to the reference site ($p < 0.05$). During the high water level, no noticeable differences in abundance and colonisation dynamics were observed between the sites (Fig. 8).

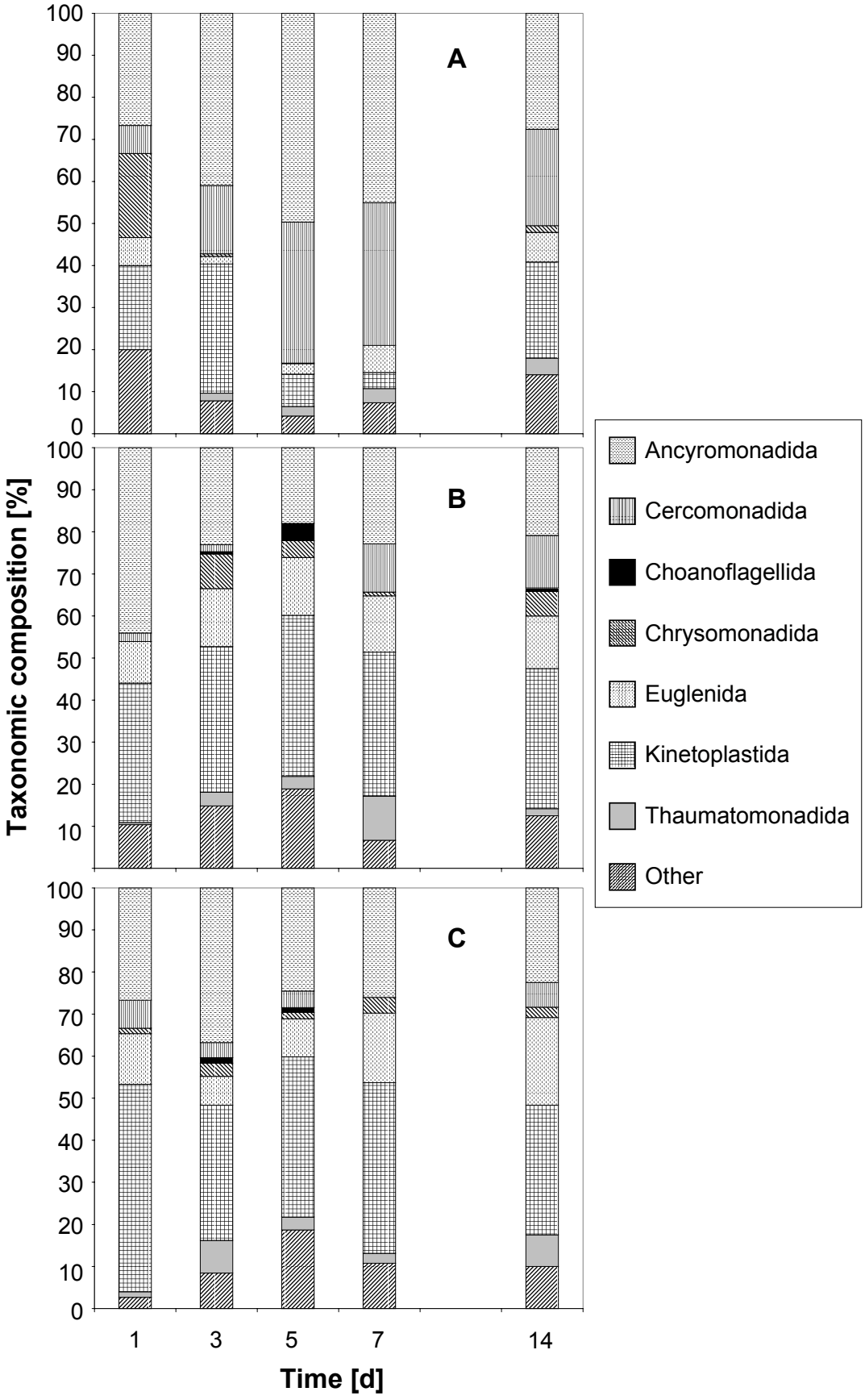

Fig. 3. Taxonomic composition (contribution of taxonomic groups to total abundance) of the heterotrophic flagellate community [%] on biofilms of the River Ilm in summer (A = Reference site, B = Reservoir site, and C = Outflow site).

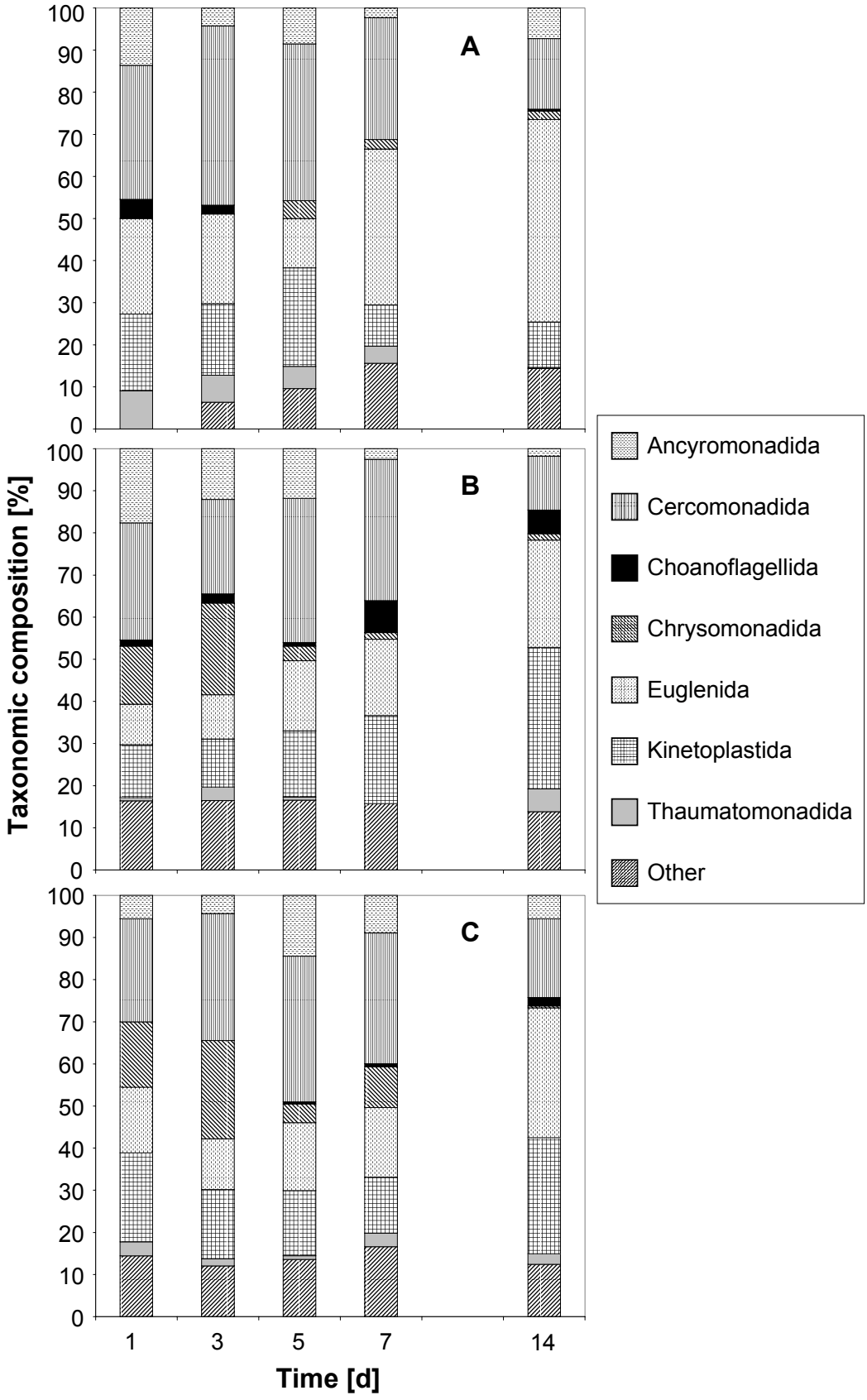

Fig. 4. Taxonomic composition (contribution of taxonomic groups to total abundance) of the heterotrophic flagellate community [%] on biofilms of the River Ilm in autumn (A = Reference site, B = Reservoir site, and C = Outflow site).

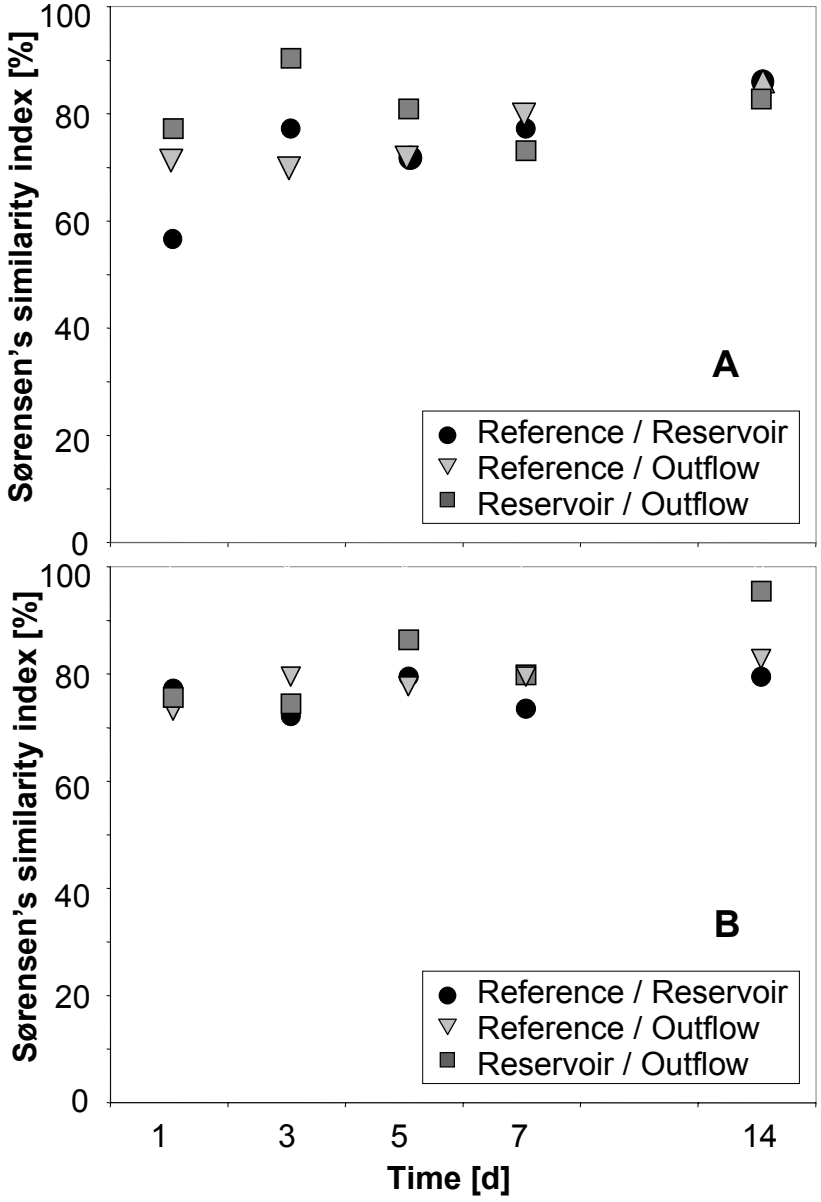

Fig. 5. Sørensen`s – Quotient of similarity [%] of the heterotrophic flagellate communities on biofilms in the River Ilm for the three sampling sites (reference, reservoir, and outflow) in summer (A) and autumn (B).

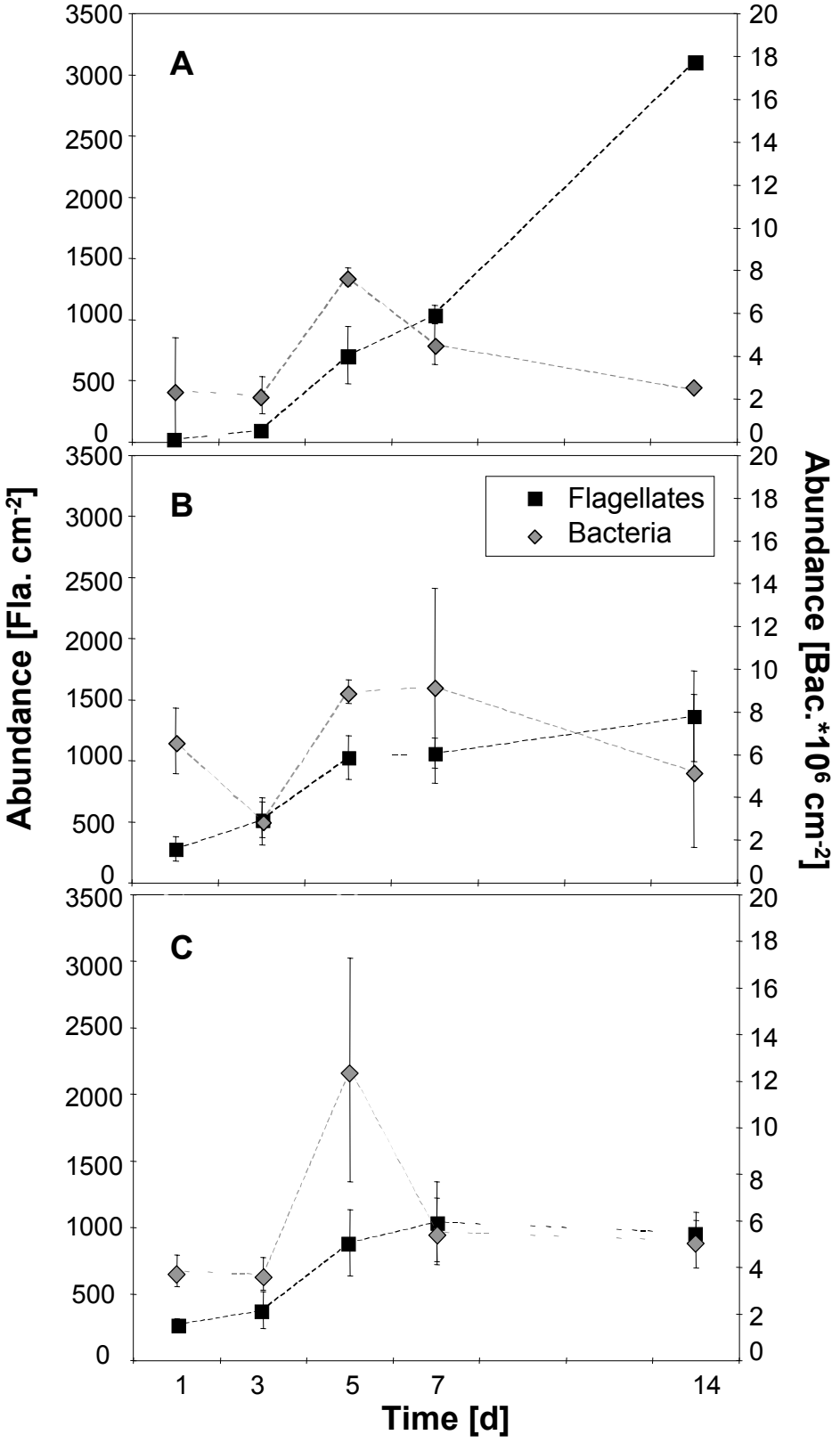

Fig. 6. Abundance of heterotrophic flagellates [Ind. cm^{-2}] and bacteria [cells * 10^6 cm^{-2}] on biofilms in the River Ilm in spring at three sampling site (A = reference, B = reservoir, and C = outflow).

A shift in the composition of the heterotrophic flagellate community was observed. Choanoflagellida had the highest number at the 1st day of the biofilm development during normal water level at all sampling sites (Fig. 9). Kinetoplastida had the highest contribution to the heterotrophic flagellate abundance during both water levels at the reference site (Fig. 9 A). At both weir sites, the abundance of Kinetoplastida was higher at high water level than at normal water level (Fig. 9 B and C). Furthermore, Chrysomonadida were more abundant at high water level than at normal water level at all sampling sites. Ancyromonadida were found at all sampling sites and water levels. The Sørensen`s similarity index was higher at normal water level than at the high water level in the stream (Fig. 10). Furthermore, the similarity increased with the time of incubation during high water level at the weir sites.

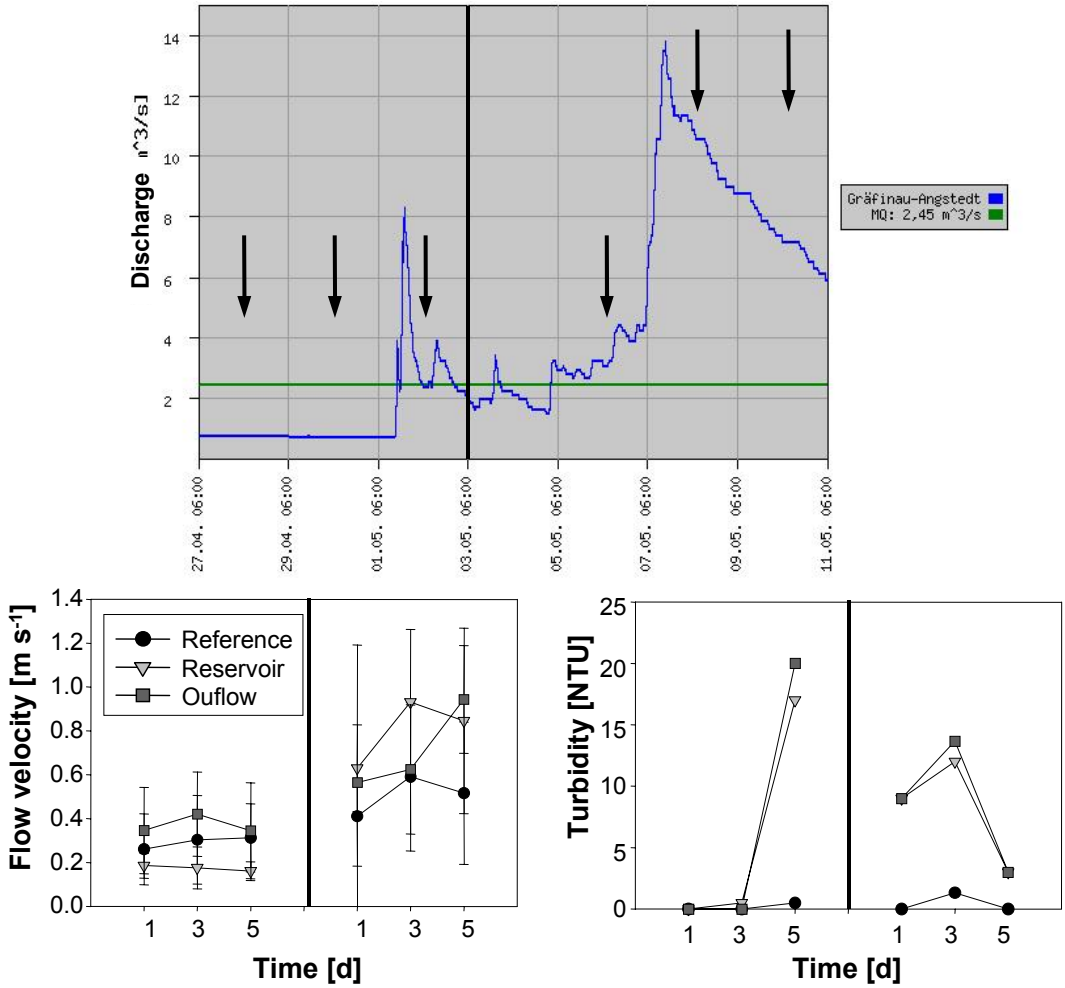

Fig. 7. Abiotic factors (discharge, flow velocity, and turbidity) of all sampling sites (reference, reservoir, and outflow) in spring. Discharge was measured at the water level gauge Gräfinau-Angstedt (117 km). Error bars indicate standard deviation (n = 3). Arrows indicate the sampling dates. Left site in each illustration = normal water level conditions and right site = high water level conditions.

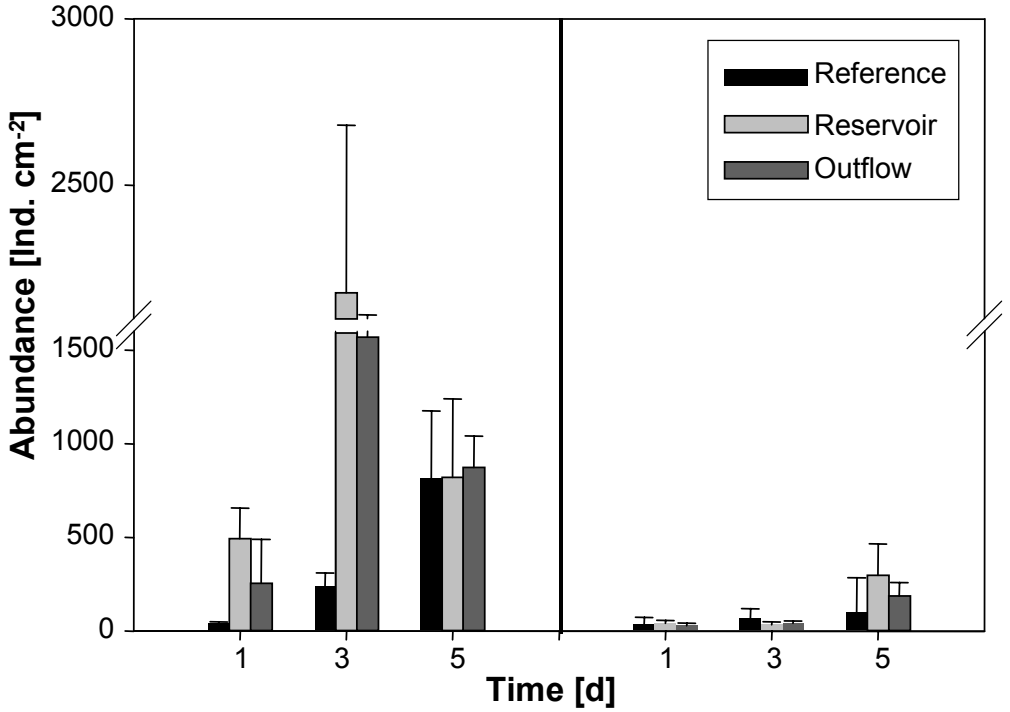

Fig. 8. Abundance of heterotrophic flagellates [Ind. cm^{-2}] at all sampling sites (reference, reservoir, and outflow) at day 1, 3, and 5 of biofilm development in the River Ilm in spring (error bars indicate standard deviation, n = 3). First part of sampling (left site) at normal water level and second part (right site) at high water level.

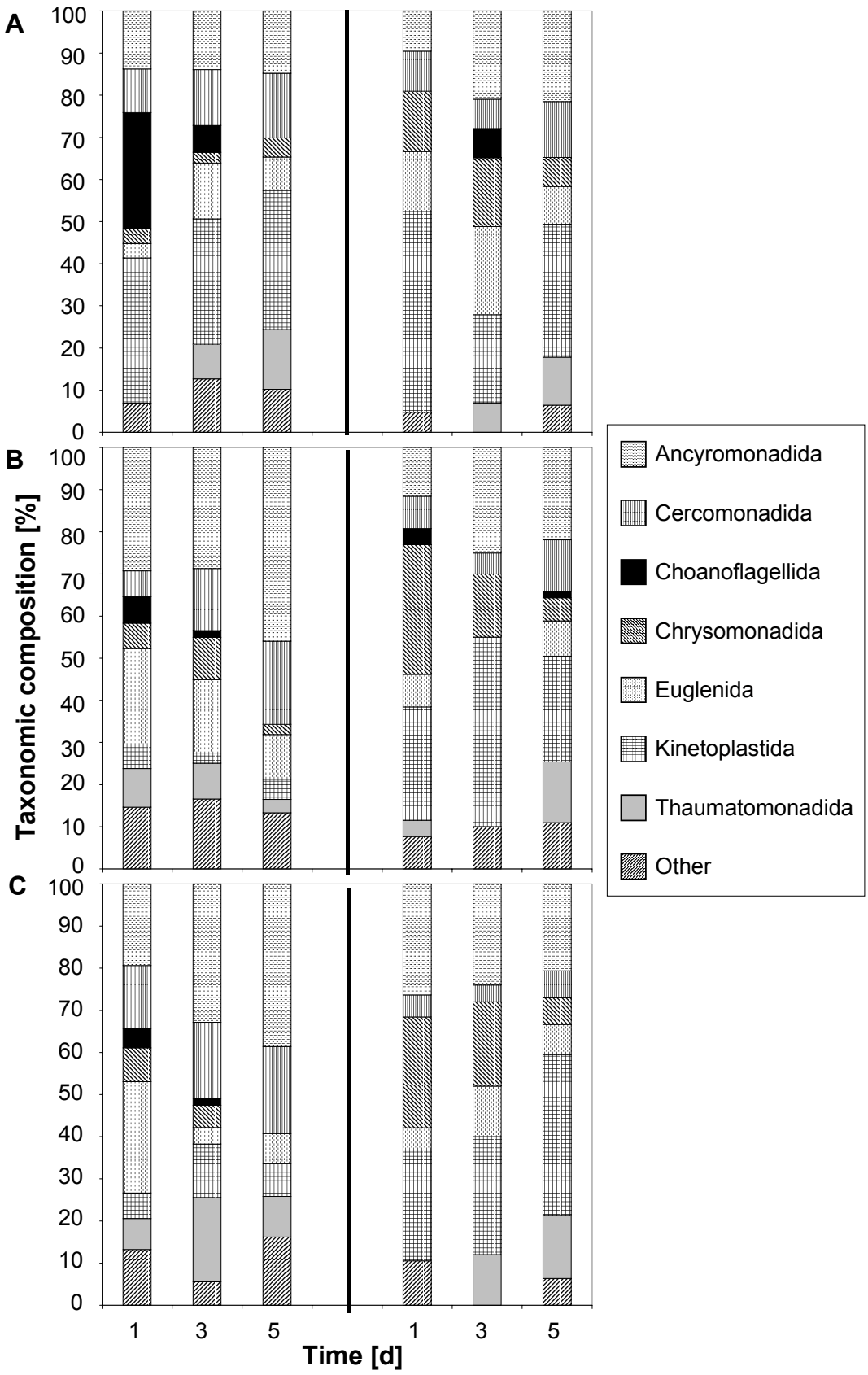

Fig. 9. Taxonomic composition (contribution of taxonomic groups to total abundance) of the heterotrophic flagellate community [%] on biofilms of the River Ilm in spring (A = Reference site, B = Reservoir site, and C = Outflow site). Left site in each illustration = normal water level conditions and right site = high water level conditions.

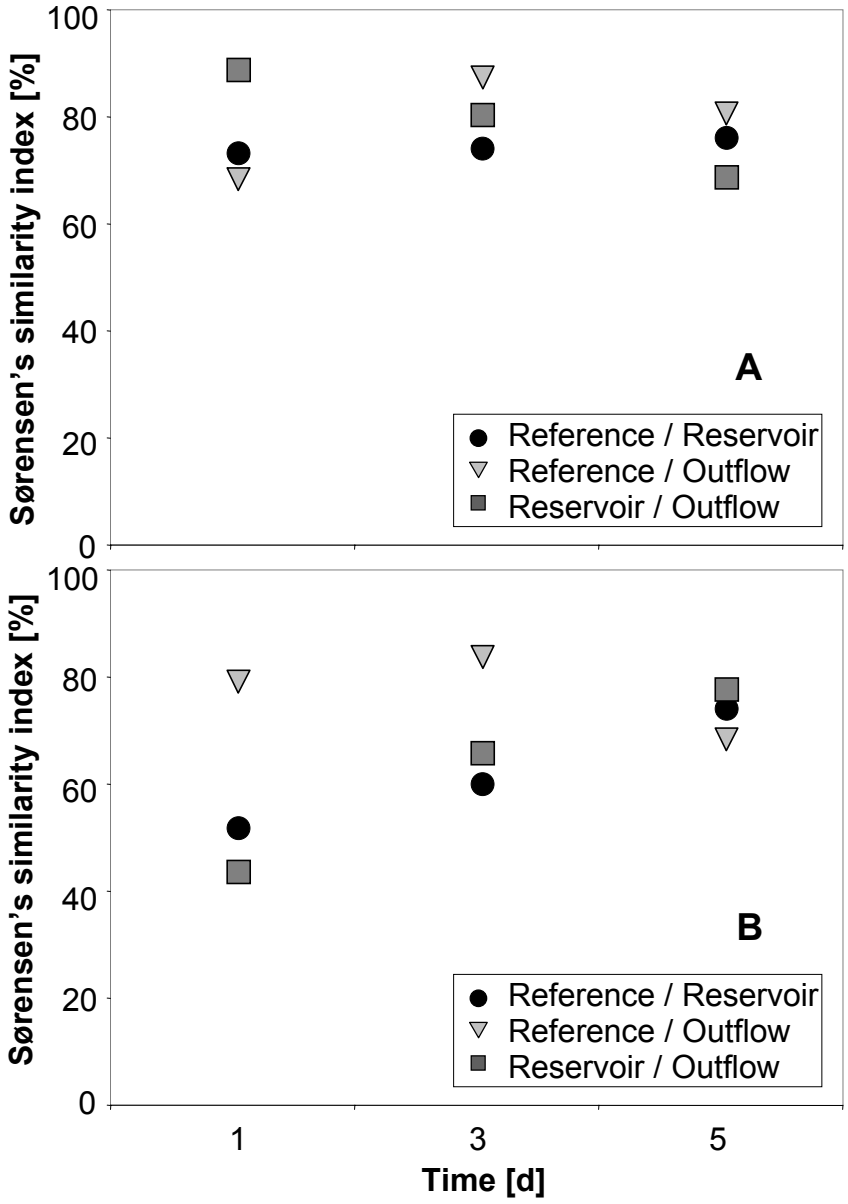

Fig. 10. Sørensen`s – Quotient of similarity [%] of the heterotrophic flagellate communities on biofilms in the River Ilm for the three sampling sites (reference, reservoir, and outflow) at normal water level (A) and at high water level (B).

DISCUSSION

The short-term colonisation process of heterotrophic flagellates was characterised on artificial substrates in the course of different water levels in a small stream.

Early processes may be crucial for further succession dynamics on biofilms. Artificial substrates like glass slides are rapidly colonised by microorganisms (Bamforth 1982). Hunt and Parry (1998) described that glass slides were exposed for a maximum of 14 days, which was sufficient for the initial colonisation of protists to reach a plateau.

The measured abiotic parameters are strongly influenced by the environmental conditions in the catchment area of the River Ilm. The highest discharge was observed in spring due to snow smelt and rainfall. Conductivity and temperature were different between the reference site and both weir sites. This difference can be explained by the location of the reference site in the middle of the Thuringian Forest. Therefore, values were lower than at the weir. In this study, the higher flow velocities at the reference site might have lead to a higher abundance of organisms due to a higher probability of organisms to get entrapped (Characklis & Marshall 1990, Hunt & Parry 1998). The recovery rate depends on age and taxonomic composition of communities as well as on flow velocity (Railkin 1998). The reason that heterotrophic flagellate abundance did not increase in our incubations during the first experiment at the slow flowing site after 5 days might be the result of sloughing. Sloughing, which is the detachment of large biofilm fragments, was observed at laminar flow or low shear stress (Characklis & Marshall 1990). On the other hand, sloughing can also occur by increasing flow velocity on entire biofilms (Beyenal & Lewandowski 2002). This might be the reason for the fact that both weir sites had a similar heterotrophic flagellate abundance in this study.

It is well-known that biofilms grown at higher flow velocities are denser than those at lower flow velocities (Characklis & Marshall 1990, Gantzer et al. 1991). In the present study at high water level, it seems that the biofilm development was not in a stage that the biofilms could resist this very high flow velocities. Schmitz (1985) observed that ciliates could not be found on biofilms at velocities higher than 0.8 m s^{-1}. The optimal growth of *Cladophora* was observed between 0.5 an 0.8 m s^{-1} in the River Ilm (Schönborn 1996). During the high water level the flow velocities were between 0.5 and 1.0 m s^{-1}. In accordance with the investigations of Schmitz and Schönborn the abundance of heterotrophic flagellates as well as chlorophyll a and seston on biofilms were reduced to more than 70 % at the high water level compared to the study at the

normal water level (data for chlorophyll a and seston are not shown in this chapter).

The knowledge on the role of substrate associated protozoans, especially heterotrophic flagellates, is still low. There are more studies available on the diversity of ciliates than for heterotrophic flagellates on riverine biofilms (e.g. Foissner et al. 1992). Recent studies considered population dynamics and diversity of heterotrophic flagellates on riverine biofilms, too (e.g. Weitere et al. 2003, Esser 2006, Willkomm 2006). During the present investigation a shift in the diversity of heterotrophic flagellates could be documented in the course of the incubation time at higher flow velocities. Kinetoplastida and Chrysomonadida were more abundant than at normal water level. Detailed investigations on the influence of flow velocities on different morphotypes of heterotrophic flagellates will be discussed in Chapter 5. The species *Ancyromonas sigmoides, Bodo designis, Bodo saltans, Petalomonas pusilla* and *Rhynchomonas nasuta* - which were found in this investigation - belong to the 20 most commonly reported species with a probable world wide distribution (Patterson & Lee 2000).

There is a close relationship between bacteria and heterotrophic protozoans due to grazing (Arndt et al. 2000). In the present investigation, the losses of bacteria on the biofilm (Fig. 6) could be explained by the grazing of heterotrophic flagellates. Assuming a grazing rate of 30 bacteria per flagellate and hour (Boenigk & Arndt 2002) multiplied by the respective flagellate abundance, the estimated grazing losses of bacteria would be in the same range as the observed decline of bacteria. In addition, ciliates should have exerted a significant grazing pressure on bacteria.

Knowledge of microbial food webs in lotic environments is still scarce relative to that in lentic environments. For the future, it will be required to study the interactions between microorganisms in lotic environments. Also, it should be put more focus on detailed investigations on biofilm developments of heterotrophic flagellates (abundance and structure) to understand more about the function of biofilms in streams.

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CHAPTER V:

EFFECTS OF MICROCURRENTS IN THE BOUNDARY LAYER ON THE ATTACHMENT OF BENTHIC HETEROTROPHIC NANOFLAGELLATES

ABSTRACT: Surfaces in running water are covered by a boundary layer. Virtually nothing is known about the importance of water currents in the microenvironment of nanofauna. Many questions have been partially answered concerning the effect of surface topography on the hydrodynamics in the vicinity of macrofauna; however, investigations of the 2 to 5 µm water layer where nanoprotists live have been neglected. In the present study, we show that the flow velocity at a distance of a few micrometers from the substrate is high enough to be very effective regarding the detachment of nanoprotists. We analysed the impact of flow velocity (detachment from substrate) on 8 nanoflagellate taxa (*Entosiphon, Cercomonas, Codonosiga, Anthophysa, Bodo, Neobodo, Apusomonas, Spumella*) with different abilities to crawl and attach to the surface. A Plexiglas disc was used to generate a defined flow velocity on the surface of a Petri dish microcosm. Laminar flow in the boundary layer was found in the layer between 0 and 700 µm above the substratum. The effect of 4 different flow velocities on heterotrophic flagellates was investigated (0.3, 0.6, 0.9 and 1.2 m s^{-1} at 5 mm above the substratum, corresponding to flow velocities of 0.001 to 0.004 m s^{-1} at 10 µm above the substratum). The colourless, gliding euglenid *Entosiphon sulcatum* showed the highest resistance towards high flow velocities. Another species, the crawling cercomonad *Cercomonas crassicauda*, had the weakest attachment. Small changes in the micro-topography of the substrate (e.g. *Ancylus* shells) may significantly influence spatial distribution of nanoflagellates.

Key Words: heterotrophic nanoflagellates, boundary layer, microcurrents, biofilm, flow velocity, topography

INTRODUCTION

It is commonly agreed that flow velocity can be an important factor for the structure and dynamics of aquatic ecosystems, especially in running waters and tidal regions (Battin et al. 2003 a, b). It is assumed that the flow velocity decreases asymptotically from the surface towards the bottom. A few millimetres above the bottom, the flow velocity is very low (Ambühl 1959). This region is called boundary layer and serves as a refuge for torrenticole macrofauna (Vogel 1981, Koehl 1984, Prandtl et al. 1984). Only a very few reports on the flow velocity in the microenvironment and the diffusion rates around microfauna and bacteria exist (e.g. Silvester & Sleigh 1985, Lazier & Mann 1989, Shimeta et al. 2001, 2002, Vopel et al. 2002, 2005, Willkomm 2006). Virtually nothing is known about the importance of water currents in the microenvironment of nanofauna. All of the questions that have partially been answered concerning the effect of surface topography on the hydrodynamics in the vicinity of macrofauna. Although some information is available on the vicinity of macrofauna (e.g. Ambühl 1959, Statzner & Holm 1989, Hunt 2004), the 2 to 5 µm water layer in which nanoprotists live remains to be investigated. To understand microbial interactions on biofilms, several questions have to be answered, e.g. to what extent do water currents affect organisms in a size range of a few micrometres? Could the roughness of bacterial biofilms form a refuge for organisms of the nanofauna? Can meio- and macrofauna burrows and houses create hydrodynamically specific habitats for nanoflagellates?

We investigated the flow velocity in the micro-layer above the substrate and its impact on nanoflagellate species with different abilities to crawl and attach on the substrate in order to address at least some of the above-mentioned questions.

MATERIALS AND METHODS

Flow velocity measurements on a microscale. The effect of microcurrents on the attachment of heterotrophic flagellates was investigated in experimental microcosms. The microcosms consisted of Petri dishes with a diameter of 13.5 cm. These were filled with about 75 ml of flagellate cultures

(WC medium is MBL; Guillard & Lorenzen 1972) to reach a water column with a height of 5 mm. By changing the current supply of a 12 V motor, the speed of a rotating Plexiglas disc on top of the fluid was regulated, generating a specific flow velocity in defined regions of the Petri dish (the regions had a distance of about 46 mm from the centre of the dish). To observe these regions, the bottom of each Petri dish contained 4 windows with cover slips (50 x 25 mm) to allow the observation of the surface-associated flagellates by inverse microscope at high magnifications (up to 400x; Zeiss Axiovert 100; Fig. 1). The inner surface of the Petri dish (including cover slips) was flush to avoid any disturbances of the water current. For the identification of the flow velocity in the microcosms, the fluid was spiked with a suspension of 10 µm neutrally buoyant hollow glass spheres (cf. Røy 2003; kindly provided by Hans Røy, Max Planck Institute for Marine Microbiology, Bremen, Germany). Flow speed of particles was estimated from video image sequences of 50 frames s^{-1}. This is a classical method of visualising the flow fields under laboratory conditions (e.g. Riedl & Forstner 1968, Oakey et al. 2002). The flow velocity was determined in different layers of the water established by a calibrated fine drive of the microscope.

The flow velocities were determined in the different layers of the circular flow chambers between 0 and 5000 µm above the substrate (Fig. 1). At a disc velocity of 0.3 m s^{-1}, the flow velocity ranged between 0.001 m s^{-1} (0 - 10 µm) and 0.3 m s^{-1} (5000 µm). In the layer between 0 and 700 µm, a linear increase of the flow velocity was detected. A slight increase of flow velocity was recorded for the layer between about 1000 and 4000 µm above the dish surface, while a linear approach to the velocity of the disc was registered in the upper layer (4000 to 5000 µm above the dish surface).

Flagellate cultures. Eight different species of heterotrophic nanoflagellates (monoxenic cultures) were used in the experiments (Fig. 2). Two species formed colonies at the end of a stalk: *Codonosiga botrytis* (total length of the colony: 45 to 95 µm; Choanoflagellida; isolated from the Rhine River by M. Weitere) and *Anthophysa vegetans* (total length of the colony: 65 to 125 µm; Chrysomonadida; isolated from the Rhine River by M. Weitere). *Spumella sp.* (cell length: 3 to 7 µm; Chrysomonadida; isolated from Lake Schöhsee by A. P. Mylnikov) was attached to the substratum by a protoplasmid thread. The euglenid *Entosiphon sulcatum* (20 to 25 µm; obtained from CCAP

1220/1A), the apusomonad *Apusomonas proboscidea* (8 to 12 µm; isolated from a lake near Borok, Russia, by A. P. Mylnikov), the kinetoplastid *Neobodo designis* (4 to 7 µm; isolated from the Rhine River by M. Weitere) and the cercomonad *Cercomonas crassicauda* (15 to 20 µm; isolated from a lake near Borok, Russia, by A. P. Mylnikov) are tectic flagellates that glided over the surface of the Petri dish. The kinetoplastid *Bodo saltans* (5 to 6 µm; isolated from a lake near Borok, Russia, by A. P. Mylnikov) was attached to the surface of the Petri dish by the tip of the posterior flagellum.

Effects of flow velocity on smooth surfaces. The 8 species of heterotrophic flagellates mentioned above, with different morphologies and types of attachment to the surface, were exposed in separate experiments to different flow velocities on a smooth surface (Petri dish). Flagellate cultures were inoculated to the Petri dish microcosms 12 h before the start of the experiments. Preliminary studies showed that this pre-incubation served as an adaptation time, allowing a comparative study of the different species. The flow velocity was increased stepwise with intervals of 0.1 m s^{-1} every 5 s until the target velocity was reached. At this point the investigation period started. During this 10 min period comparative data for all species were collect. The time until detachment (passive pull off) of flagellates was determined at flow velocities of 0.3, 0.6, 0.9 and 1.2 m s^{-1} (measured at a height of 5 mm above the surface of the Petri dish). At the height of 10 µm above the surface of the Petri dish, the flow velocities were 0.001, 0.002, 0.003 and 0.004 m s^{-1}. Then, 5 to 8 replicate experiments with different cultures of the same flagellate strain were carried out. The height of organisms indicated (Fig. 2) was estimated at conditions without flow using a calibrated fine drive of the microscope.

Effects of flow speed on the distribution of flagellates depending on surface topography. Two of the above-mentioned heterotrophic flagellates, *Entosiphon sulcatum* and *Cercomonas crassicauda*, were introduced into Petri dishes with shells of the basommatophoran snail *Ancylus fluviatilis* (length: 3.1 mm, width: 1.9 mm and height: 1.1 mm; average dimensions) glued on the bottom of the dishes. The shells were used to mimic the roughness of the biofilm due to the presence of macrofauna. *A. fluviatilis* is a typical inhabitant of biofilms in rivers (Nielsen 1950, Elser 1999), where most of the flagellates used in this study were isolated. The spatial distributions of the 2 flagellate species

were investigated in the area behind the shells. This area was divided into 20 fields (92 x 100 µm). Experiments were carried out at a flow velocity of 0.3 m s^{-1} (at a height of 5 mm above the surface of the Petri dishes). The direction of the current and the flow velocity were determined in every field (methods see above). The abundance of the heterotrophic flagellates was determined every minute for each single field by an inverse microscope (video-microscopy with a ZEISS Axiovert 100; 25x magnification). The average abundance of flagellates in each field was determined for a period of 10 minutes. The maximum abundance in each experimental set up was considered as 100 %. Four replicate experiments were carried out for each species. Pearson rank correlation was used to analyse the correlation between abundance and flow velocity.

RESULTS

Effects of flow velocity on nanoflagellates on smooth surfaces

When the disc velocity changed from 0.1 to 1.4 m s^{-1}, the flow velocity above the substrate changed from about 0.0003 to 0.0045 m s^{-1}. The question arose whether these changes of more than an order of magnitude in flow velocities might affect the behaviour of nanoflagellates living close to the substrate. The 8 different nanoflagellate species were exposed to 5 different flow velocities, and the time until detachment was measured. All species differed significantly regarding their type of attachment, the substrate and the mean distance from the substrate (Fig. 2). *Codonosiga botrytis* and *Bodo saltans* withstood the maximal target time (10 min) up to a velocity of 0.6/0.002 m s^{-1} (at a distance of either 5 mm or 10 µm from the substrate; Fig. 3). At the highest velocity of 1.2/0.004 m s^{-1}, *Entosiphon sulcatum* (7:25 min) reached the longest attachment time of all 8 flagellates followed by *B. saltans* (5:53 min), *C. botrylis* (5:24 min), *Anthophysa vegetans* (2:45 min), *Apusomonas proboscidea* (1:35 min), *Neobodo designis* (0:31 min), *Spumella sp.* (0 min) and *Cercomonas crassicauda* (0 min). No species withstood the flow for the maximum target time of 10 min at the highest velocity. *Spumella sp.* and *C. crassicauda* showed the least resistance to the flow, numbers of attached

specimens decreased very rapidly already when low flow velocities were applied (Fig. 3). There was no correlation between the distance of the individual flagellate cells from the substrate and the period of attachment (cf. Fig. 2).

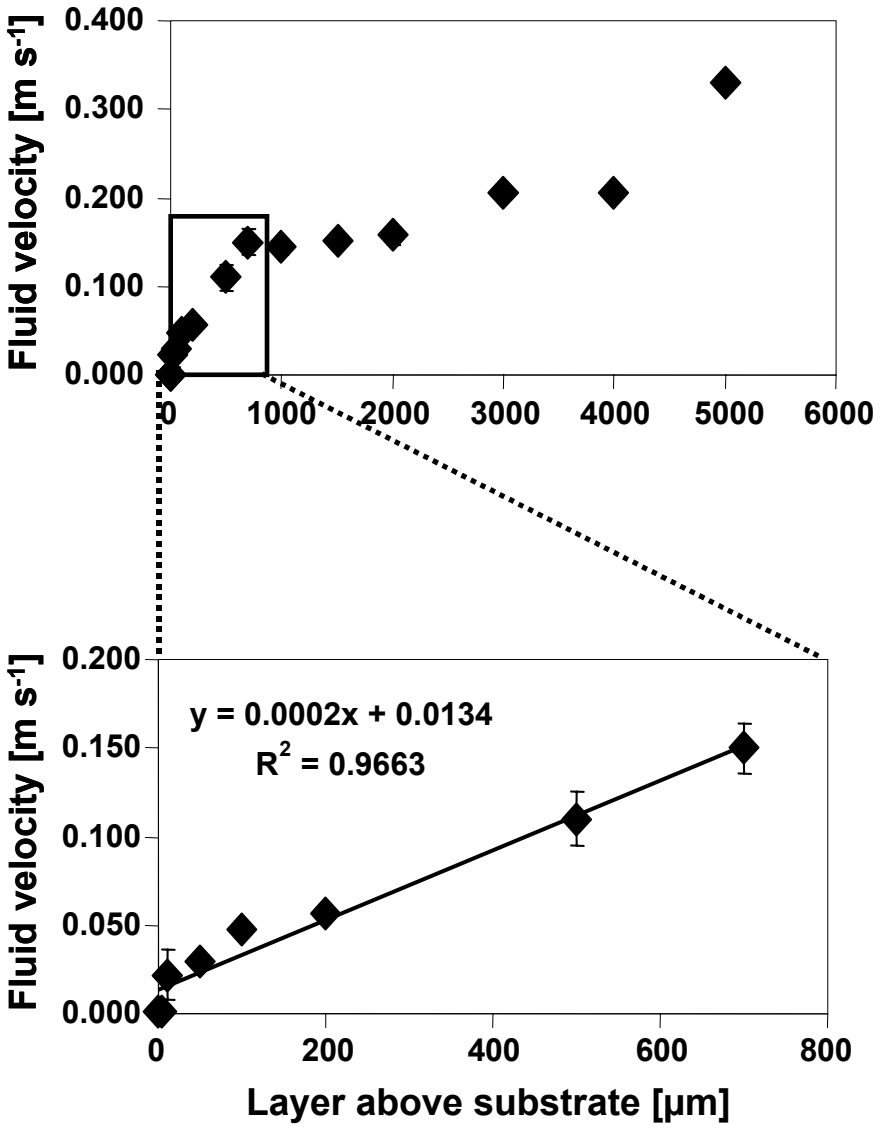

Fig. 1. Flow velocity at different layers of the microcosm (in µm above the substrate) at a speed of the rotating disc of 0.3 m s^{-1}. Error bars indicate the standard deviation. The lower graph is an enlargement for the region of linear increase of flow velocity (n= 2 – 5)

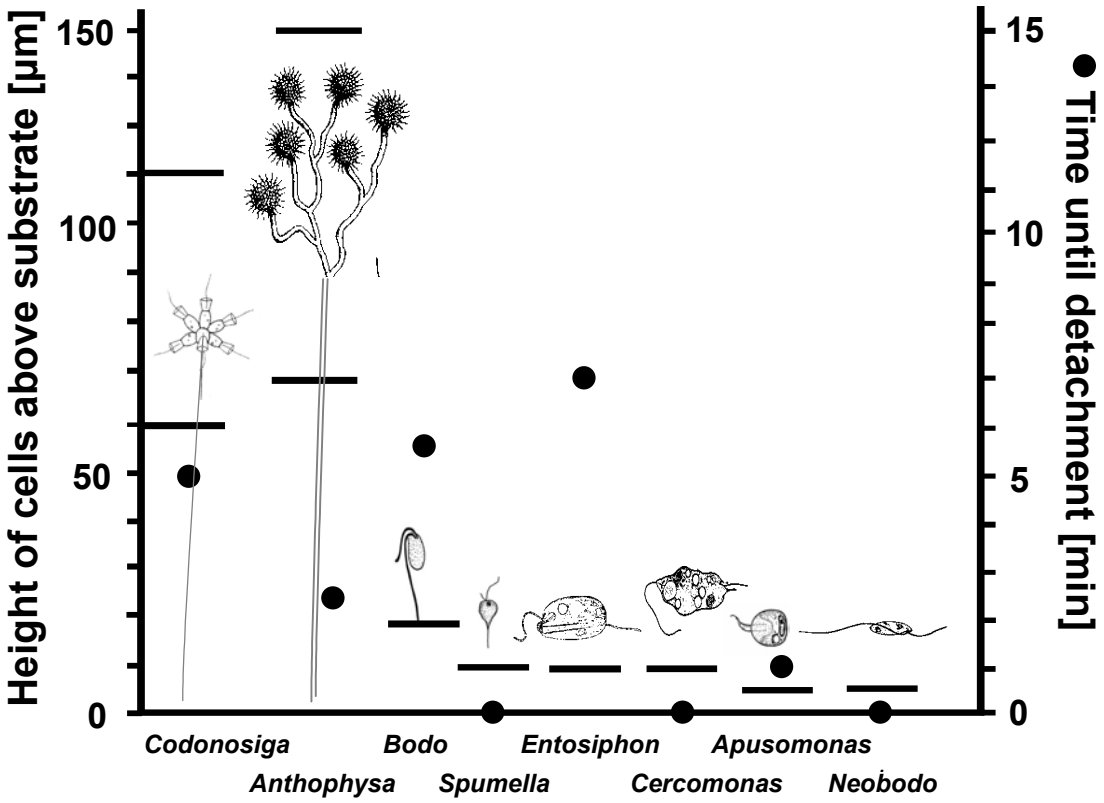

Fig. 2. Relationship between the morphology and the detachment time of 8 heterotrophic nanoflagellates. Horizontal bars indicate the mean distance of single cells from the substrate (for *Codonosiga* and *Anthophysa* a range is given for the colonies); black dots stand for the mean time until detachment at a disc velocity of 1.2 m s^{-1} (cf. Fig. 3)

Effects of flow velocity on the distribution of flagellates in relation to surface topography

The effect of substrate topography in the microscale using shells of the snail *Ancylus fluviatilis* as a model was studied by analysing the distribution patterns of 2 flagellate species, *Entosiphon sulcatum* and *Cercomonas crassicauda*. The highest abundance of *Entosiphon* was found close to the shell, on the lee side, in the region where flow velocities were lowest. *Cercomonas* showed a similar distribution pattern (Fig. 4). There was a significant negative correlation ($p < 0.05$) between the abundance in the investigated fields of observation and the corresponding flow velocity.

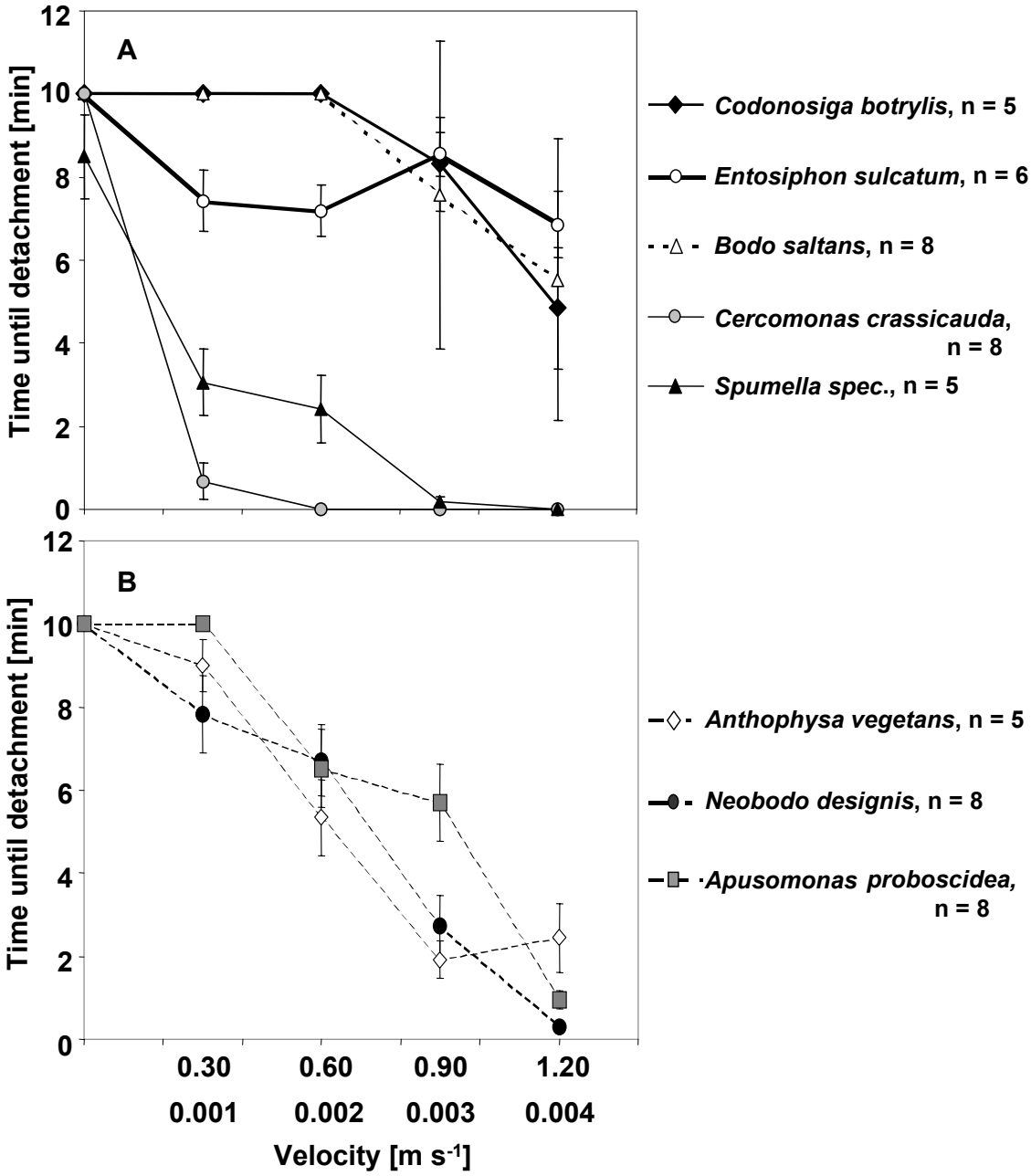

Fig. 3. Time until detachment of 8 different heterotrophic nanoflagellates at different flow velocities. The upper line of the abscissa gives the disc velocity (5 mm above the substrate), while the lower line shows the corresponding flow velocities above the substrate (0 to 10 µm). The maximum investigation time was 10 min. A: *Codonosiga botrylis, Entosiphon sulcatum, Bodo saltans, Cercomonas crassicauda* and *Spumella sp.* B: *Anthophysa vegetans, Neobodo designis, Apusomonas proboscidea*

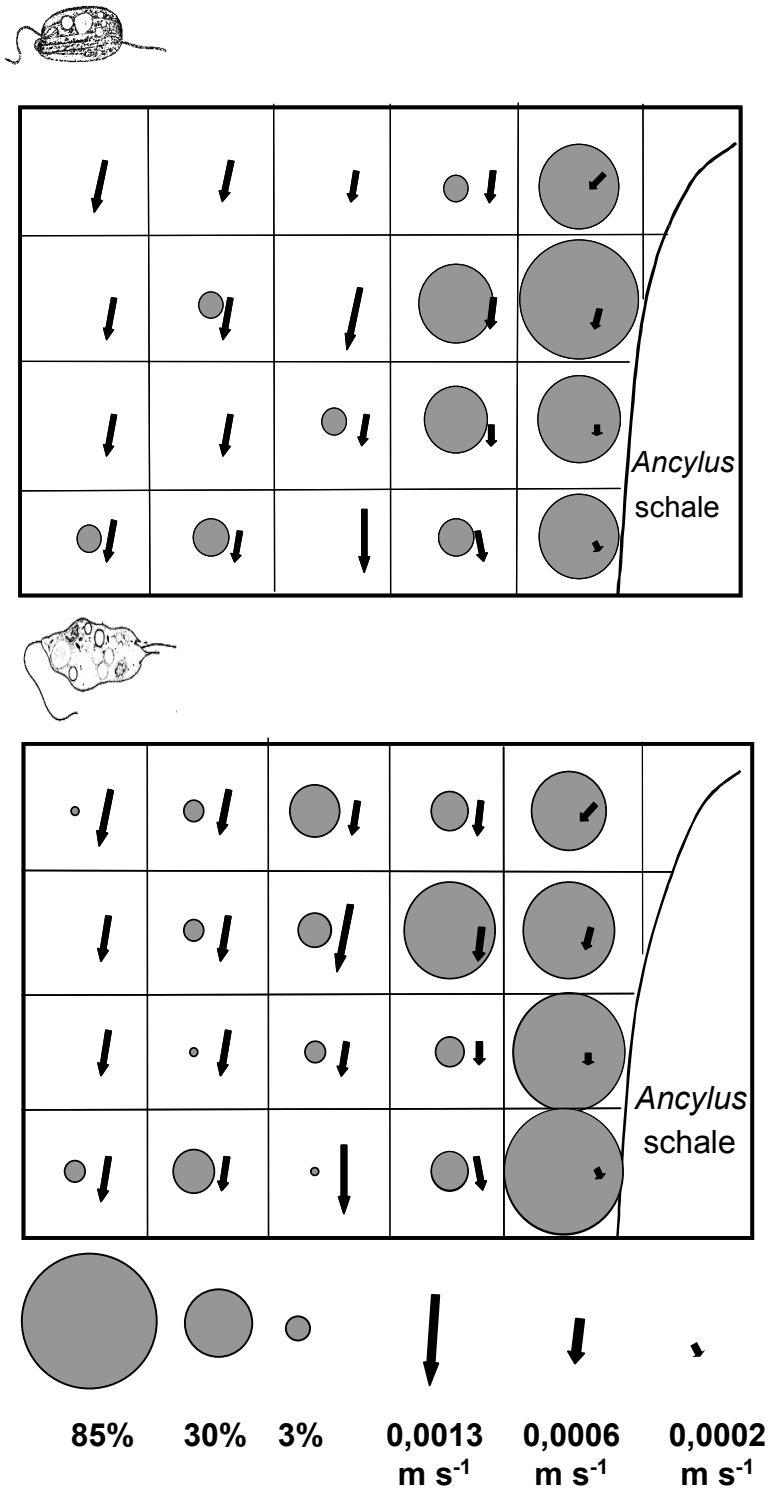

Fig. 4. (A) *Entosiphon sulcatum* and (B) *Cercomonas crassicauda*. Distribution behind an *Ancylus* shell exposed to flow for 10 min at a disc velocity of 0.3 m s^{-1} (mean of 4 experiments for both species). The diameter corresponds to the mean abundance in the fields of observation (92 x 100 µm). The arrows indicate the direction of the water current and the flow velocity above the substrate (in the 0 to 10 µm layer)

DISCUSSION

We used glass slides as an artificial substrate in order to compare the different behaviour of heterotrophic nanoflagellate species. The addition of snail shells gave an idea of the potential effects of natural micro-topographies on biofilms.

It is known that boundary layer flow characteristics in the sub-millimetre range may control pathways and magnitudes of material and gas exchange in the surface micro-layer of sediments which depend on the topography of the sediment surface (e.g. de Beer et al. 1994, 1996, Huettel et al. 2003, Røy et al. 2005). Even today, only very few studies have considered the effects on microfauna (e.g. Jakobsen 2001, Vopel et al. 2002, 2005) and the influence of flow velocity on nanofauna (< 20 µm) has not yet been studied. Our microscopic technique gave us the opportunity to study the effect of flow on heterotrophic nanoflagellates in the laminar layer at intermediate Reynolds numbers (0.91 to 12.42; e.g. Smith 1975, Vogel 1981). We found significant effects on the attachment of heterotrophic flagellates already at very low flow velocities directly above the substrate (0.0003 to 0.004 m s^{-1}). We hypothesised that when such small flow velocities affect the occurrence of different nanoflagellates, small changes in the surface topography significantly influence the distribution patterns of flow-sensitive flagellates. Our experiments with *Entosiphon* and *Cercomonas* supported this hypothesis.

Another important result was that flow speeds of only a few millimetres s^{-1} had significantly different effects on the attachment of diverse flagellate species. Heterotrophic nanoflagellates may differ significantly with regard to their ecology and behaviour (Fenchel 1987, Arndt et al. 2000). The large *Cercomonas* species showed the lowest resistance towards the flow. This species lives generally within thick sediment layers and is not adapted to attach closely to the surface. *Spumella* has a low resistance as well. The thin plasmatic thread by which the single cells are attached to the substratum is suitable for attaching to suspended particles, but it does not allow withstand strong turbulence well. This is in agreement with observations of field biofilms (e.g. from rivers: M. Willkomm unpubl. data), where *Spumella* forms only a minor part of the biofilm community, in contrast to its major contribution to the

plankton community. The calculation of drag according to Silvester & Sleigh (1985) revealed a force of 1.6 x 10^{-15} Nm to be strong enough to detach *Spumella* from surface, while *Bodo saltans* seems to be adapted to withstand a 2 to 3 times higher drag force. The other investigated colourless chrysomonad *Anthophysa* is much better protected from high flow velocities. Colonies are attached via a flexible, excreted, extracellular polymer matrix. Though the flow velocity increases with increasing distance from the substrate, colonies are pressed by the flow towards the substrate, thus avoiding high flow velocities. Similar behaviour was described by Vopel et al. (2005) for peritrich colonies; when colonies are disrupted by high flow velocities, whole colonies or single cells break free and swim individually in the water column until they find more suitable habitats for attachment. *Codonosiga*, the other colony forming flagellate, has a much more rigid stalk made of proteinaceous substances, allowing this choanoflagellate species to withstand even high flow velocities. This species regularly populates biofilms of rivers in high densities (M. Willkomm & M. Esser unpubl. data). The speciality of the kinetoplastid *B. saltans* is its strong attachment by the tip of the posterior flagellum, which explains its attachment even at relatively high flow velocities. This species occurs at extremely high turbulences due to artificial aeration in waste water treatment plants. The colourless, gliding euglenid *Entosiphon* showed the highest resistance towards high flow velocities. It has a dorsoventrally flattened pellicle and has a shape reminiscent of the body shape of torrenticolous insects. The shape seems to be optimised for a current-sheltered life on surfaces.

Statzner (1981) and Statzner & Holm (1989) characterised the flow field around shells of *Ancylus fluviatilis,* a typical rheophilous organism of running waters (Elser 1999). Our experiments in the microscale showed that there is still water motion behind the shells, though at very low flow velocities. However, even these low flow velocities (0.0002 to 0.002 m s^{-1}) lead to characteristic micro-distributions of *Entosiphon* and *Cercomonas* accumulating in the region with lowest flow velocities (0.0002 - 0.0005 m s^{-1}). We conclude that the structure of biofilms and specific flow fields around objects and organisms inside the laminar flow region (e.g. sand grains, invertebrates, 3-dimensional structures of bacterial and/or algal biofilms) influences the micro-distribution and community structure of nano- and microfauna communities. We observed a

behaviour typical of *Entosiphon* and *Cercomonas* in relationship to the flow velocity: at high flow velocities, the flagellates actively searched for areas of low flow velocity. Furthermore, in the absence of structures, both flagellates stopped movement at high flow velocities until detachment. For planktonic ciliates, it is known that they may react to flow velocities created, e.g. by the filter current of copepods (Jakobsen 2001).

The bulk of microbial activity in streams occurs on biofilms (Bryers 1982), and colonisation processes seems to be strongly influenced by surface micro-topography, which, in turn, should govern the community structure of nano- and microfauna in lotic environments. Understanding the interactions between physical and biological processes in the micro-scale seems to be an important prerequisite in order to analyse microbial life in the water.

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CHAPTER VI:

NON-PREDATORY INDUCED LYSIS OF HETEROTROPHIC FLAGELLATES: PARAMETER ESTIMATES OF POPULATION DYNAMICS OF THE EUGLENID *ENTOSIPHON SULCATUM* AT DIFFERENT TEMPERATURES

ABSTRACT. Estimates of the mortality of Protozoa are difficult since most aloricate forms burst at the moment of dying and cannot be recognized after death. Several causes of mortality of heterotrophic flagellates may occur in the field. Generally, only the mortality due to predation is considered. Here we show that there may be a considerable non-predatory mortality. We studied all parameters of population dynamics of the euglenid *Entosiphon sulcatum* (rates of division, growth and mortality) at eight different temperatures ranging from 0 to 40 °C. *Entosiphon* served as a model organism using the remaining empty pellicle as an indication of mortality and lysis events, so mortality could be calculated independent of the growth rate. *Entosiphon* showed continuous division at all temperatures. Especially at high temperatures the division rates were much higher than the growth rates. The mortality of *Entosiphon* lay between 0 to 7% per day at 0 – 25 °C and increased up to 18 - >1000% per day at 30 - 40 °C. Life spans of *Entosiphon* ranged between >30 days at 0 °C and less than three hours at 40 °C. Most heterotrophic flagellate populations should suffer from a significant non–predatory mortality. Production estimates based on determinations of growth rates should underestimate the real turnover rates of heterotrophic flagellates.

Key Words: Division rate, growth rate, life span, mortality, protozoa.

INTRODUCTION

In contrast to studies on the taxonomy, distribution and feeding ecology of heterotrophic flagellates, the knowledge about the population ecology of heterotrophic flagellates is still limited (e.g. Fenchel 1982 a, b; Goldman and Caron 1985; Berninger et al. 1991; Burkholder and Glasgow 1997; Arndt et al. 2000). Despite the importance of heterotrophic flagellates for the functioning of aquatic ecosystems (e.g. Azam et al. 1983) population dynamics are not well understood. To our knowledge, there are no data available on mortality and division rates for heterotrophic flagellates. It is assumed that protozoa multiply by division into two daughter cells and production is generally estimated from the growth rate. If there would be no mortality this procedure would give reliable estimates (Fenchel 1968, 1974). However, as for metazoans, mortality of heterotrophic flagellates should not only be due to predation but also due to parasitism and viral lysis, as well as cell death due to unfavourable abiotic (e.g. temperature, pressure, oxygen concentration) and biotic conditions (e.g. food concentration and food quality).

Population ecological studies of metazoans would never ignore mortality as an important factor of population loss. Remains of dead animals are easily to be recognised and production estimates are often based on growth-rate independent estimates (e.g. egg number, increase of body weight). Why should mortality of protists be ignored? Many laboratory data on population dynamics in the absence of predators, even the very classical ones, clearly indicate the occurrence of population declines and point to a non-predatory loss.

Encystment could be one reason for a loss of protist cells in populations. For planktonic ciliates, seasonal changes in encystment rates were found under field conditions (e.g. Müller and Wünsch 1999). Only very little quantitative information on cyst formation and hatching are available for heterotrophic flagellates (e.g. Leadbeater and Karpov 2000). Another factor of population loss is non-predatory mortality. This type of loss has not yet been considered as a factor of population loss in heterotrophic nanoflagellate populations. This is probably due to the fact that most nanoflagellates may disintegrate during lysis leaving no remains. For ciliates and testaceans, Schönborn (1977, 1982 a, b, 1992) has estimated mortality under field conditions and obtained values of

non-predatory mortality of 0 - 10% d^{-1}. If there is significant mortality of growing flagellate populations, production estimates based on growth rates would considerably underestimate real production.

The aim of the present study was to quantify the rate of non-predatory mortality (= all kinds of mortality except predation) of heterotrophic flagellate population dynamics. *Entosiphon sulcatum* (Euglenida) was used as a model organism, since direct determinations of the mortality are possible due to the fact that this species possesses a thick pellicle (cell cortex stabilised by proteins, Triemer and Farmer 1991) that remain after death.

MATERIALS AND METHODS

Study organisms. The euglenid *Entosiphon sulcatum (DUJARDIN) STEIN* (kindly provided by the Culture Collection of Algae and Protozoa, CCAP-No.1220/1A, Windermere, UK) was used as a model organism for the experiments. The monoxenic cultures of the phagotrophic *Entosiphon* were kept in SPL-medium (Sigma cereal leaf – Prescott Liquid medium) prepared either of 1.0 g Cerophyll (Sigma, C-7141) or 1.0 g dried nettle powder in 1.0 l PJ-solution (Prescott`s & James's Solution). PJ-solution was prepared of three stock solutions: 1) 0.433 g / 100 ml $CaCl_2$+$2H_2O$ and 0.162 g / 100 ml KCl, 2) 0.512 g / 100 ml K_2HPO_4 and 3) 0.280 g / 100 ml $MgSO_4$+$7H_2O$. An equal amount of each stock solution (1 ml) was added to 1 litre of distilled water. The mixtures of Cerophyll and the PJ-solution were boiled for five minutes. The solution was then filtered through a folded filter paper (Whatman, 2V) and filled up to the original volume with distilled water. The medium was autoclaved before inoculation.

Experimental set up. Changes in the numbers of living flagellates and pellicles were used to calculate the growth rate and mortality, respectively. The population dynamics of *Entosiphon* were investigated at eight temperatures. The experiments were carried out either in tissue culture plates (96 – well flat bottom suspension cells; 5, 10, 20 and 25 °C $\pm$ 0.5 °C; n = 8) or in replicate tissue culture flasks (20 ml medium; 0, 30, 35 and 40 °C $\pm$ 0.5 °C; n = 8). Abundance of living *Entosiphon* and pellicles were investigated either at an

inverted microscope (Zeiss Axiovert S 100, magnification 200X; 96 well cells) or in 3 µl subsamples (tissue culture flasks) at an upright microscope using phase contrast optics (Zeiss Axioskop, magnification 200X).

Experiments were started by transferring inocula from *Entosiphon* cultures (15 °C long-term adaptation) to 0, 5, 10, 20, 25, 30, 35 and 40 °C (± 0.5 °C), respectively. Cultures were investigated in the course of several days except for exposures at 40 °C where counting was carried out every 1.5 hours due to the short life span of organisms.

Entosiphon received a surplus food concentration of macrophyte detritus offering the SPL-medium (non-food limited culture). Population growth rates were investigated for *Entosiphon* cultures receiving a daily exchange of the food medium with those receiving no exchange of medium (at 20 °C). Since there was no effect on population growth rates we assumed that food was not limiting in continuous cultures. *Entosiphon sulcatum* was observed feeding preferably on the offered Cerophyll detritus, in addition co-occurring bacteria (about 10^7 bact./ml) were used as a food source in the culture vessels. All experiments were conducted in the dark. The subsamples were taken under sterile conditions every 24 hours (exception: 40 °C).

To correct the number of pellicles for those disappearing in the course of incubation due to bacterial degradation the loss rate of pellicles was estimated in tissue culture plates (n = 8) at 5, 10 and 20 °C (Fig. 1A). The loss rates estimated at these temperatures were extrapolated for the whole range of experimental temperatures (Table 1) using a polynomial equation obtained from the fitting of data ($Y = -0.0002x^2 - 0.0009x - 0.0372$; $R^2 = 0.99$; Fig. 1B). The different stages of degradation of the pellicles were not distinguished.

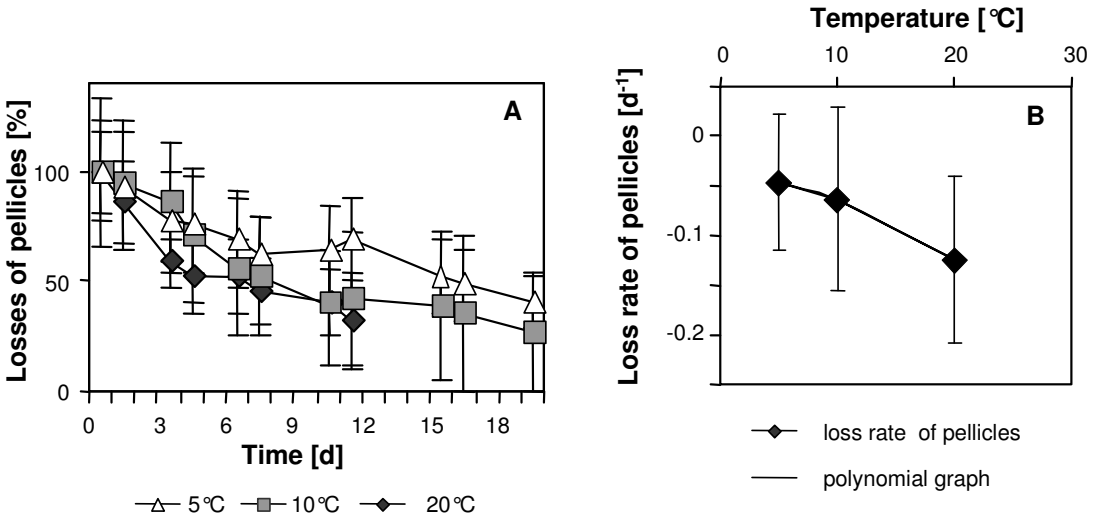

Fig. 1. Temporal changes in the losses of pellicles in culture medium free of living *Entosiphon sulcatum* at 5, 10 and 20 °C, respectively (A). Losses are given as percentages compared to the beginning of the degradation experiments (n = 8). The polynomial graph fitting to the data of loss rates is shown in the right graph (B).

Table 1. Estimated loss rates (ε, d^{-1}) of pellicles of *Entosiphon sulcatum* at different temperatures.

Temperature [°C]	0	5	10	15	20	25	30	35	40
Loss rates of pellicles [d^{-1}]	-0.04	-0.05	-0.07	-0.10	-0.14	-0.19	-0.24	-0.31	-0.39

Determination of parameters of population dynamics. The parameters were estimated assuming exponential growth, using the formula:

$$r = (\ln N_t - \ln N_0)/t,$$

where r is the intrinsic rate of increase per day, N_0 is the initial density of cells (ind. ml^{-1}) and N_t is the density of cells at time t (days) (Fenchel 1974). For the calculation of the mortality, we developed a set of equations:

$$m = (P_p/(EXP((\ln N_0 + \ln N_t)/2)/t),$$

where m is the mortality rate (d^{-1}) and P_p is the number of pellicles produced per time interval. The number of pellicles (pellicles per ml) present after the

respective time interval was corrected by the factor ε, the loss rate of pellicles due to destruction of pellicles in the time interval. This was taken from Table 1 for the respective temperature. Assuming exponential changes of pellicle numbers the losses of pellicles (P_l) were calculated by

$$P_l = ((EXP(lnP_t + lnP_0))/2)*\varepsilon ,$$

where P_0 is the number of pellicles at t_0 and P_t is the numbers of pellicles at time t. To calculate the produced pellicles (P_p) within the time interval, the number of lost pellicles was added to P_t to correct for the degraded pellicles:

$$P_p = (P_t + P_l - P_0).$$

The division rate b (d^{-1}) and generation time T (d) were calculated according to Fenchel (1968):

$$b = r + m$$

$$T = ln2/r.$$

The mortality (M) in % per day was calculated according to Schönborn (1977):

$$M = ((P_t - P_0)/(P_t - P_0 + N_t)) * 100 [\% d^{-1}].$$

Rates were calculated for the time interval for which the maximal growth rate was observed.

Calculated rates were considered different from zero when the 95% confidence intervals were above zero.

RESULTS

Parameters of population dynamics showed a similar pattern for temperatures in the range of 0 - 25 °C characterised by a low mortality throughout the period of observation and decreasing growth and division rates (Fig. 2 a-c). At 30 °C, despite high division rates at the end of the period of observation, population densities rapidly declined due to a high mortality. At 35 and 40 °C, high division rates could not compensate the high mortality.

The range of temperature tolerance was considered as the range of temperatures at which a positive growth rate (0 - 30 °C) could be observed. At 40 °C the population survived for only three hours. The maximal growth rate increased at temperatures from 0 to 25 °C (Fig. 3).

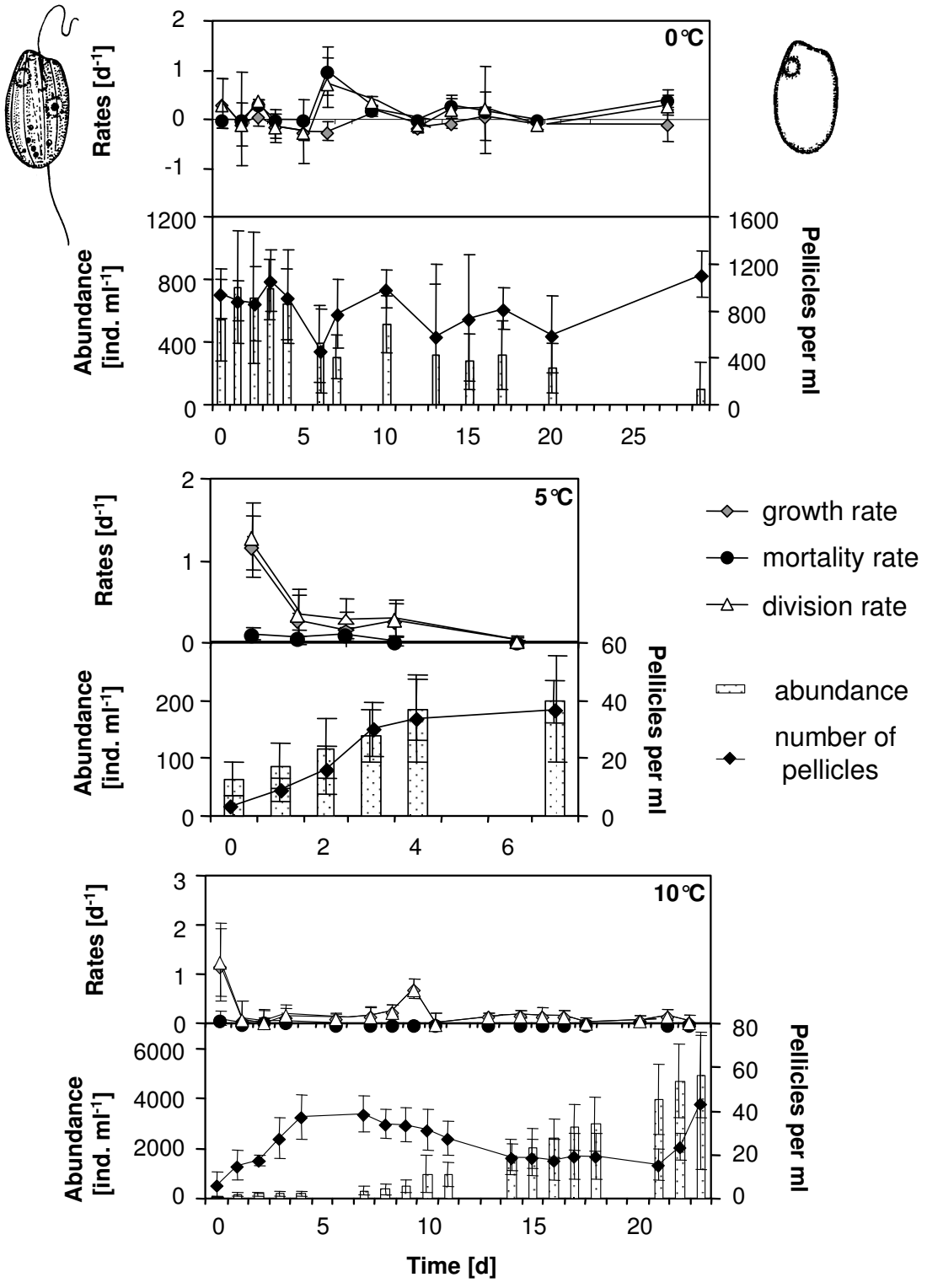

Fig. 2a. Parameters of the population dynamics of *Entosiphon* (growth rate [d^{-1}], mortality rate [d^{-1}], division rate [d^{-1}]), abundance [ind. ml^{-1}] and number of pellicles [ml^{-1}] at 0, 5 and 10 °C. Error bars indicate standard deviation (n = 8).

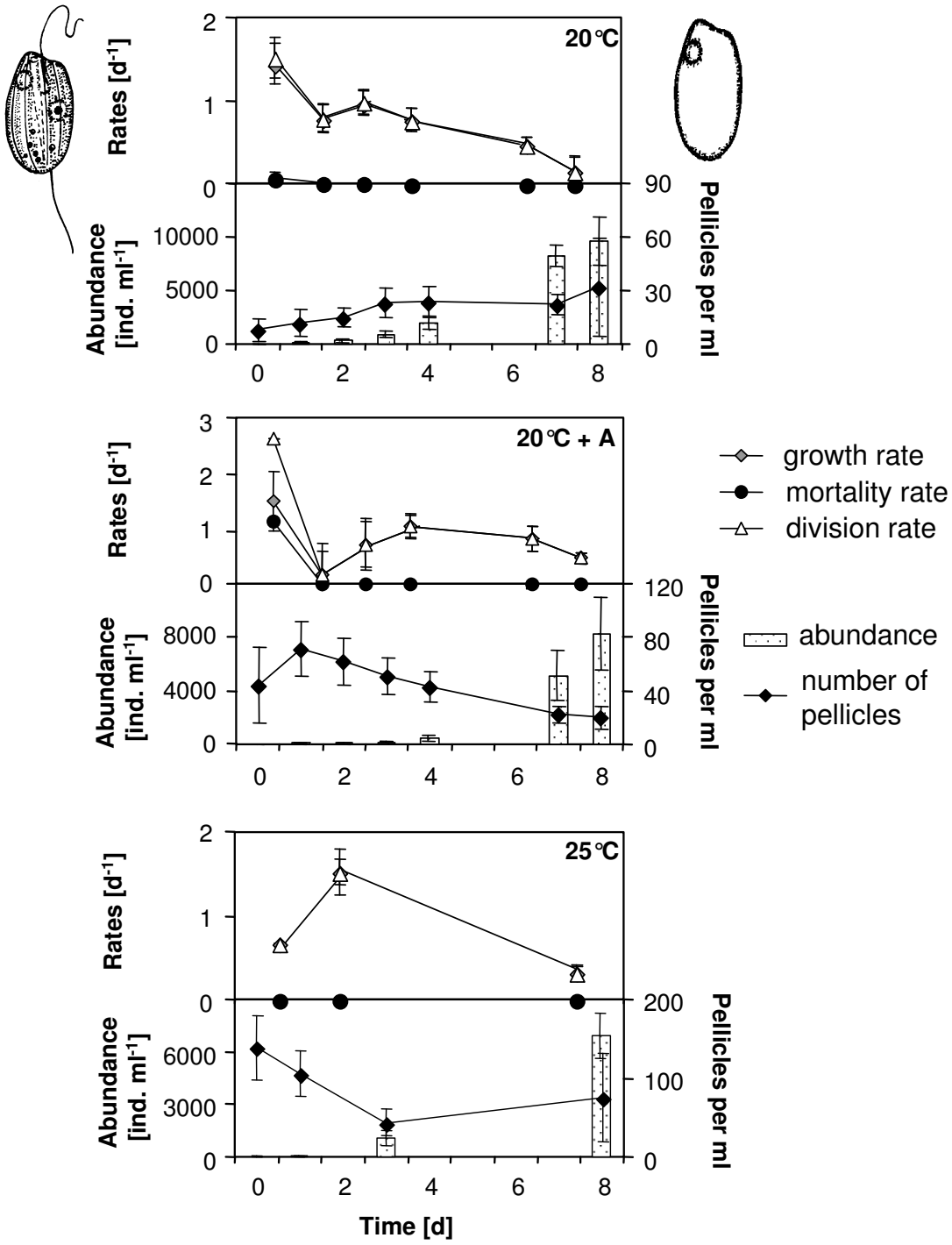

Fig. 2b. Parameters of the population dynamics of *Entosiphon* (growth rate [d^{-1}], mortality rate [d^{-1}], division rate [d^{-1}]), abundance [ind. ml^{-1}] and number of pellicles [ml^{-1}] at 20 and 25 °C. Error bars indicate standard deviation (n = 8). At 20 °C a second set of experiments (20 °C+A) was investigated with a daily exchange of medium.

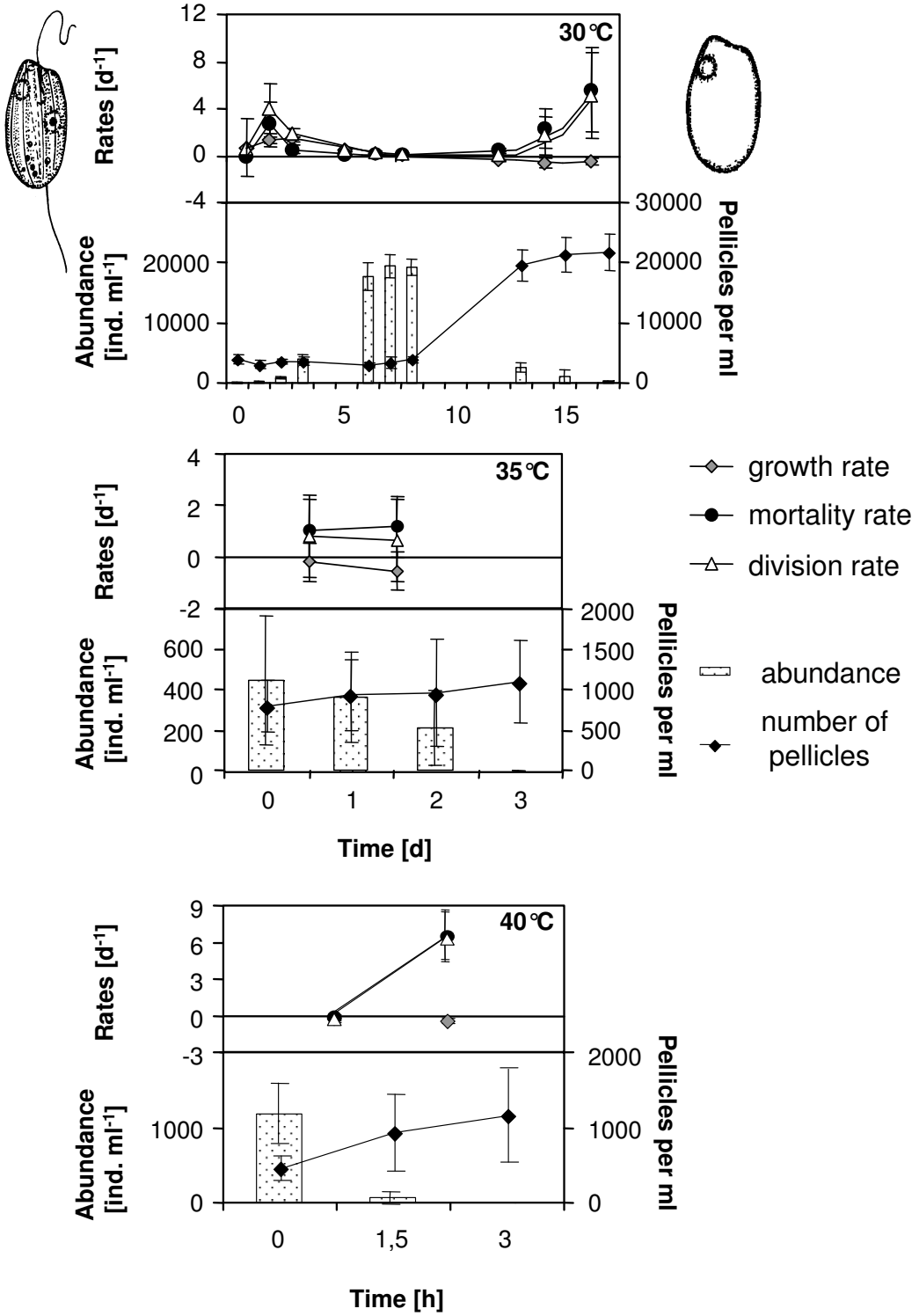

Fig. 2c. Parameters of the population dynamics of *Entosiphon* (growth rate [d^{-1}], mortality rate [d^{-1}], division rate [d^{-1}]), abundance [ind. ml^{-1}] and number of pellicles [ml^{-1}] at 30, 35 and 40 °C. Error bars indicate standard deviation (n = 8).

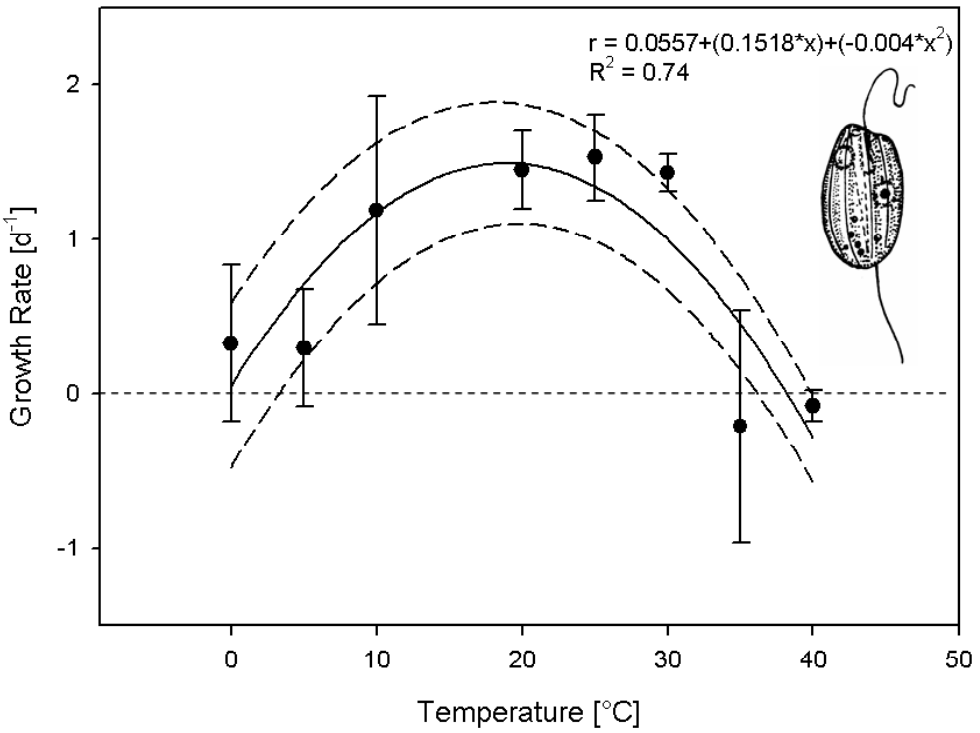

Fig. 3. The relationship between growth rate [d^{-1}] and temperature [°C] for *Entosiphon sulcatum*. The 95% confidence interval is indicated by dashed lines.

The analysis of dead organisms due to the counting of empty pellicles allowed the estimate of mortality rates and corresponding division rates (Table 2). The mortality of *Entosiphon* laid between 0 to 7% per day at 0 - 25 °C and increased to 18 - 1359% per day at 30 - 40 °C. Individual life spans were often longer than the periods of observation and reached a life span of more than 30 days at 0 °C. The generation time calculated on the basis of division rates was mostly lower than the generation time traditionally calculated on the basis of the growth rate. At 0 - 30 °C, generation times calculated on the basis of division rates were on average about 10% shorter than those calculated on the basis of growth rates.

Table 2. Summary of the parameters of population dynamics of *Entosiphon sulcatum* at 0, 5, 10, 20, 25, 30, 35 and 40 °C ± 0.5 °C. Rates and mortality were calculated for the interval of maximum growth rate (mean ± 95% C.I.). The generation time T_r was estimated on the base of the growth rate whereas the generation time T_b was calculated on the base of the division rate.

	0°C	5°C	10°C	20°C	25°C	30°C	35°C	40°C
Observation time [d]	>30	>7	>23	>8	>8	>21		
Extinction time of the population [d]							3	0.125
Max. growth rate [d^{-1}]	0.33±0.35	0.30±0.26	1.19±0.51	1.45±0.17	1.53±0.19	1.43±0.08	-0.21±0.52	-0.08±0.07
Division rate [d^{-1}]	0.33±0.35	0.38±0.28	1.29±0.52	1.52±0.17	1.53±0.10	1.94±0.26	0.81±1.09	-0.01±0.54
Mortality rate [d^{-1}]	0	0.08±0.05	0.10±0.10	0.07±0.04	0	0.51±0.25	1.00±0.83	0.07±0.55
Mortality [% d^{-1}]	0	7.01±3.33	7.25±5.25	3.53±1.89	0	18.06±9.15	31.09±41.74	1359±234
Generation time T_r[h]	51.2	54.7	14.0	11.5	10.9	11.7	---	---
Generation time T_b[h]	51.2	43.8	12.9	10.9	10.9	8.6	20.5	---

DISCUSSION

Population ecology. The major aim of this study was to determine non-predatory mortality rates for a heterotrophic flagellate. Despite the fact that Schönborn (1977, 1982 a, b, 1992) has determined mortality rates of ciliates and testaceans under field conditions of up to 10% d^{-1}, it seems that our study is the first for a heterotrophic flagellate where the mortality rate could be calculated independent of the growth rate. In most studies except for a few short-term investigations (e.g. Holen and Boraas 1991; Boenigk 2002) mortality could not be separated from growth. The fact that most heterotrophic flagellates generally burst at the moment of dying (e.g. Matz et al. 2004) makes the measurement of mortality very difficult. The euglenid *Entosiphon sulcatum* was

selected as a model organism to investigate non-predatory mortality, because the cell cortex of pellicles of most euglenids is stabilised by proteins (Triemer and Farmer 1991) and remains after death. We considered only complete pellicles in the counts to be sure that they were just in the beginning of their degradation.

Schönborn (1977, 1982a, b, 1992) interpreted the numerous empty, undamaged shells of testaceans and loricate ciliates appearing in experimental studies as the result of non-predatory mortality. Predators would have destroyed the shells (Schönborn 1992). Using the equations of Schönborn (1992), we have estimated the mortality for *Entosiphon* of up to about 7% d^{-1} for a temperature range from 0 to 25 °C. This is comparable to published data for other protists (see Table 3). At higher temperatures (30 – 40 °C) the mortality of *Entosiphon* increased from 18 to more than 1000% d^{-1}. According to a comparison of the observed non-predatory mortality rates of *Entosiphon* with estimates of predatory mortality in field populations of flagellates in temperate lakes (e.g. Weisse 1991; Arndt et al. 2000) non-predatory mortality might be of considerable importance for population dynamics of flagellates especially during late summer and autumn.

Under laboratory conditions we could exclude predator induced mortality, however, the importance of the mortality caused by parasites (e.g. bacteria and viruses) could not be determined. For future investigations, it would be desirable to develop techniques which allow statements about the different causes of mortality.

Table 3. Mortality estimates of protozoans under field and laboratory conditions.

Organisms and habitat	Mortality (% d^{-1})	Author
Heterotrophic flagellates		
Entosiphon sulcatum, laboratory studies		this study
0°C	0	
5°C	7.0	
10°C	7.3	
20°C	3.5	
25°C	0	
30°C	18.1	
35°C	31.1	
40°C	1359	
Testaceans		
River Saale, α-mesosaprobic		
Slides	5.0	Schönborn (1977)
In mosses (*Mnium cuspidatum)*	3.1	
Soil, moder, only Euglyphidae		
Beech forest	6.1	Schönborn (1975)
Coniferous forest	6.9	
Soil, moder-raw humus (aspen woodland)		
Litter	10.0	Lousier & Parkinson (1984)
Humus	10.9	
Soil (*Fraxinus-Acer*)		
Mull	8.5	Schönborn (1982b)
Soil, raw humus, *spruce (Picea)* forest		
Litter and fragmented litter	8.8	Schönborn (1986)
Humus	11.0	
Ciliates		
River Saale, slides, α-mesosaprobic site		
Non-loricate species	6.0	Schönborn (1982a)
Loricate species	5.0	

Temperature tolerance. *Entosiphon* tolerated a range of 0 to 30 °C. The general pattern of temperature tolerance observed for *Entosiphon* is typical for many organisms: population growth rate increases with temperature, reaches a maximum and may remain relatively constant at intermediate temperatures, and is then reduced at high temperatures (e.g. Lee and Fenchel 1972; Wickham and Lynn 1990; Choi and Peters 1992). Unfortunately, there are only a very few studies on the temperature tolerance of heterotrophic flagellates. Choi and Peters (1992) found a clonal variation in temperature adaptation of strains of *Paraphysomonas* isolated from arctic and temperate regions. Boenigk et al.

(2006) investigated chrysomonads of the genus *Spumella* and documented a correlation of the maximum tolerated temperate with the ambient temperature of the original habitat, with Antarctic isolates forming an exception to this rule. The range of temperatures tolerated by various protozoa seems to lie between –2 and ~50 °C (Finlay 1990). There are a few records of protozoa living above 50 °C. But there is no evidence except for that of Dallinger (1887) that they can grow and divide at these high temperatures (Kahan 1972; Nisbet 1984). One thermophilic ciliate grows well up to a temperature of 52 °C (Baumgartner, Stetter, and Foissner 2002). The optimal temperatures allowing highest population growth rates were between 20 and 30 °C in our study of *Entosiphon*. The temperature optimum of most freshwater protozoan seems to lie in the same range. Several freshwater ciliates have a temperature optimum from 25 - 32 °C (e.g. Pheps 1956; Prescott 1957; Fenchel 1968), the amoeba *Vannella sp.* grows best at 20 - 25 °C (Baldock, Baker, and Sleigh 1980).

Entosiphon showed positive growth rates or at least a long life span even at temperatures of only 0 °C. This seems to be typical for many heterotrophic flagellates. Psychrophilic strains of the flagellate *Paraphysomonas* could grow even at temperatures below zero (Choi and Peters 1992). Field studies revealed a high diversity and high abundance of flagellates during winter under ice (e.g. Dietrich and Arndt 2000).

A problem that has not yet been addressed in the present investigation is the influence of short-term fluctuations of temperature which was shown to play a significant role in ciliates (e.g. Lee and Fenchel 1972; Fenchel 1987; Weisse and Montagnes 1998). Montagnes and Weisse (2000) investigated the growth rates of planktonic ciliates under daily fluctuating temperatures in laboratory experiments indicating positive effects on the growth rates for some ciliate strains, but negative effects for others. The estimates of growth and mortality rates in experiments with a one-week adaptation to either 5 °C or 15 °C and a following stepwise acclimation to the experimental temperature did not show any deviation from the data presented here (MW. and HA., unpubl. data). This may support the idea that *Entosiphon* is well adapted to the temperature range from about 0 - 25 °C.

Consequences for production estimates. The direct determination of the non–predatory mortality of *Entosiphon* in relation to temperature showed

that this part of mortality can play a significant role for the population dynamics of this flagellate species, especially at high temperatures. Normally the production of heterotrophic flagellates or other protozoa is estimated using determinations of growth rates (Fenchel 1968). However, the studies of Schönborn (1992) for some ciliates and testate amoebae and our study on a heterotrophic flagellate indicate that determinations of temporal changes in densities of individuals or biomass make production estimates difficult as long as the mortality is unknown. At all temperatures, *Entosiphon* showed continuous division. At higher temperatures the division rates were much higher than the growth rates which may lead to a significant underestimation of production. Probably, all flagellate populations suffer from a significant non–predatory mortality. Thus, production estimates based on determinations of growth rates should underestimate the values of the real turnovers of heterotrophic flagellates. Food web models require mortality estimates of heterotrophic flagellates for a better understanding of their role in the carbon transfer (e.g. Baretta-Bekker, Baretta, and Koch 1995).

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ABSTRACT

The importance of biofilms for the self-purification of streams and the high availability of nutrients in biofilms was recognised during the last decades. The knowledge of the colonisation of microorganisms (rotifers, protozoans, bacteria) on biofilms, especially of heterotrophic flagellates, is still limited. Until now most studies are dealing with ciliate communities or determine only the abundance of heterotrophic flagellates but not the taxonomic diversity on riverine biofilms.

One important factor determining the colonisation process of microorganisms is the flow velocity. In undisturbed rivers pool and riffle sections are created by different flow velocity conditions due to a heterogenic substrate at the river bed. An anthropogenic enlargement of these sections, especially pool sections, is caused by weirs. Another important parameter influencing the dynamics of microorganisms (e.g. growth rate, division rate and mortality) in biofilms is the temperature which may influence the colonisation speed and carrying capacity on riverine biofilms, too. Detailed investigations on the dynamics of biofilm-dwelling heterotrophic flagellates are still scarce even though these organisms often show the highest abundance compared to other protozoans on riverine biofilms.

Field and laboratory experiments were conducted to answer the question on how colonisation and dynamics of heterotrophic flagellates are affected by environmental parameters. Different flow conditions (reservoir and outflow) were created by weirs in field experiments (River Ilm, Thuringia, Germany). The abundance and diversity of heterotrophic flagellates were determined during short-term (between 1 and 14 days) and long-term (14 days) exposures under these different flow velocity conditions.

For a detailed insight in the question on how flagellates are affected by different flow velocities (between 0 and 1.2 m s^{-1}) a new method was developed for direct observations under laboratory conditions. In a second series of laboratory experiments, the impact of temperature on the population dynamics of the benthic heterotrophic flagellate, *Entosiphon sulcatum*, was investigated. Here, a new method was designed to directly determine the mortality of this flagellate. The euglenid *Entosiphon* was chosen as a model organism due to the fact that the thick pellicle of this species remains after death and its cell does not burst like the other protozoans.

The field investigations revealed that the abundance of heterotrophic flagellates was higher in a riffle section than at both weir sites of a river. At flow velocities higher than 0.5 m s^{-1}, abundances of heterotrophic flagellates were significantly lower than abundances at velocities under 0.5 m s^{-1}. In pool sections as well as in riffle sections, ten groups of heterotrophic flagellates were observed during the field experiments (Ancyromonadida, Cercomonadida, Choanoflagellida, Chrysomonadida, Euglenida, Kinetoplastida, Thaumatomonadida, Apusomonadida, Ciliophryida and Cryptomonadida).

In the laboratory experiments regarding the behaviour of flagellates under different flow velocities, *Entosiphon sulcatum* (a gliding euglenid) had the highest resistance towards higher flow velocities. *Cercomonas crassicauda* (crawling cercomonad) showed the weakest resistance. Laboratory experiments confirmed the field observations regarding the impact of flow velocity on the different morphotypes of heterotrophic flagellates.

Investigations of population dynamics regarding the model organism (*Entosiphon*) revealed a continuous division at temperatures ranging between 0 and 40°C. The division rate at higher temperatures between 25 and 40°C was much higher than the growth rate. This may lead to a significant underestimation of production due to high mortality.

The results of this thesis show that flow velocity as well as temperature may influence the colonisation and population dynamic parameters of biofilm-dwelling heterotrophic flagellates. These studies may serve as first steps for a better understanding of the dynamics of heterotrophic flagellates on riverine biofilm.

KURZZUSAMMENFASSUNG

Die Bedeutung der Biofilme für die Selbstreinigung von Flüssen und die hohe Verfügbarkeit von Nährstoffen in Biofilmen wurde während der letzten Jahrzehnte erkannt. Das Wissen über die Besiedlung der Mikroorganismen auf Biofilmen, besonders durch heterotrophe Flagellaten, ist immer noch begrenzt. Bis jetzt beschäftigen sich die meisten Untersuchungen mit der Ciliaten-gemeinschaft oder ermitteln nur die Abundanz der heterotrophen Flagellaten, aber nicht die taxonomische Diversität auf den Biofilmen in Fließgewässern.

Ein wichtiger Faktor, der den Besiedlungsprozess der Mikroorganismen bestimmt, ist die Fließgeschwindigkeit. In ungestörten Fließgewässern werden Pool- und Riffle-Bereiche durch die unterschiedlichen Fließgeschwindigkeiten, aufgrund heterogenes Substrat auf dem Flussbett, geschaffen. Eine anthropogene Vergrößerung dieser Bereiche, besonders der Pool-Bereiche, wird durch Wehre verursacht. Ein anderer wichtiger Parameter, der die Dynamiken der Mikroorganismen (z.B. Wachstumsrate, Teilungsrate und Mortalität) auf den Biofilmen beeinflusst, ist die Temperatur, die auch die Besiedlungsgeschwindigkeit und das Fassungsvermögen von Biofilmen in Fließgewässern beeinflussen kann. Detaillierte Untersuchungen der Dynamiken der auf den Biofilm lebenden heterotrophen Flagellaten sind noch immer selten, obwohl sie im Vergleich mit anderen Protozoan auf Biofilmen im Fließgewässer häufig die höchste Abundanz zeigen.

Feld- und Laborexperimente wurden durchgeführt, um die Frage zu beantworten wie die Besiedlung und Dynamiken von heterotrophen Flagellaten durch Umweltparameter beeinflusst werden. Verschiedene Fließ-geschwindigkeiten wurden durch Wehre (Staubereich und Ausfluss) in den Feldexperimenten verursacht (Ilm, Thüringen, Deutschland). Die Abundanz und Diversität der heterotrophen Flagellaten wurden während einer Kurzzeit- (zwischen 1 und 14 Tagen) und einer Langzeit- (14 Tage) Exponierung unter diesen unterschiedlichen Fließgeschwindigkeiten bestimmt.

Für einen detaillierten Einblick zu der Frage, wie die Flagellaten durch unterschiedliche Fließgeschwindigkeiten (zwischen 0 und 1,2 m s^{-1}) beeinflusst werden, wurde eine neue Methode zur Direktbeobachtung unter Laborbedingungen entwickelt. In einer zweite Serie von Laborexperimenten wurde der Einfluss der Temperatur auf die Populationsdynamiken des benthischen, heterotrophen Flagellaten, *Entosiphon sulcatum*, untersucht. Hier

wurde eine neue Methode zur Direktbestimmung der Mortalität des Flagellaten entwickelt. Die Euglenide, *Entosiphon*, war als Modelorganismus ausgewählt worden, da die starke Pellikula von dieser Art nach dem Absterben zurückbleibt und die Zelle nicht platzt wie bei den anderen Protozoan.

Die Felduntersuchungen zeigten auf, dass die Abundanz der heterotrophen Flagellaten im Riffle-Bereich höher waren als an beiden Wehrseiten in dem Fluss. Bei Fließgeschwindigkeiten über 0,5 m s^{-1} waren die Abundanzen der heterotrophen Flagellaten signifikant niedriger als die Abundanzen bei Geschwindigkeiten unter 0,5 m s^{-1}. Sowohl in den Pool-Bereichen als auch in den Riffle-Bereichen wurden zehn Gruppen von heterotrophen Flagellaten während der Feldexperimenten beobachtet (Ancyromonadida, Cercomonadida, Choanoflagellida, Chrysomonadida, Euglenida, Kinetoplastida, Thaumatomonadida, Apusomonadida, Ciliophryida und Cryptomonadida).

In den Laborexperimenten hinsichtlich dem Verhalten von Flagellaten unter verschiedenen Fließgeschwindigkeiten, hatte *Entosiphon sulcatum* (eine sich gleitend bewegende Euglenide) die höchste Resistenz gegen höhere Fließgeschwindigkeiten. *Cercomonas crassicauda* (kriechende Cercomonade) zeigte die niedrigste Resistenz. Die Laborexperimente bestätigten die Feldbeobachtungen bezüglich des Einflusses der Fließgeschwindigkeit auf die verschiedenen Morphotypen heterotropher Flagellaten.

Die Untersuchungen der Populationsdynamiken hinsichtlich des Modellorganismus (*Entosiphon*) zeigten eine kontinuierliche Teilung bei Temperaturen, die sich zwischen 0 und 40°C bewegten, auf. Die Teilungsraten bei hohen Temperaturen zwischen 25 und 40°C waren viel höher als die Wachstumsraten. Dies kann zu einer signifikanten Unterschätzung der Produktion wegen der hohen Sterblichkeit führen.

Die Ergebnisse dieser Doktorarbeit zeigen, dass sowohl die Fließ-geschwindigkeit als auch die Temperatur die Besiedlung und die populationsdynamischen Parameter der auf den Biofilmen lebenden, heterotrophen Flagellaten beeinflussen können. Diese Studien können als erste Schritte für ein besseres Verständnis der Dynamiken der heterotrophen Flagellaten auf Biofilmen in Fließgewässern dienen.

Kooperationspartner:

Für die Auswertung in der vorliegenden Arbeit wurden teilweise Daten Dritter zur Verfügung gestellt bzw. Daten mit Hilfe Dritter erhoben. Dies war im einzelnen:

- Die Durchflussrate der Ilm wurde durch das Staatliche Umweltamt Erfurt routinemäßig an der Messstation Gräfinau-Angstedt ermittelt.

Erklärung

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit - einschließlich Tabellen, Karten und Abbildungen -, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie - abgesehen von den auf der folgenden Seite angegebenen Teilpublikationen - noch nicht veröffentlicht worden ist sowie, dass ich solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde.
Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Hartmut Arndt betreut worden.

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Teilpublikationen:

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Anteil an der 2. Und 3. Publikation:

Zu 2.: Die Publikation entstand im Rahmen des Graduiertenkollegs „Funktions- und Regenerationsanalyse belasteter Ökosysteme“, 266/2, an der Friedrich-Schiller-Universität Jena. Meine Ergebnisse zu den Untersuchungen der heterotrophen Flagellaten auf den Biofilmen wurden in die Publikation eingebunden. Bei der Erstellung des Konzeptes der Publikation habe ich mit geholfen und auch Literatur eingebracht. Alle Versionen wurden Korrektur gelesen.

Zu 3.: Die Hauptuntersuchungen wurden von mir durchgeführt. Die Publikation wurde auch von mir geschrieben. Annette Schlüssel und Ellen Reiz erhoben während eines Praktikums, welches von mir betreut wurde, die Daten zur Verteilung von *Cercomonas crassicauda*, mit und ohne Beeinflussung der *Ancylus*-Schale, und *Entosiphon sulcatum*, ohne Beeinflussung der *Ancylus*-Schale.

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