Colonization dynamics and grazing activity of ciliates in stream biofilms

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Ganz unerwartet geraten wir Abenteurer in einen ungeheuren, wimmelnden Mikrobenschwarm. Unzählige Bakterien und kleine Urtiere kriechen, springen und rasen durcheinander. Manche sausen wie blitzende Geschosse, denen das Auge nicht folgen kann, andere drehen sich im possierlichen Tanz oder schrauben sich in eleganten Windungen spiralig durch die Flut. Es ist ein wildes, wirbelndes Spiel! ... Alle diese Mikroben sind hier zu einem festlichen Geschmause versammelt.

Robert Nachtwey aus „Unsichtbare Lebenswunder“ 1938

CONTENTS

INTRODUCTION

Characteristics of lotic ecosystems

Lotic ecosystems are characterized by four dimensions namely the longitudinal (upstream / downstream), lateral (channel / margins), vertical (channel / underground), and temporal exchange processes of water and nutrients (Ward 1989). The temporal scale displays the continuous unidirectional transport of water and dissolved nutrients from the spring towards the sea (Ward 1989). The importance of running waters in the hydrological cycle is obvious since they equalize the difference between evaporation and precipitation from oceans (see Allan & Castillo 2007). Physical and chemical parameters gradually change along running water systems and biotic communities respond by temporal species replacement to the given environmental conditions as proposed by the river continuum concept (RCC) (Vannote *et al.* 1980). Downstream transport limits the residence time of biodegradable particulate matter and dissolved nutrients leading to a spiraling rather than cycling of nutrients and carbon (Newbold *et al.* 1982). Hence, downstream communities profit from the inefficiency of upstream processes (Vannote *et al.* 1980).

Flow velocity in natural systems such as streams is mainly turbulent (except in the porous system of sediments). At surfaces, turbulent flow is slowed down due to the frictional resistance creating a region of exponentially decreasing flow velocity (Ambühl 1959; Oertel *et al.* 2001). The so-called boundary layer can be divided into the viscous sublayer, a region of laminar flow, situated directly above surfaces and the logarithmic layer, where flow velocity is reduced compared to bulk water flow but with a turbulent character (Dade *et al.* 2001). Boundary layer flow is not uniform but is composed of a mosaic of rolling motions (Corino & Brodkey 1969; Boudreau 2001). The thickness of the boundary layer decreases exponentially with increasing flow velocity of the bulk water. Depending on the length of the overflown area and roughness, the thickness of the boundary layer is less than 1 mm at a flow velocity of 1 m s^{-1} (Schönborn 1992a). However, water flow occurs also in biofilm voids and channels (de Beer *et al.* 1994; Stoodley *et al.* 1994).

The above mentioned river continuum concept and nutrient spiraling concept both deal with free flowing stream ecosystems. Stream regulation by dams, weirs, and straightening was necessary for discharge regulation, drinking water, and electric power supply. The serial discontinuity concept (Ward & Stanford 1983; Ward & Stanford 1995) illustrates the effect of

large, deep-release storage dams that alter discharge regime, interrupt sediment transport, and disconnect upstream / downstream habitats of stream communities (Ward & Stanford 1979; Petts 1984). On the other hand, small low head dams with a hydraulic head of < 5 m and an impounded area of < 20 ha, that are far more numerous than large dams (Poff & Hart 2002), alter local flow velocity patterns and subsequently sediment composition as well as particulate organic matter budgets. The most serious consequence for stream ecosystems is the 'barrier effect' of dams (Watters 1996; Pringle 2003). Especially migratory fishes are threatened when dams prevent them from reaching upstream habitats (Morita & Yamamoto 2002). Up to now, no investigation focused on the impact of dams on the distribution of biofilm associated ciliates. Under unfavorable conditions, ciliates are able to form cysts, which contribute to their dispersal. The question arises whether the dam itself effects ciliates distribution or rather the alteration of flow velocity and sedimentation dynamics.

Ciliates in stream biofilms

In small headwater streams, heterotrophic processes dominate due to shading and input of allochthonous matter from the riparian vegetation. Submerged leaves and driftwood will shortly be covered by biofilms initializing the decay. Biofilms, which are surface-associated communities of bacteria, protists, and micrometazoa living in a matrix of exopolymeric substances (Lock *et al.* 1984), occur at any kind of moistened interface (liquid-solid, liquid-gas). In small streams, stone surfaces serve as stationary habitats for organisms that are otherwise rapidly transported downstream due to faster flow velocities and short water residence time. Due to the low organic matter and nutrient content of stream water, biofilms are hot spots for the turnover of organic matter. The majority of bacteria and algae live attached to the streambed displaying even higher metabolic rates compared to suspended cells (Geesey *et al.* 1978; Fletcher 1986; Romaní & Sabater 1999). Heterotrophic flagellates occur within a few minutes, followed by autotrophic algae, ciliates, and amoeba (Arndt *et al.* 2004). As described for still water conditions, the attachment of protists occurs rapidly due to their chemosensory potential (Fenchel & Blackburn 1999; Fenchel 2004).

Ciliates are important in biofilms since they can reach abundances of 10^4 cells cm^{-2} (Taylor 1983; Harmsworth *et al.* 1992; Harmsworth & Sleigh 1993; Schönborn 1996; Franco *et al.* 1998; Fukuda *et al.* 2004; Ribblett *et al.* 2005; Norf *et al.* 2007). They can make up 75% of the protozoan production in biofilms (Sleigh *et al.* 1992). In chalk stream biofilms, the total annual ciliate production is of the same magnitude as that by *Gammarus* and even more as

that by fish (Sleigh *et al.* 1992). The metabolism of biofilm associated ciliates is 20 times higher than that of the macrozoobenthos (Schönborn 1992b). Ciliates contribute to the self-purification ability of streams since they utilize the bacterial biomass (Liebmann 1962). Besides bacteria, ciliates utilize also algae, flagellates, other ciliates, and even detritus particles (Parry 2004; Scherwass *et al.* 2005) and transfer carbon and energy to the meio- and macrofauna (Schönborn 1987; Bott & Borchard 1999). The carbon and energy flow is enhanced due to the stimulation of bacterial production (Sherr & Sherr 1984), and maintenance of the bacterial population in a productive state (Johannes 1965). The decomposition of leave litter, which is an important carbon pool in small streams, is enhanced in the presence of both flagellates and ciliates (Ribblett *et al.* 2005).

In small streams, studies about colonization dynamics of biofilm associated ciliates at different flow velocities are rather rare. The stabilization time considering ciliate species number is prolonged and less species occur at faster flow velocities (0.2 -0.7 m s^{-1}) in biofilms of a small mountain stream near Zagreb (Primc & Habdija 1987). In the River Rhine, ciliate abundance on glass slides exposed for 2 weeks is higher near the bottom where flow velocities were lower compared to near surface sites (Schmitz 1985). In flow channel experiments focusing on the relation between peptone mineralization and ciliate community composition, total ciliate abundance and species number correlate negatively with flow velocity (Bick & Schmerenbeck 1971; Schmerenbeck 1975). Contrastingly, the ciliate abundance in biofilms of the River Saale is not always reduced due to faster flow velocities during flood events (Schönborn 1982). Special morphological features to delay or avoid detachment might be advantageous at faster flow velocities. Besides special fixation and retention mechanisms, the torrenticole macroinvertebrates are small and dorso-ventrally flattened (Ambühl 1959). It is postulated for ciliates that a flattened cell shape in combination with the reduction of cilia, to the cell side that faces the surface, and a permanent attachment with stalk anchorage are advantageous for living in biofilms at faster flowing sites (Buitkamp 1997). However, the combination between ciliate morphotypes and colonization of surfaces at different flow velocities has rarely been studied (Ribblett *et al.* 2005). No investigation was found considering detachment of ciliate morphotypes at different flow velocities. Behavioral features such as the haptic response are described to minimize the risk of detachment from a surface and avoid drift to unknown areas (Buitkamp 1997). Positive rheotaxis, which is the oriented movement against the direction of the flow, is a strategy of invertebrates to

compensate drift. In ciliates, the proportionally modulated rheotactic behavior is a kind of adaptive response (Ricci *et al.* 1999). Few species are extensively studied while the question remains if the above described motility changes are used by a great variety of biofilm associated ciliates at faster flow velocities.

Motility and especially grazing by protists effects bacterial biofilm morphology (Lawrence & Snyder 1998; Matz *et al.* 2004; Huws *et al.* 2005; Weitere *et al.* 2005; Queck *et al.* 2006). The long time accepted opinion that bacteria are protected against grazing by biofilm formation (Costerton *et al.* 1981) has been disproved (Pederson 1990). Specific mouth structures as the cyrtopharynx of the Phyllopharyngia enables ciliates to graze on attached prey particles like bacteria and algae (Foissner *et al.* 1991; McCormick 1991; Balczon & Pratt 1996). Defense strategies of biofilm bacteria might play a significant role in bacterial survival and persistence (Matz & Kjelleberg 2005). Microcolony formation is stimulated by early biofilm colonizers (Matz *et al.* 2004; Weitere *et al.* 2005). Regarding ciliates, investigations focused mainly on the impact of the filter feeder *Tetrahymena* sp. on biofilm morphology (Weitere *et al.* 2005; Parry *et al.* 2007). Filter feeders temporally associate with surfaces to increase their clearance efficiency (Fenchel 1986) but utilize mainly suspended bacteria (Parry 2004). Since the lifestyle and food uptake of gulper feeders are strongly associated to surfaces, they might influence biofilm morphology more than the investigated filter feeders.

THESIS AIM

The project was integrated in the Graduate Research School 'Restoration and regeneration of disturbed ecosystems'. The main objective of this thesis was to investigate the impact of flow velocity, which is altered at small low-head dams, on colonization dynamics of biofilm associated ciliates. In addition, detachment, grazing activity, and behavioral changes of ciliates were studied at increased flow velocities. At slow flow velocities, protists with different feeding modes and their effect on the morphology of a bacterial biofilm were examined. Further, ciliate communities with respect to functional feeding modes were studied during a seasonal cycle and at three different small low-head dam sites. The following hypotheses were tested:

(1) The colonization of virgin surfaces by ciliates is faster at slow compared to faster flow velocities in a small stream.
(2) Vagile flattened gulper and filter feeders dominate and stay longer attached at a surface at faster flow velocities than vagile round filter feeders.
(3) Increased flow velocities induce a positive rheotaxis and a larger displacement rate irrespective of feeding mode and cell morphology, and effects grazing activity.
(4) Gulper feeders alter biofilm morphology more than filter feeders and cause a higher biofilm surface area / biofilm volume ratio and porosity.

THESIS STRUCTURE

The **first chapter** of the thesis intended to illustrate the effects of flow velocity on the colonization dynamics of biofilm associated ciliate morphotypes in a small stream. The study focused on initial biofilm colonization since the stream sediment bed is frequently relocated due to flow velocity fluctuations. A fast re-colonization of surfaces is crucial to regain the stream's self-purification activity. To study different flow velocity conditions in headwater streams, natural riffle and pool structures could be used. However, in hydro-dynamically highly fluctuating systems, like small streams, alternating flood (high flow velocity) and draught (slow flow velocity) periods relocate natural riffles and pool structures. Man-made low-head dams (hydraulic head < 5 m) create similarly slow (reservoir) and faster (outlet) flowing sections, but weaken discharge peaks of flow velocity fluctuations. Colonization of

biofilm associated ciliates was observed at a slow flowing reservoir and faster flowing outlet and natural sites between 1 and 14 days. However, additional environmental effects, like sedimentation processes in reservoirs, might influence ciliate colonization patterns. Thus, field experiments at the 3rd order stream Ilm (Thuringia, Germany) were complemented by flow channel experiments to study, under more controlled conditions in the laboratory, the effects of different flow velocities and sediment contents on biofilm colonization dynamics by ciliate morphotypes.

The impact of flow velocity on detachment and behavioral responses is addressed in the **second chapter**. Eleven ciliate species were investigated that can be grouped according to their feeding modes and morphological features into sessile filter feeder, vagile flattened and round filter feeder, and vagile flattened gulper feeder. The ciliates were isolated or enriched from the 3rd order stream Ilm and from a small pond in Cologne. Circular flow channels, where flow velocities were produced by a rotating disc on top of the culture medium, were used as microcosms. This flow channel device was placed directly under the microscope allowing the live observation of ciliates and simultaneously the recording of motility tracks.

Chapter three is dealing with the influence of grazing protists on the spatial morphology of a bacterial biofilm at slow flow velocities. Protists were co-cultivated with multispecies bacterial biofilms in small flow cells. These small flow cells are advantageous since they can be placed directly under the microscope to investigate undisturbed biofilms. Confocal laser scanning microscopy, which allows the optical thin sectioning, is a powerful tool to visualize the undisturbed, spatial biofilm morphology. Analysis of image stacks was performed with the novel computer program ‘daime’ that integrates digital image analysis and three-dimensional visualization functions.

To extrapolate the described patterns for ciliate distribution at small low-head dams (chapter 1), the **fourth chapter** illustrates the ciliate abundance, diversity, and functional feeding groups during a seasonal cycle and at three nearby located dams that differ in size. Since all major rivers worldwide are influenced by human impacts, the **fifth chapter** reviews the present knowledge regarding the effect of dams on physical, chemical and biological conditions in streams and rivers. Removal of small low-head dams, which is the fundamental measure in management plans and restoration activities, is critically discussed.

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COLONIZATION DYNAMICS OF BIOFILM ASSOCIATED CILIATE MORPHOTYPES AT DIFFERENT FLOW VELOCITIES

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Abstract

The impact of flow velocity on initial ciliate colonization dynamics on surfaces were studied in the 3rd order stream Ilm (Thuringia, Germany) at a slow flowing site (0.09 m s^{-1}) and two faster flowing sites (0.31 m s^{-1}) and in flow channels at 0.05, 0.4, and 0.8 m s^{-1}. At the slow flowing stream site, surfaces were rapidly colonized by ciliates with up to 60 cells cm^{-2} after 24 h. In flow channels, the majority of suspended ciliates and inorganic matter entered the biofilm within 4.5 h at 0.05 m s^{-1}. At 0.4 m s^{-1} the increase in ciliate abundance was highest between 72 and 168 h and approximated 3 cells cm^{-2} h^{-1}. Faster flow velocities were tolerated by vagile flat ciliates that live in close contact to the surface. Vagile flat and round filter feeder preferred biofilms at slow flow velocities. Addition of inorganic particles (0, 0.6 and 7.3 mg cm^{-2}) did not affect ciliate abundance in flow channel biofilms, but small ciliate species dominated and species number was lowest (16 species cm^{-2}) in biofilms at high sediment content. Although different morphotypes dominated the communities at contrasting flow velocities, all functional groups contributed to initial biofilm communities implementing all trophic links within the microbial loop.

Keywords: ciliate morphotypes, flow velocity, inorganic sediment, stream, biofilm

Introduction

Biofilm associated ciliates can reach high abundances in streams (i.e. Harmsworth and Sleigh 1993; Schmitz 1985; Schönborn 1982). They enhance the carbon and energy flow due to the stimulation of bacterial production (Sherr and Sherr 1984). Ciliates play a significant role in the decomposition of leave litter (Ribblett et al. 2005) and during the self-purification process in lotic ecosystems (Liebmann 1962; Schönborn 1984). By transferring carbon and energy to the meio- and macrofauna, ciliates display an important link in the aquatic food web (Bott and Borchard 1999; Schönborn 1987).

The colonization of surfaces in streams and rivers is advantageous for protists because their downstream transport is prevented and food concentrations (i.e. bacteria, algae) can be several orders of magnitude higher in the biofilm than in the surrounding water (Geesey et al. 1978; Romani and Sabater 1999). Ciliates can rapidly colonize surfaces with attached bacteria due to their chemosensory potential (Arndt et al. 2003; Fenchel and Blackburn 1999; Franco et al. 1998). Associated with surfaces, ciliates utilize a variety of food particles, such as bacteria, algae, flagellates, small ciliates (Parry 2004), and even detritus particles (Scherwass et al. 2005). General modes of food acquisition in ciliates are either the concentration of food particles by filter feeders or the active search for food particles by gulper feeders (Hausmann 2002). Feeding modes and morphological features are closely related. Sessile ciliates stay firmly attached to the surface by stalks and filtrate the surrounding water for mainly bacteria, whereas vagile ciliates with a flattened cell shape use food items associated with the surface (Lawrence and Snyder 1998). Vagile round species swim and casually associate with the biofilm to filtrate the near vicinity of the biofilm (Fenchel 1986). Filter feeders associated with the biofilm have higher clearance rates than free swimming ciliates because they can create stronger feeding currents (Fenchel 1986).

Ciliates that colonize biofilms in streams live in the boundary layer, where the flow velocity decreases exponentially (Ambühl 1959; Oertel et al. 2001). However, even the laminar flow in the boundary layer can become turbulent in streams (Oertel et al. 2001); so that special adaptations of the biofilm associated ciliates are advantageous to maintain attachment at varying flow velocity conditions. The colonization of biofilm associated ciliates at different flow velocities have been rarely studied with respect to ciliate morphotypes and feeding modes (Franco et al. 1998). Besides boundary layer characteristics, the flow velocity

influences the colonization of surfaces by altering the contact rate of suspended cells with the surface (Hunt and Parry 1998) and the detachment of single cells or biofilm fragments (Characklis 1984). Further the sedimentation of particulate organic and inorganic matter is determined by the flow velocity. Differences in the biofilm sediment content might affect the biofilm associated community due to compaction and changes in the nutrient supply.

In a large river, the total ciliate abundance in 14 days old biofilms is lower at slow compared to fast flow velocities (Schmitz 1985). However, increased flow velocities during flood events do not always decrease the total ciliate abundance in biofilms (Schönborn 1982). At fast flow velocities, the ciliate species number in biofilms of a small stream is low and a prolonged time is necessary to reach higher species numbers (Primc and Habdija 1987). Flood events, which regularly disturb small streams, move the stream bed sediment, relocate riffle and pool structures, and lead to an abrasion of biofilm communities (Schönborn 1992; Schwoerbel 1999). A fast re-colonization of the newly created surfaces is crucial for stream ecosystems to regain their self-purification activity but studies about the colonization dynamics of biofilm associated ciliates in hydrodynamically variable streams are rare.

The present study, therefore aims to compare the effects of different flow velocities on the colonization dynamics and community structure of biofilm associated ciliates in a small stream. Field experiments were complemented by flow channel experiments to study under more controlled conditions the effects of flow velocity and sediment addition on biofilm colonization by different ciliate morphotypes. We hypothesize (i) that ciliates colonization of surfaces is faster at slow flow velocities and (ii) that species attached by stalks and vagile flattened species that live in close contact to the surface predominate fast flow velocities.

Methods

Sampling site. The Ilm is a typical mountain stream which arises about 800 m above sea level in the northern part of the Thuringian forest (Germany) and meets the River Saale, a tributary of the River Elbe, after 137 km. The discharge varied from 0.2 to 78.4 $m^3 s^{-1}$ during the whole investigation period with an annual mean of 2.45 $m^3 s^{-1}$ (DGJ 2003, 2005, 2007). Discharge peaks occurred either following the snow melt from December till March or as consequence of precipitation maxima from June till November. 56 small low-head dams with a hydraulic head < 5 m (0.7 - 3.1 m) and an impounded area of < 3 ha were built

approximately every 2.3 km to weaken discharge peaks. The capacity and length of the reservoirs are small caused by the high slope of the stream (3.16 %) and material aggregation. The sediment of the headwater consists of gravel and cobble which starts to move at base flow conditions of 1.12 $m^3 s^{-1}$ (TLU 1996).

Samples were obtained from a slow flowing reservoir and the corresponding faster flowing outlet of a low-head dam (50°44'58"N, 11°02'14"E) and a faster flowing natural site at the stream Ilm (50°40'03"N, 10°51'32"E), where no dams were present 10 km upstream. Physical-chemical measurements indicated significant different flow velocities between sites measured 5 - 10 cm above the stream bed (FLO-MATE 2000, Marsh McBrunney Inc., Hyattsville, MD, USA) ($F_2 = 35.137$, $p < 0.001$). Over the year, flow velocities were highly variable by seasonally changing discharge regimes of the stream, but the range was much smaller during periods of sampling. Flow velocity at the reservoir (0.04 - 0.13 m s^{-1}, mean 0.09 ± 0.03 m s^{-1}) was slower compared to the faster flowing outlet (0.16 - 0.48 m s^{-1}, mean 0.32 ± 0.10 m s^{-1}) and natural site (0.23 - 0.39 m s^{-1}, mean 0.30 ± 0.06 m s^{-1}). Temperature (4.4 - 18.5°C), oxygen content (6.9 - 13.4 mg l^{-1}), pH (6.3 - 7.7), conductivity (0.25 - 0.42 mS cm^{-1}), and turbidity (0 - 4 NTU) (U10, Horiba, Kyoto, Japan) were not altered by the low-head dam. Oxygen concentration obtained by a data logger (O_2-Log550, Driesen + Kern GmbH, Bad Bramstedt, Schleswig-Holstein, Germany) over a 21 day period near the stream bed of the reservoir varied between 60 and 106 % (12 - 18 °C) showing that the near bottom zone of the reservoir was oxic.

Stream biofilms. Biofilm samples were obtained in April, July, and November 2003 at all three sites after 24, 72, 120, 168, and 336 h of biofilm development. Glass slides (7.6 x 2.6 cm) were placed in cylinders made of perforated stainless steal (16 x 8.3 cm, perforation ø 0.8 cm) (Alfreider et al. 1997), and horizontally exposed near the stream bed. These cylinders had to be used to avoid destruction of the glass slides by floating stones. Due to the flow resistance of the perforated cylinders, flow velocity will be reduced inside the cylinders. Measurements with the FLO-MATE 2000 inside the cylinders indicated a decrease up to 50%, but differences between sites remained the same. For biofilm analysis, glass slides from three separate cylinders served as replicates. Triplicates of 250 ml surface water were also taken at every sampling to determine ciliate abundance and species number.

Flow channel biofilms grown at different flow velocities. Water from the stream Ilm (outlet of the small dam) was sampled in March 2006. The water was enriched with organisms scraped off from stones to ensure the presence of also biofilm associated species. Each flow channel (160 x 11 x 18 cm) (Augspurger et al. 2008) was equipped with horizontally exposed, precombusted clay tiles (4.7 x 4.7 cm) and filled with 10 l of the sampled water. To study the effect of flow velocity, the slopes of the flow channels were adjusted to meet 0.05, 0.4, and 0.8 m s^{-1}. Three replicate flow channels per treatment were kept in a climate chamber at 13 °C and 13 h light / 11 h dark (photosynthetic active radiation: 96 µmol m^{-2} s^{-1}). Three and two clay tiles out of one flow channel were harvested after 1, 2, 4.5, 8, 12, 24, 48, 72, and 168 h to determine ciliates and particulate matter, respectively. In addition, 250 ml of flow channel water were collected at the start and end of the experiment to determine suspended ciliates.

Flow channel biofilms grown at different sediment content. Stream water was sampled in March 2007 as described above and filled in flow channels (50 x 16 x 4 cm) equipped with 24 glass slides. To assure maximum sedimentation of inorganic sediment particles with a grain size of 0.5 - 10 µm (silicon dioxide 99%, about 80% of particles 1 - 5 µm, Sigma-Aldrich, Taufkirchen, Germany) the flow velocity was reduced to 0.01 m s^{-1}. Sediment particles were suspended in filtered and autoclaved stream water and 1 ml of the solution was added successively during five following days into the flow channel water to reach a theoretical final sediment load of 1 and 10 mg cm^{-2}. 1 ml of filtered and autoclaved stream water without sediment particles was added to the control treatment. The flow was stopped for 15 minutes to add the sediment suspension. After 168 h, two glass slides were sampled to enumerate ciliates. Two other glass slides were sampled to determine biofilm accumulated inorganic matter as difference in dry weight before (24 h at 105 °C) and after ashing (2 h at 550 °C).

Determination of ciliates. The biofilm was scraped off the glass slides or clay tiles, and washed with 15 ml of filtered and sterilized stream water. Ciliate samples of biofilm and water column were fixed with Bouni's solution (final concentration 5%) and stained following the quantitative protargol staining procedure (QPS) (Montagnes and Lynn, 1987, Skibbe, 1994). Field biofilm samples were washed two times with Bouin's solution. Depending on the concentration of ciliates, sub samples were extracted from the original. After 24 - 36 h the concentrated material was filtered through cellulose nitrate filters (1.2 µm, Sartorius, Goettingen, Germany), which were mounted in Canada balsam after the staining procedure to guarantee their permanence. Ciliates were identified using Foissner et al. (1991,

1992, 1994, 1995) and Kahl (1935) and quantified with differential interference contrast at 400times magnification (Axioplan; Zeiss, Jena, Germany). For each sample, at least 50 individuals or the whole filter was scanned.

According to morphological features and feeding modes ciliates were divided into three groups: (i) sessile species which stay firmly attached to a surface by stalks and filtrate the surrounding water mainly for bacteria, (ii) vagile species with a flattened cell shape and cilia that are located only at one cell side and either filtrate or actively search for surface-associated food, and (iii) vagile species with a round cell profile which swim freely in the near vicinity of biofilms and casually associate with the biofilm for filtration.

Data analysis. Total ciliate abundance and species number of stream biofilms and flow channel biofilms of the flow velocity experiment were analyzed after log(x+1) transformation using a two-way analysis of variance (ANOVA) and a repeated measures ANOVA, respectively (SPSS 13.0, SPSS Inc., Chicago, Illinois, USA). One-way ANOVA with the Bonferroni post-hoc test for multiple comparisons were used to test for differences in environmental parameters between stream sites and to test the effect of sediment addition on ciliates. Spearman rank correlation analyses were carried out to reveal relationships between abundance of most important ciliate species and inorganic sediment content. Communities between different sediment treatments were compared using the Soerensen-Quotient (number of shared species are considered), the Wainstain-Index (relative frequencies of shared species are considered), and the number of Renkonen (measured value of similarity of two communities according to their species dominance relations) calculated with the mean values of three replicate flow channels. To characterize stream and flow channel biofilms according to their ciliate community structure a principle coordinate analysis (PCoA, Canoco 4.5) (ter Braak and Smilauer 2002) with the Bray-Curtis distance measure was used. Data are shown as a Euclidean representation where distances between the symbols (sampling points) approximate the dissimilarity in species composition.

Results

Effect of flow velocity on biofilm associated ciliates in a small stream

To weaken flow velocity variations in this hydrodynamically fluctuating stream, biofilms were grown in regulated stream areas (a slow flowing reservoir and a faster flowing outlet of a small low-head dam) in addition to a faster flowing natural site. In the water column ciliate abundances varied between 0.2 and 3.9 cells ml^{-1}. The colonization rates of biofilm associated ciliates differed significantly between sites in April 2003 (Table 1). However, replicates (different glass slides) were very heterogeneous and similar trends were observed at all samplings (Fig. 1). Ciliates colonized glass slides faster at the slow flowing reservoir and reached 2 to 4 times higher abundances (up to 60 cells cm^{-2}) after 24 h compared to both faster flowing sites. In the reservoir, the maximal abundance was always reached after 120 - 168 h independent of the month of sampling. In contrast, the abundance of biofilm associated ciliates at both faster flowing sites steadily increased during 14 days of exposition.

Species number in the water column was low and approximated 5 to 26 species ml^{-1}. In total, 68 and 83 different ciliate species were detected in the water column and in the biofilm, respectively. In general, slightly more species were found at the reservoir (max. 77) compared to the faster flowing outlet (max. 71), and natural site (max. 67). In the reservoir, up to 20 species cm^{-2} (40% of total species number) were present after 24 h in contrast to faster flowing sites (5 - 15 species cm^{-2}) (Fig. 1). More species were exclusively found at the slow flowing reservoir (18) than at the faster flowing outlet (10). 11 species occurred first in the reservoir before they were found in the outlet. Shannon diversity index and evenness varied between 1.41 to 3.05 and 0.50 to 0.96, respectively, with no differences according to flow velocity.

Similarly to the prolonged colonization shown for total ciliate abundance and species number at both faster flowing sites (Fig. 1), multivariate analysis of the ciliate community structure displayed a delay in the colonization process in stream biofilms caused by faster flow velocities (Fig. 2). Older biofilms of the fast flowing natural site (120 h) and outlet (72 h) grouped with younger biofilms of the slow flowing reservoir (24 h). Structure of biofilm ciliate communities at different flow velocities became more similar with increasing biofilm age (reservoir 120 - 336 h, outlet 120 - 336 h and natural site 336 h).

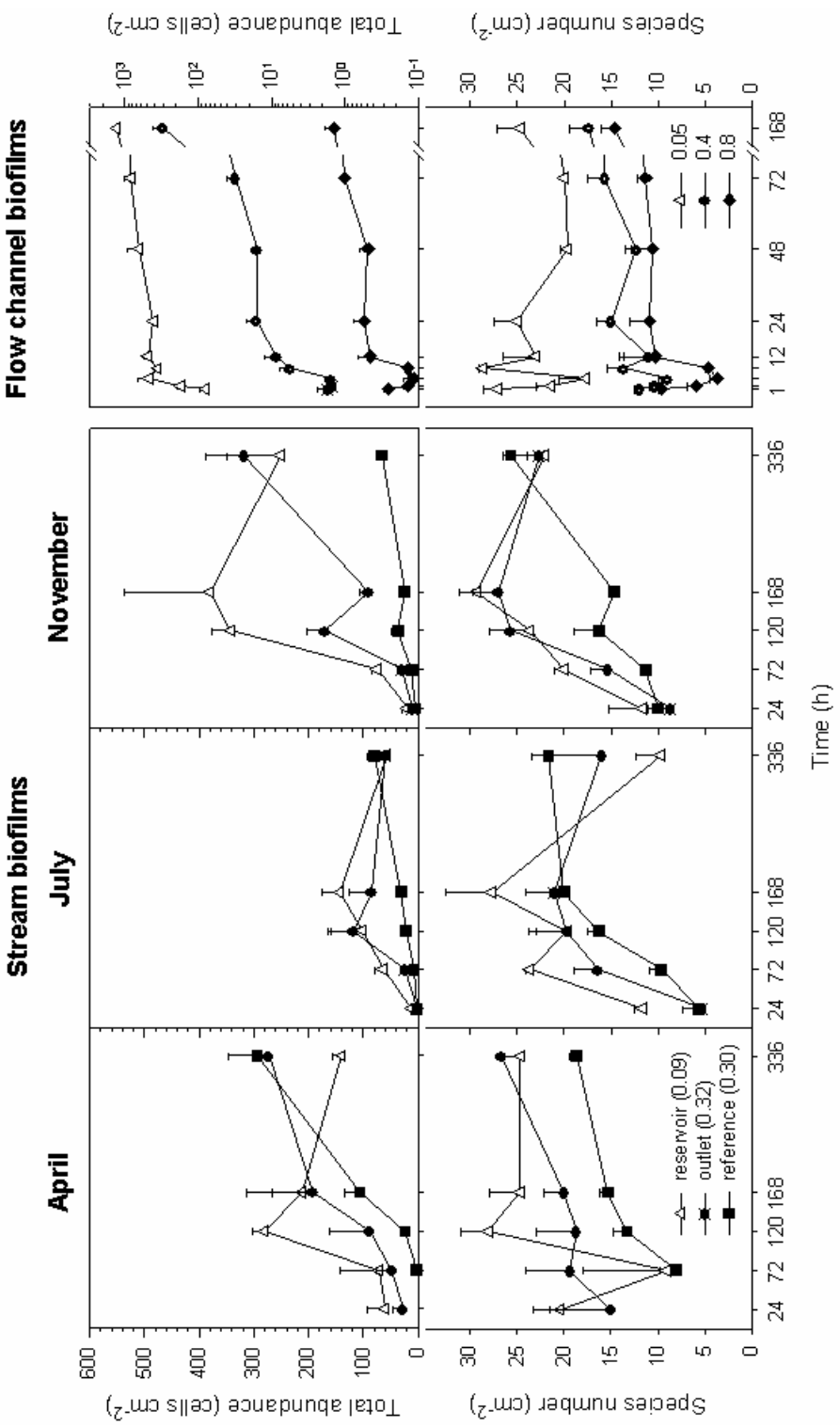

Fig. 1. Colonization dynamics and spatial variation of total ciliate abundance (cells cm^{-2}) (upper graphs) and species number (cm^{-2}) (lower graphs) in stream and flow channel biofilms at different flow velocities (mean + SE, n = 3). Please note the logarithmic scale of ciliate abundance of the flow velocity experiment and the break in the time axis. [abc] Significant differences between stream sites and flow channel treatments using a one-way ANOVA and Bonferroni post-hoc test ($p < 0.05$). Flow velocity treatments were significantly different at every time point.

Table 1. *F*-values of the two-way ANOVA are shown for total ciliate abundance and species number in stream biofilms. All variables were log(x+1) transformed, d.f. represents the degree of freedom; significant effects are represented by *.

		Time	Site	Time * Site
		4 d.f.	2 d.f.	8, 30 d.f.
Total ciliate abundance	April 2003	16.35***	5.85**	2.49*
	July 2003	17.35***	8.21**	1.64
	November 2003	52.65***	51.16***	1.89
Species number	April 2003	9.03***	7.62**	5.32***
	July 2003	12.32***	1.91	2.88*
	November 2003	26.51***	8.10**	2.96*

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

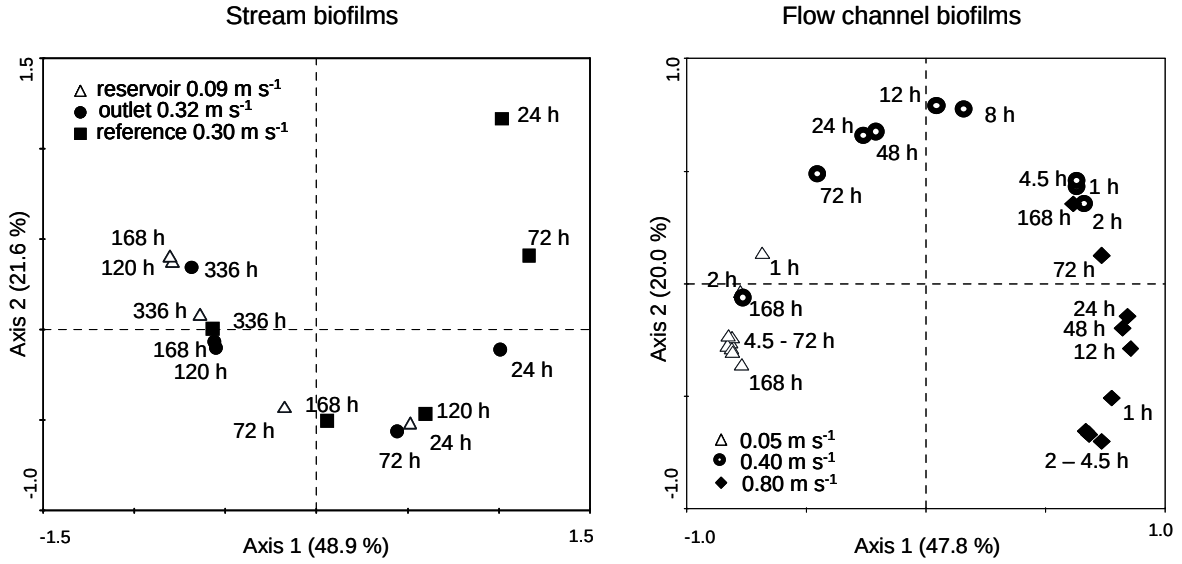

Fig. 2. Euklidean representation of the PCoA based on ciliate abundance data from stream biofilms (1 to 336 h, sum of three samplings in April, July, and November 2003, right graph) and flow channel biofilms (1 to 168 h, left graph). Numbers refer to the sampling time (h).

Effect of flow velocity on biofilm associated ciliates in flow channels

To ensure defined stable flow velocities and to exclude additional environmental effects, biofilm associated ciliate community development was also studied in flow channels. We hypothesized that at slow flow velocities all functional groups were present already at early stages of biofilm development, because all food sources were immediately present due to passive sedimentation.

Ciliate abundances in the flow channel water ranged from 20.5 to 58.9 cells ml^{-1}, with no significant differences between treatments ($F_2 = 2.478$, $p = 0.164$) and decreased to 1.7 - 17.5 cells ml^{-1} till the end of the experiment. Flow velocity significantly influenced total ciliate abundance and colonization rate in flow channel biofilms (Fig. 1, Table 2). After 1 h, ciliates were 40 and 245 times more abundant at 0.05 m s^{-1} compared to 0.4 and 0.8 m s^{-1}, respectively. At 0.05 m s^{-1}, the highest increase in abundance occurred between 2 and 4.5 h (115.8 cells cm^{-2} h^{-1}). An initial lag-phase occurred at faster flow velocities lasting for 4.5 and 8 h at 0.4 and 0.8 m s^{-1}, respectively. At 0.4 m s^{-1}, ciliate abundance displayed the highest increase between 72 and 168 h (2.8 cells cm^{-2} h^{-1}). Ciliate abundance remained below 1.5 cells cm^{-2} during the experiment at 0.8 m s^{-1}.

54 of the 83 species found in stream biofilms were also detected in flow channel biofilms. Irrespective of flow velocity, a total of 28 species determined in biofilms after 1 h were not recorded during the following 3.5 h, caused by i.e. *Chilodonella uncinata*, *Dexiostoma campylum*, and *Glaucoma scintillans*. The mainly free swimming *Rimostrombidium humile* occurred later frequently in biofilms at 0.05 m s^{-1} but not at 0.4 and 0.8 m s^{-1}. Significantly more species were found at 0.05 m s^{-1} compared to 0.4 and 0.8 m s^{-1} (Fig. 1, Table 2). After 72 h, species number increased more rapidly at 0.4 m s^{-1} diminishing the differences to 0.05 m s^{-1} (Table 2). Dominant species missing in the beginning at 0.4 and 0.8 m s^{-1} appeared after 2, 4.5, or 8 h and after 24 or 48 h, respectively (Table 3). 41.5% of species that occurred first or exclusively at 0.05 m s^{-1} belonged to bacterivorous ciliates. Other species first detected at 0.05 and 0.4 m s^{-1} and later at 0.8 m s^{-1} mainly belonged to bacteri-algivorous ciliates. In general, distribution and colonization dynamics of functional groups were not influenced by flow velocity. Relative abundance of bacteri-algivorous ciliates increased towards 168 h.

Table 2. *F*-values of the repeated measures ANOVA are shown for total ciliate abundance, species number, and important ciliate species in flow channel biofilms of the flow velocity experiment. All variables were log(x+1) transformed, d.f. represents the degree of freedom, significant effects are represented by *.

	Between-subjects effects	Within-subjects effects	
	Flow	Time	Time vs. Flow
	2, 6 d.f.	8 d.f.	16, 48 d.f.
Total ciliate abundance	465.46***	56.38***	13.77***
Species number	87.02***	6.31***	2.26*
Vagile flat ciliates			
Chilodontopsis depressa (gf,a)	178.72***	12.46***	4.50***
Litonotus alpestris (gf,b)	253.68**	2.14	0.72
Litonotus cygnus (gf,c)	372.26**	5.87***	6.14***
Litonotus lamella (gf,c)	55.62***	23.93***	6.94***
Chlamydonella alpestris (gf,a)	26.63***	7.67***	5.57***
Thigmogaster potamophilus (gf,a)	205.43***	14.10***	4.37***
Trithigmostoma cucullulus (gf,a)	296.77***	42.95***	13.51***
Trochilia minuta (gf,b)	495.06***	12.14***	5.20***
Aspidisca lynceus (ff,b)	15.13***	3.74**	1.51
Vagile round ciliates			
Dexiostoma campylum (ff,b)	10.98*	1.21	0.67
Uronema nigricans (ff,b)	221.72***	19.07***	5.11***
Sessile ciliates			
Carchesium polypinum (ff,b)	30.29***	7.89***	4.00***
Vorticella aquadulcis (ff,b)	256.10***	4.48***	3.70***
Vorticella convallaria (ff,b)	44.94***	1.50	2.13*

gf: gulper feeder, ff: filter feeder, b: bacterivorous, a: algi-bacterivorous, c: carnivorous.
*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Table 3. Dominance values (%) of ciliate species which made up >2 % of the total ciliate community in stream and flow channel biofilms calculated with the sum of all time points. Classification of species characteristics is based on Foissner et al. (1991, 1992, 1994, 1995) and Hausmann (2002). Numbers in parenthesis indicate first occurrence (h) of species in flow channel biofilms. Division of dominance classes (Engelmann, 1978): eudominant 32 - 100 % (bold and italics), dominant 10 - 31.9 % (bold), subdominant 3.2 - 9.9 %, recedent 1 - 3.1 %, subrecedent 0.32 - 0.9 %, and sporadic <0.32 %.

	Dominance value (%)								
	Stream biofilms			Flow channel biofilms					
Species	reservoir (0.09 m s^{-1})	outlet (0.32 m s^{-1})	reference (0.30 m s^{-1})	0.05 m s^{-1}		0.4 m s^{-1}		0.8 m s^{-1}	
Vagile flat ciliates									
Chilodontopsis depressa (gf,a)	2.9	4.0	1.3	3.6	(1)	2.8	(1)	1.4	(24)
Litonotus alpestris (gf,b)	7.2	7.4	6.8	1.2	(1)	2.2	(1)	3.7	(1)
Litonotus cygnus (gf,c)	5.2	5.9	6.7	0.9	(1)	0.1	(8)	0.8	(48)
Litonotus lamella (gf,c)	5.4	6.4	3.4	3.2	(1)	4.6	(1)	3.3	(1)
Chlamydonella alpestris (gf,a)	4.9	2.6	**23.6**	2.1	(1)	0.4	(4.5)	2.1	(24)
Thigmogaster potamophilus (gf,a)	**12.1**	**11.2**	4.3	7.9	(1)	9.2	(1)	**11.2**	(1)
Trithigmostoma cucullulus (gf,a)	**19.5**	**20.5**	2.2	**27.3**	(1)	***56.4***	(1)	**20.4**	(1)
Trochilia minuta (gf,a)	**12.2**	**11.8**	**17.6**	2.5	(1)	2.5	(1)	4.4	(1)
Aspidisca lynceus (gf,a)	**10.0**	7.1	2.2	2.6	(1)	1.5	(1)	2.9	(1)
Vagile round ciliates									
Dexiostoma campylum (ff,b)	3.3	4.7	9.7	0.3	(1)	0.8	(1)	2.5	(1)
Uronema nigricans (ff,b)	***39.0***	**19.9**	**22.2**	6.2	(1)	4.8	(1)	**20.0**	(1)
Sessile ciliates									
Carchesium polypinum (ff,b)	2.0	0.9	0.3	5.6	(1)	2.6	(2)	0.3	(1+2)
Vorticella aquadulcis (ff,b)	4.5	3.2	1.7	**18.2**	(1)	3.9	(1)	9.7	(1)
Vorticella convallaria (ff,b)	4.1	4.5	3.1	2.4	(1)	0.8	(1)	2.4	(1)

gf: gulper feeder, ff: filter feeder, b: bacterivorous, a: algi-bacterivorous, c: carnivorous.

Effect of inorganic sediment particles on biofilm associated ciliates in flow channels

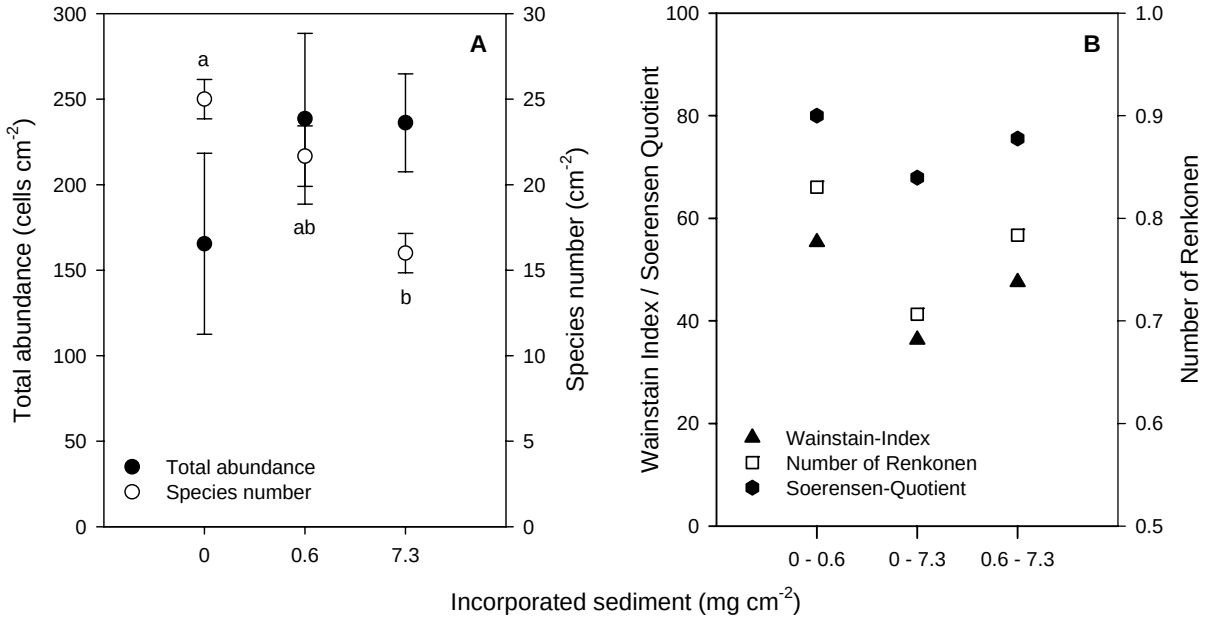

Fig. 3. Ciliate abundance (cells cm^{-2}) and species number (cm^{-2}) shown as mean ± SE, n = 3 (A), and ciliate communities comparison (B) of flow channel biofilms with different inorganic sediment content. ^{ab} significant differences between treatments calculated with a one-way ANOVA and Bonferroni post-hoc test ($p < 0.05$).

Inorganic sediment particles accumulated rapidly in flow channel biofilms of 0.05 m s^{-1} reaching 0.6 mg cm^{-2} after 1 h and 4.4 mg cm^{-2} after 168 h. At 0.4 m s^{-1} the inorganic matter reached 0.6 mg cm^{-2}, whereas no inorganic matter accumulated at 0.8 m s^{-1} in biofilms. Thus, the high sediment content at slow flow velocity conditions could have additionally affected the abundance of biofilm associated ciliates and probably favor specific morphotypes or species with a small cell size.

The addition of 1 or 10 mg cm^{-2} inorganic sediment to the water of flow channels during biofilm growth at 0.01 m s^{-1} yielded a sediment load of 0.6 and 7.3 mg cm^{-2}, respectively. No inorganic matter accumulated in control biofilms within 168 h. Total ciliate abundance in biofilms was not significantly affected by the added sediment ($F_2 = 0.848$, $p = 0.474$) but the species number ($F_2 = 10.750$, $p < 0.01$) with fewer species present in biofilms of the 7.3 mg cm^{-2} compared to the 0.6 or 0 mg cm^{-2} treatment (Fig. 3). 17 of the observed 37 species did not occur in biofilms with 7.3 mg cm^{-2} sediment, i.e. *Aspidisca*

lynceus (Table 4) and *Epistylis* sp. Some of the small species showed high dominance values and abundances at 7.3 mg cm^{-2} (Table 4), whereas others were not influenced by higher sediment content. Larger species correlated negatively with inorganic sediment content. The relative contribution of functional groups was not altered by sediment addition (data not shown). Comparison of the community structure of different sediment treatments (Fig. 3) showed greatest similarity between 0 and 0.6 mg cm^{-2} and lowest similarity between 0 and 7.3 mg cm^{-2}.

Table 4. Characteristics (based on Foissner et al. (1991, 1992, 1994, 1995) and Hausmann (2002)) and dominance values (%) of important ciliates in flow channel biofilms with different inorganic sediment content. Spearman Rank correlation coefficients (R^2, *significant correlations $p < 0.05$) of species abundance and inorganic sediment content were calculated. Division of dominance classes (Engelmann, 1978): dominant 10 - 31.9 % (bold), subdominant 3.2 - 9.9 %, recedent 1 - 3.1 %, subrecedent 0.32 - 0.9 %, and sporadic <0.32 %.

Species	Length (µm) (from Foissner et al. 1991-1995)	Dominance value (%) 0.0 mg cm^{-2}	0.6 mg cm^{-2}	7.3 mg cm^{-2}	R^2
Vagile flat ciliates					
Chilodontopsis depressa (gf,a)	50 - 80	0.3	0.3	0.0	-0.666
Litonotus alpestris (gf,b)	30 - 50	3.0	2.7	2.1	0.226
Litonotus cygnus (gf,c)	200 - 300	8.5	6.8	2.5	-0.418
Litonotus lamella (gf,c)	50 - 100	6.8	9.6	**13.6**	0.745
Chlamydonella alpestris (gf,a)	25 - 35	1.0	1.6	1.1	0.175
Thigmogaster potamophilus (gf,a)	20 - 30	1.5	0.8	3.6	0.628
Trithigmostoma cucullulus (gf,a)	80 - 160	**12.1**	**12.2**	5.0	-0.328*
Trochilia minuta (gf,b)	15 - 40	1.7	1.7	3.2	0.672
Aspidisca lynceus (ff,b)	35 - 50	1.8	0.7	0.0	-0.718*
Vagile round ciliates					
Uronema nigricans (ff,b)	25 - 50	5.4	8.9	**17.5**	0.65
Dexiostoma campylum (ff,b)	35 - 90	0.0	1.1	2.1	0.636
Sessile ciliates					
Carchesium polypinum (ff,b)	80 - 140	**11.7**	5.1	5.7	-0.368
Vorticella aquadulcis (ff,b)	25 - 55	5.1	2.0	2.5	-0.351
Vorticella convallaria (ff,b)	40 - 95	3.8	3.4	3.2	0.253*

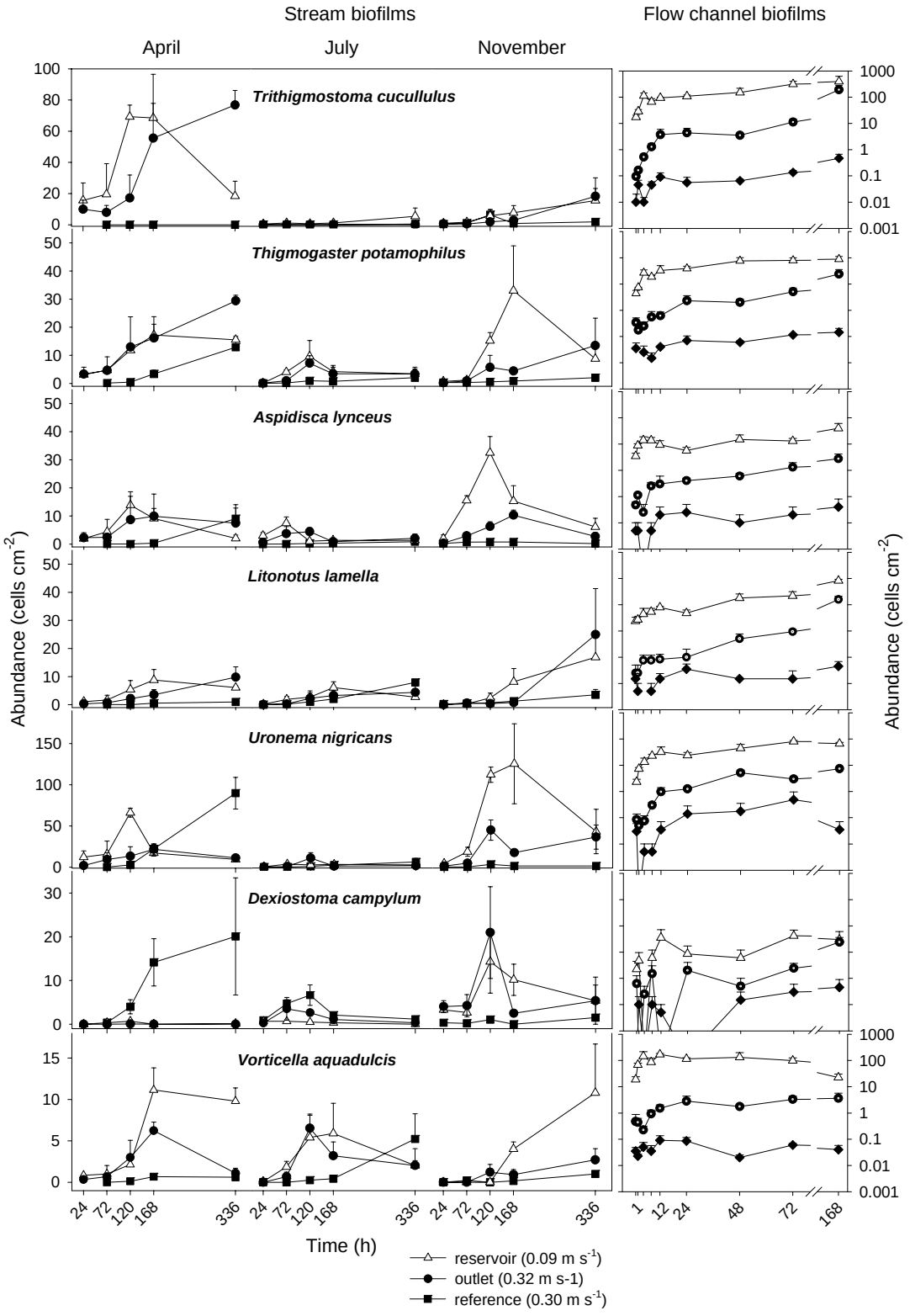

Fig. 4. Colonization dynamics and spatial variation of vagile flat (Trithigmostoma cucullulus, Thigmogaster potamophilus, Aspidisca lynceus, Litonotus lamella), vagile round (Uronema nigricans, Dexiostoma campylum), and sessile (Vorticella aquadulcis) ciliates (cells cm^{-2}) of stream and flow channel biofilms (mean + SE, n = 3).

Ciliates morphotypes

A total of 92 ciliate species, reflecting 12 subclasses and 72 genera, were found in stream and flow channel biofilms (see Table S2 for species list). 71 species (flattened: 38, rounded: 37), were vagile, 18 were sessile, and 4 were planktonic. 14 ciliate species made up 72 % and 86 % of stream and flow channel biofilm communities, respectively, and will be discussed in more detail.

*Vagile flat ciliates***.** Phyllopharyngia made up 38% of ciliate communities in stream and flow channel biofilms. Dominant species in the stream at both faster flowing sites and in flow channel biofilms at 0.4 and 0.8 m s^{-1} were the algivorous *Trithigmostoma cucullulus* and *Thigmogaster potamophilus* (Fig. 4, Table 3). However, their colonization rates were delayed at faster flow velocities in flow channels (Table 2). The algivorous *Chilodontopsis depressa*, the bacterivorous *Litonotus alpestris*, and the carnivorous *L. cygnus* and *L. lamella* showed no preference to different flow velocities (Fig. 4, Table 3). The slope of the colonization rates of bacterivorous *Aspidisca lynceus* in flow channel biofilms were similar at all flow velocity treatments (Table 2), but higher abundances were reached at the slow flowing reservoir and 0.05 m s^{-1} (Fig. 4, Table 3).

*Vagile round ciliates***.** *Uronema nigricans* reached higher abundances at the slow flowing reservoir and in flow channels of 0.05 m s^{-1}. Surprisingly, this small scuticociliate reached higher abundances at the faster flowing natural site in April 2003 (Fig. 4) and contributed with up to 48% to the ciliate community between 24 and 72 h in flow channel biofilms at 0.8 m s^{-1} (Table 2, 3). Despite its large size *Dexiostoma campylum* reached high abundances in stream biofilms at faster flowing sites (Fig. 4).

*Sessile ciliates***.** *Vorticella aquadulcis*, which possesses a contractile stalk, reached low abundances in streams (Fig. 4). Only at 0.05 m s^{-1}, *V. aquadulcis* was dominant (Table 3) and reached the maximal abundance within 12 h (Fig. 4), while at 0.4 m s^{-1} abundances steadily increased towards 168 h. Similarly, the colony forming sessile ciliate *Carchesium polypinum* reached its maximal abundance at 0.05 m s^{-1} and at the slow flowing stream site. *C. polypinum* did not occur at 0.8 m s^{-1} (Table 2, 3).

Discussion

Effect of flow velocity on colonization dynamics

Initial colonization processes occur often in frequently disturbed ecosystems such as streams that are influenced by floods and flow velocities >1 m s^{-1}, where either suspended particles scrub off the biofilm (Schönborn 1992) or the whole sediment bed is relocated (TLU 1996). Freshly exposed clay tiles or glass slides were colonized more rapidly by ciliates at slow compared to faster flow velocities. The lag-phase observed during the initial ciliate colonization at faster flow velocities and the delayed occurrence of several ciliate species was similar to results obtained from leave bags exposed at a pool (0.06 - 0.07 m s^{-1}) and a riffle site (>1.2 m s^{-1}) (Franco et al. 1998). However, slower initial colonization of microorganisms at faster flow velocities serving as food for phagotrophic ciliates can not explain the observed differences in ciliate colonization dynamics in the stream Ilm, because the abundance of bacteria and heterotrophic nanoflagellates was similar at the slow flowing reservoir and the faster flowing outlet during the first three days of exposure (Pohlon and Willkomm pers. comm.). Thus, faster flow velocities appeared to inhibit directly ciliate attachment to virgin surfaces, a process which often occur in small streams especially after streambed movement. Nonetheless, all functional groups of ciliates were present during the initial colonization period suggesting that only the extent of carbon channeling by ciliates within the microbial loop was affected.

Total ciliate abundance and biofilm community structure became more similar after 120 to 336 h at all sites in the stream Ilm. In flow channel biofilms grown at 0.05 and 0.4 m s^{-1} ciliate community structure became more similar after 168 h. A direct comparison of the biofilm associated ciliate community at the faster flowing outlet and natural sites with flow channel biofilms grown at 0.4 m s^{-1} is not appropriate, due to flow velocity fluctuations in the Ilm during sampling and to a lowered flow velocity inside the cylinders. The high increase in abundance at faster flow velocities after the lag-phase might be due to entrapment of water column ciliates, which is enhanced at higher flow velocities, because more organisms per unit time pass the biofilm (Hunt and Parry 1998). Biofilm structures like microcolonies and ripples observed in 4-day-old biofilms (Battin et al. 2003; Stoodley et al. 1999) may even enhance entrapment of water column ciliates. The increased advection of prey particles at fast flow velocities allows a high carbon turnover by the biofilm associated community.

Effect of sedimentation on colonization dynamics

In flow channel biofilms grown at 0.05 m s^{-1} the majority of suspended ciliates entered the biofilm within 4.5 h. Passive sedimentation of ciliate cells appeared to be negligible, because 63% of ciliates were in the size range of 20 - 50 µm, which is too small for deposition (Lampert and Sommer 1999; Ma et al. 2007; see Hjulstrom diagramm in Schwoerbel and Bredelberger 2005). However, the high load of particles accumulated on the clay tiles after 1 h might enable particle associated sedimentation. Suspended particles which can be colonized by a variety of protists (Wörner et al. 2000; Zimmermann-Timm 2002) can accumulate at sites protected from high flow velocities, e.g. pools or reservoirs (Reynolds and Carling 1991) and serve as inoculum for biofilm colonization. Reservoirs of small weirs can be key zones of transient storage for organic matter (Pohlon et al. 2007). However, loosely attached sediment particles colonized by ciliates might be easily resuspended at hydrodynamically fluctuating conditions in small streams.

Flow channel biofilms treated with high sediment additions showed a negative correlation with the number of ciliate taxa. A positive correlation between abundance and sediment addition was displayed by small (i) filter feeders that utilize bacteria (*D. campylum, U. nigricans*), (ii) gulper feeders that utilize algae and bacteria (*T. potamophilus*, *T. minuta*), and (iii) gulper feeders that utilize other ciliates (*L. lamella*). Larger ciliates preferred biofilms with low sediment contents. Movement of larger ciliates seems to be inhibited by the lack of space in estuarine sediments (Hamels et al. 2005) which might be similar to biofilms with high inorganic sediment load. Thus, besides the mode of food uptake also the ciliate cell size appeared to play a crucial role.

Ciliates morphotypes at different flow velocities

Despite the different surface properties of smooth glass slides and rough clay tiles, the colonization dynamics and the structure of the ciliate community in stream and flow channel biofilms were similar which was displayed in the Soerensen Quotient of 74%.

On the functional group level the effect of flow velocity was negligible. According to ciliate morphotypes, differences suggested that the bacterivorous sessile ciliates and vagile flat gulper feeder, including bacteri-algivorous species (*Trithigmostoma cucullulus,*

Thigmogaster potamophilus) and carnivorous species (*Litonotus* spp.) can tolerate faster flow velocities. Vagile flat ciliates are positively thigmotactic, which is the tendency to settle in an area of maximal and persistent contact to solid surfaces (Hausmann and Hülsmann 1996). Due to their flattened cell shape and thigmotactic cilia (Foissner et al. 1991) they are enabled to stay attached to surfaces also at faster flow velocities. The bacterivorous and carnivorous species of the genus *Litonotus* that are typical stream species (Foissner et al. 1995) were not affected by different flow velocities in this study.

The low abundance of sessile filter feeders at faster flow velocities during the initial colonization process suggested that the attachment of these cells was inhibited. However, in later stages of biofilm development Peritrichia might benefit from the faster ambient flow velocity by filtering the water for drifting bacteria (Fenchel 1986; Shimeta et al. 2001). In more mature biofilms, sessile filter feeders appear to be important for channeling the organic carbon from the water column (Augspurger et al. 2007). Thus, sessile filter feeders contribute to a tight coupling between water column and biofilm (Weitere et al. 2003), and this coupling is particularly important in small streams due to the large stream bed surface area compared to the water column.

Small vagile round ciliates, like the bacterivorous *Uronema nigricans* (filter feeder, 25 - 50 µm) which swims in the surrounding of a biofilm and exploits transitory patches of bacteria (Fenchel 1980), was more abundant at slow flow velocities, but occurred at faster flow velocities in later stages of biofilm development. The vagile round *Cyclidium glaucoma* (14 -30 µm) is one of the dominant species at flow velocities of 0.5 - 0.8 m s^{-1} in biofilms of the River Rhine (Schmitz 1985). The small size of both species might be advantageous in the exploitation of small micro-niches where flow velocities are close to zero. Micro-niches of slow flow velocity formed by the presence of *Ancylus* sp. shells are preferably inhabited by small sized flagellates (15 - 25 µm) (Willkomm et al. 2007). Despite its small size, the bacterivorous vagile flat *Aspidisca lynceus* (filter feeder, 35 - 50 µm) was more abundant at slow flow velocities. The movement with cirri enlarges the distance between cell and surface which might promote detachment. Feeding currents produced by filter feeders especially those by *A. lynceus* that are oriented towards the biofilm might contribute to transport processes of nutrients and gases into biofilms at slow flow velocities. Enhanced O_2 and nutrient transport into the biofilm was shown for vagile as well as sessile filter feeder (Glud and Fenchel 1999; Vopel et al. 2005). The supply of nutrients is further enhanced by the

increased spatial heterogeneity in biofilms (Lawrence and Snyder 1998) due to the feeding on recently attached bacterial cells (Parry 2004).

Thus, morphologically different species with similar food spectra contributed to functional groups at contrasting flow velocities. Pool-riffle structures in small streams increase the habitat diversity and create optimal conditions for different biofilm associated ciliate species. The maintenance of a rich species pool will enhance the resilience (Steiner et al. 2006) of stream ecosystems to recover after perturbations.

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DETACHMENT AND MOTILITY OF BIOFILM ASSOCIATED CILIATES AT INCREASED FLOW VELOCITIES

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Abstract

Though seldom investigated, the microcurrent environment may form a significant part of the ecological niche of protists in stream biofilms. We investigated if specific morphological features and feeding modes of ciliates are advantageous for a delayed detachment at increased flow velocities. Three sessile filter feeders (*Vorticella, Carchesium, Campanella*), six vagile filter feeders (*Aspidisca, Euplotes, Holosticha, Stylonychia, Cinetochilum, Cyclidium*) as well as two vagile gulper feeders (*Chilodonella, Litonotus*) were studied. A rotating disc on top of the culture medium generated different flow velocities in Petri dishes. All tested sessile species stayed attached at the fastest investigated flow velocity (4100 µm s^{-1}). *Vorticella convallaria* (Peritrichia) remained about 45% of the observed time in a contracted state at >2600 µm s^{-1}. Hence, filtration activity of sessile ciliates seemed to be inhibited at fast flow velocities. Among the vagile filter feeders, flattened species which extended more than 60 µm into the water column and round species showed the lowest resistance to fast flow velocities. Only the vagile flattened gulper feeder *Chilodonella uncinata* (Phyllopharyngia) withstood flow velocities ≥2600 µm s^{-1}. Considering the behavior of ciliates, all studied vagile species had a higher displacement rate and showed a positive rheotactic creeping behavior between 300 and 1100 µm s^{-1}. Thus, dispersion and positive rheotaxis might allow vagile species to colonize more favorite habitats and balance the drift caused by the unidirectional flow of water.

Keywords: ciliate morphotypes, biofilm, flow velocity, boundary layer, motility, rheotaxis

Introduction

In stream ecosystems, the majority of bacteria and protists are associated with stationary surfaces that are characterized by the unidirectional flow of water (Geesey et al. 1978, Schwoerbel 1994). The boundary layer at the liquid-solid interface is thought to be an important habitat for the lotic microbial and invertebrate communities (Ambühl 1959, Schwoerbel 1994). Although the flow velocity decreases exponentially at the interface, the laminar flow at the surface can become turbulent due to surface roughness (Oertel et al. 2001). Even within a biofilm, the microcurrent in voids can reach up to 90% of the flow velocity measured 2 mm above the biofilm surface (de Beer et al. 1994, Stoodley et al. 1994). Thus, morphological adaptations of surface associated organisms might be advantageous to avoid drift. Heterotrophic flagellates are detached when the microcurrent increases above certain values (Willkomm et al. 2007).

Biofilm associated ciliates contribute to the carbon and energy transfer from bacteria and protists to the meio- and macrofauna. Due to their grazing activity, ciliates keep bacteria in the exponential growth phase which stimulates the decomposition of coarse particulate matter such as leave litter (Ribblett et al. 2005). The clearance and feeding rates of sedimentary ciliates are positively correlated to increased flow velocities (Shimeta et al. 2001). Besides, flow velocity influences the distribution of ciliates according to their feeding modes. In addition, specific morphological features might be advantageous at fast flow velocities.

Motility of ciliates on surfaces can be described as random walk (Berg 1993), where the direction of movement is randomly related to a gradient. Under still water conditions, ciliates increase the probability to feed in the inhabited food patch by lowering the walking speed and increasing the frequency of tumbling (Jonsson & Johansson 1997, Stock et al. 1997, Lawrence & Snyder 1998, Fenchel & Blackburn 1999). According to the flow direction, ciliates show either positive or negative rheotactic responses (Jennings 1906, Ricci et al. 1992, Ricci et al. 1999). Ciliates motility can be affected by increased flow velocities leading to inhibition of food uptake, or to dispersion out of the preferred food patch. Thus, it is important to study the motility pattern of ciliates at different flow velocities to evaluate the role of microcurrents in the aquatic microbial food web of lotic habitats (Fenchel & Blackburn 1999, Fenchel 2004).

It was the objective of this study to investigate detachment and motility of eleven ciliate species with different morphological features at increased flow velocities. We hypothesized that (1) vagile flattened gulper feeders with cilia reduced to one cell side are not detached at fast flow velocities, and (2) increased flow velocities induce positive rheotaxis and larger displacement rates of all vagile ciliates.

Materials & Methods

Ciliate cultures. Ten ciliate species with different morphological features and attachment mechanisms (Table 1) were isolated or enriched from the stream Ilm (Thuringia, Germany). In the enrichment cultures, flagellates or other ciliates of minor abundance (<20%) were present. One sessile colony forming ciliate species, *Campanella umbellaria* (Peritrichia), was isolated from a small pond at Cologne. *C. umbellaria* developed 1, 4, or 8 heads, whereas *Carchesium polypinum* (Peritrichia) developed 7 - 44 heads. Batch cultures of ciliates were kept at 20 ± 2 °C in Volvic water (Le Dû-Delepierre et al. 1996) with a sterilized rice grain. The chlorophyte *Chlorogonium* sp. (freshwater soil extract medium; kindly provided by K. Eisler, Institute of Zoology, University of Tübingen) was used as additional food source (added twice a week) for cultures of *Euplotes patella* (Hypotrichia), *Stylonychia pustulata* (Stichotrichia), and *Holosticha monilata* (Stichotrichia). *Litonotus cygnus* (Haptoria) was kept in mixed cultures together with *Vorticella convallaria* (Peritrichia) and *Cinetochilum margaritaceum* (Hymenostomatia).

Experimental setup. Petri dishes (Ø 13.5 cm) were filled with 75 ml of ciliate culture to reach a water column height of 0.5 cm. A rotating disc on top of the culture medium generated the flow velocity (Willkomm et al., 2007). The Petri dishes were directly placed under an inverse microscope (Axiovert S100, Zeiss) and ciliates were observed alive. The observation area ranged from a distance of 4 to 6 cm from the Petri dish centre. All observations started at a distance of 4.5 cm from the Petri dish centre. The three sessile species (Peritrichia) were pre-grown on glass slides. Glass slides were fixed with Baysilone (silicon paste, VWR) in the Petri dishes. Ciliate cultures of vagile species were filled into the Petri dishes unfiltered to avoid food limitation during the experiment. A pre-incubation of at least 12 hours served as time of recovery and adaptation to the environmental changes allowing the comparative study of different ciliate species.

The flow velocity was stepwise increased at intervals of 0.1 m s^{-1} (near disc velocity) every 5 s for vagile species and every 2 min for sessile species until the target velocity was reached. The ciliates were observed for a maximum of 5 min (vagile species) or 10 min (sessile species). To minimize artifacts due to adaptation of ciliates to the flow velocity environment (Machemer 1988), at least 30 minutes relaxation time was guaranteed between experiments. Ciliate cells out of three to eight different Petri dishes served as independent replicates. If separate ciliate cells were recorded in one microscopic observation field, they served as independent replicate as well. Cells were observed using phase contrast at 25x magnification, except for *Cinetochilum margaritaceum* and *Cyclidium glaucoma* which were observed at 40x and 100x magnification, respectively. All observations were recorded on videotapes (S-VHS).

The time till detachment, contraction behavior of sessile ciliates, and motility of vagile ciliates were studied at average flow velocities of 300, 1100, 2600, and 4100 µm s^{-1} which corresponded to flow velocities at the rotating disc of 0.1, 0.4, 0.8, and 1.2 m s^{-1} (Willkomm et al. 2007). In addition, intermediate flow velocities of 400, 500, and 700 µm s^{-1} were used for 4 vagile species that could not withstand velocities of ≥1100 µm s^{-1}. Still water conditions served as control. The percentage of cells remaining attached to the surface compared to still water conditions represented the species capacity to withstand detachment at different flow velocities. The elevation of cells above the surface was measured during the experiments to check behavioral adaptations of ciliates using the calibrated fine drive of the microscope.

Microscale flow velocity. Flow velocity along the diameter of the Petri dish (distance from Petri dish centre: 4, 5, and 6 cm) was measured 20 µm above the surface. For this purpose, the speed of 10 particles (10 µm neutrally buoyant hollow glass spheres) (Røy 2003) was estimated from video image sequences of 25 frames s^{-1}. Measurements revealed that the flow velocity in the Petri dishes increased towards the outer edge, especially at the two highest flow velocities. Flow velocity changes were small between 4 and 5 cm from the Petri dish centre. In this area, deviations of the projected flow velocities of 300, 400, 700, 1100, 2600, and 4100 µm s^{-1} ranged from 280 - 420, 340 - 540, 380 - 780, 700 - 1900, 2200 - 3500, and 4000 - 5300 µm s^{-1}, respectively. To assure that faster flow velocities in outer areas did not affect the motility of ciliates, motility tracks were studied in the area between 4 and 5 cm from the Petri dish centre that corresponded to video sequences within 2 min after start of the experiment.

Analysis of ciliate behavior. In case of vagile ciliates, a video sequence of 1 min was analyzed; alternatively, when ciliates detached earlier the whole sequence was used. During the observation time, the number of side stepping reactions (SSR) (Ricci et al. 1992, Barbanera et al. 2000) as a measure of cell orientation of *Litonotus cygnus* (Haptoria), *Euplotes patella* (Hypotrichia), *Stylonychia pustulata* (Stichotrichia), and *Holosticha monilata* (Stichotrichia) was counted. The SSR is characterized by a fast backwards movement followed by a reorientation of the cell (Jennings 1906). The direction of movement was recorded by means of spot (100 x 100 µm) changes towards, away from, or rectangular to the direction of the flow. With these data the relative contribution of each of the four directions was calculated where the sum of all spot changes was set 100%. The net-distance moved in a given time was measured to calculate the displacement rate. Displacement rate and length to width ratio of ciliate tracks demonstrate the effectiveness of a track in displacing the organism in space. Video sequences of the sessile *Vorticella convallaria* (Peritrichia) were examined for the number of contractions within the maximal observation time (10 min). The time needed for cell and stalk extension after contraction was measured with video frame-by-frame analysis (at 25 frames s^{-1}). The duration of the extension was estimated by dividing the number of frames it took the ciliate to extend cell and stalk by the number of frames per second. One contraction of a single *V. convallaria* cell observed during 0 - 2, 4 - 6, and 8 - 10 min after start of the experiment was used to measure the extension time.

Data analysis. The one-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons were used to observe significant differences of the examined behavioral parameters between flow velocity treatments (SPSS 15.0). Data which failed the homogeneity test (Levene-Test, $p < 0.05$) were $\log_{10}(y+1)$ transformed. Pearson correlation coefficients were calculated to test the elevation of cells above the surface and time until detachment at certain flow velocities.

Table 1. Characteristics of investigated ciliate species and relative percentage (%) of cells that remained at the surface at different near surface velocities (µm s^{-1}) during a 10 min (sessile species) or 5 min (vagile specis) observation period (values in brackets represent number of replicates). nd: not determined, s: sessile, cs: contractile stalk, as: acontractile stalk, vf: vagile flattened, vr: vagile round, gf: gulper feeder, ff: filter feeder.

Taxon	Morphological features and feeding modes	Length x Width (µm)	Elevation above surface (µm)	Cells that remained at surface (%) at different near surface velocities						
				0 µm s^{-1}	300 µm s^{-1}	400 µm s^{-1}	600 µm s^{-1}	1100 µm s^{-1}	2600 µm s^{-1}	4100 µm s^{-1}
Peritrichia										
Carchesium polypinum	s, cs, ff	50 - 100 x 40 - 70	1,645 - 2,300	100 (4)	100 (4)	nd	nd	100 (4)	100 (4)	100 (4)
Campanella umbellaria	s, as, ff	135 - 160 x 90 - 120	270 - 1,340	100 (4)	100 (4)	nd	nd	100 (4)	100 (4)	100 (4)
Vorticella convallaria	s, cs, ff	42 - 85 x 34 - 62	100 - 386	100 (16)	100 (16)	nd	nd	100 (16)	100 (16)	100 (16)
Phyllopharyngia										
Chilodonella uncinata	vf, gf	30 - 50 x 15 - 25	23 - 39	100 (8)	100 (11)	nd	nd	100 (10)	87 (8)	25 (8)
Haptoria										
Litonotus cygnus	vf, gf	165 - 230 x 27 - 31	35 - 53	100 (6)	100 (5)	nd	42 (12)	25 (12)	nd	nd
Hypotrichia										
Aspidisca lynceus	vf, ff	43 - 51 x 31 - 39	27 - 41	100 (8)	100 (8)	nd	25 (8)	0 (7)	nd	nd
Euplotes patella	vf, ff	78 - 110 x 47 - 78	50 - 105	100 (8)	0 (12)	0 (17)	0 (6)	nd	nd	nd
Stichotrichia										
Stylonychia pustulata	vf, ff	75 - 130 x 40 - 60	31 - 72	100 (7)	50 (8)		nd	0 (8)	nd	nd
Holosticha monilata	vf, ff	100 - 170 x 43 - 63	82 - 138	100 (4)	0 (4)	0 (10)	nd	0 (4)	nd	nd
Hymenostomatia										
Cinetochilum margaritaceum	vf, ff	34 - 45 x 28 - 34	18 - 35	100 (7)	83 (6)	12 (7)	0 (10)	nd	nd	nd
Cyclidium glaucoma	vr, ff	17 - 22 x 8 - 13	6 - 8	100 (6)	83 (6)	0 (10)	nd	nd	nd	nd

Results

Detachment of vagile ciliates

Chilodonella uncinata (Phyllopharyngia) was the only vagile ciliate that could stand the fastest tested flow velocity of 4100 µm s^{-1} (Fig. 1A). However, 13 and 75% of *C. uncinata* cells detached at 2600 and 4100 µm s^{-1} (Table 1), respectively. Irrespective of flow velocity, cells moved to the outer region of the Petri dish, and detachment times were representative for the time the cells stayed in the projected flow velocity area. At 1100 µm s^{-1}, the flattened *Litonotus cygnus* (Haptoria) stayed longer attached to the surface than *Stylonychia pustulata* (Stichotrichia) and *Aspidisca lynceus* (Hypotrichia). Nonetheless, the majority of cells of *L. cygnus* (58 %) and *A. lynceus* (75 %) detached at flow velocities ≥700 µm s^{-1} (Table 1). Species which extended more than 60 µm above the surface, like *Stylonychia pustulata* (50 %), *Holosticha monilata* (Stichotrichia) (100 %), and *Euplotes patella* (Hypotrichia) (100 %) detached already at 300 µm s^{-1} (Fig. 1B, Table 1). Elevation of cells above the surface of Hypotrichia and Stichotrichia and time until detachment were negatively correlated at flow velocities of 300 (R^2 = -0.996, $p < 0.01$), 700 (R^2 = -0.961, $p < 0.05$), and 1100 µm s^{-1} (R^2 = -0.803, $p = 0.197$). *H. monilata* and *E. patella* successively detached and attached again to the surface of the Petri dish during the observation time. The vagile flattened *Cinetochilum margaritaceum* and the vagile round *Cyclidium glaucoma* (Hymenostomatia) (Fig. 1A) stayed attached for >260 s at 300 µm s^{-1}. All cells of *C. margaritaceum* detached at 700 µm s^{-1}, and all cells of *C. glaucoma* detached at 400 µm s^{-1} (Table 1).

Behavioral responses of vagile ciliates

Displacement rate of *Chilodonella uncinata* (Phyllopharyngia) was significantly higher at 300 µm s^{-1} ($p < 0.05$) and 1100 µm s^{-1} ($p < 0.001$), but not at 2600 µm s^{-1} ($p = 0.142$) and 4100 µm s^{-1} ($p = 0.999$) compared to still water conditions (Fig. 2). Number of spot changes were significantly lower at 4100 µm s^{-1} ($p < 0.01$) compared to still water conditions (Fig. 3). Flow velocity did not affect displacement rate of *Litonotus cygnus* (Haptoria) (Fig. 2), but cells contracted more often (11 - 13 min^{-1}) than at still water conditions (8 min^{-1}). In contrast, vagile species walking with ventral cirri along the surface as *Euplotes patella* (Hypotrichia), *Stylonychia pustulata* (Stichotrichia), and *Holosticha monilata* (Stichotrichia) showed higher

displacement rates and higher number of spot changes at 300 to 1100 µm s^{-1} (Fig. 2, Fig. 3). Both parameters varied greatly displaying large differences between single cells. Side stepping reactions (SSR) in *E. patella* ($F_2 = 6.651$, $p < 0.01$), *S. pustulata* ($F_2 = 19.156$, $p < 0.001$), and *H. monilata* ($F_3 = 0.159$, $p = 0.922$) increased as well at flow velocities ≥300 µm s^{-1} (Table 2) compared to still water conditions. The elevation of *E. patella* above the surface was significantly reduced ($p < 0.01$) at 300 µm s^{-1} (Fig. 4). Displacement rates ($p < 0.01$) and number of spot changes ($p > 0.05$) of *Aspidisca lynceus* (Hypotrichia) were higher at 700 and 1100 µm s^{-1} compared to still water conditions and 300 µm s^{-1}.

The behavioral responses of the dorso-ventrally flattened *Cinetochilum margaritaceum* and round *Cyclidium glaucoma* (Hymenostomatia) were not altered at 300 µm s^{-1}. *C. margaritaceum* showed similar responses as vagile flattened species walking with cirri, displaying a larger displacement rate ($p = 0.343$) at 700 µm s^{-1} compared to still water conditions (Fig. 2). *C. glaucoma* stayed at the surface and rarely moved at still water conditions remaining 150 s out of 300 s in one spot of 100 x 100 µm. During flow velocity treatments, many of the observed cells oriented their anterior-posterior axis in the flow direction. Length to width ratios of all observed ciliate tracks increased due to flow velocity impact from a mean of 1.9 - 3.1 to 4.9 - 14.0, except tracks of the rarely creeping *C. glaucoma*.

A positive rheotactic response showed *Chilodonella uncinata* (Phyllopharyngia), *Litonotus cygnus* (Haptoria), *Euplotes patella* (Hypotrichia), *Stylonychia pustulata*, *Holosticha monilata* (Stichotrichia), and *Cinetochilum margaritaceum* (Hymenostomatia) at 300 µm s^{-1} with 33 to 44% of spots changed against the flow direction (Fig. 3). At 1100 µm s^{-1} significantly more spots were changed against the flow direction by *C. uncinata* (68 %, $p < 0.05$) and *Aspidisca lynceus* (Hymenostomatia) (52 %, $p < 0.01$). *C. uncinata*, *L. cygnus*, *S. pustulata*, *H. monilata* (data not shown), and *E. patella* were sidetracked and crept mainly in a 90° angle in relation to the flow direction at the corresponding fastest flow velocity of each species. The main movement of cells pointed towards the outer region of the Petri dish, but a greater percentage of spot changes of *L. cygnus* pointed towards the inner region of the Petri dish.

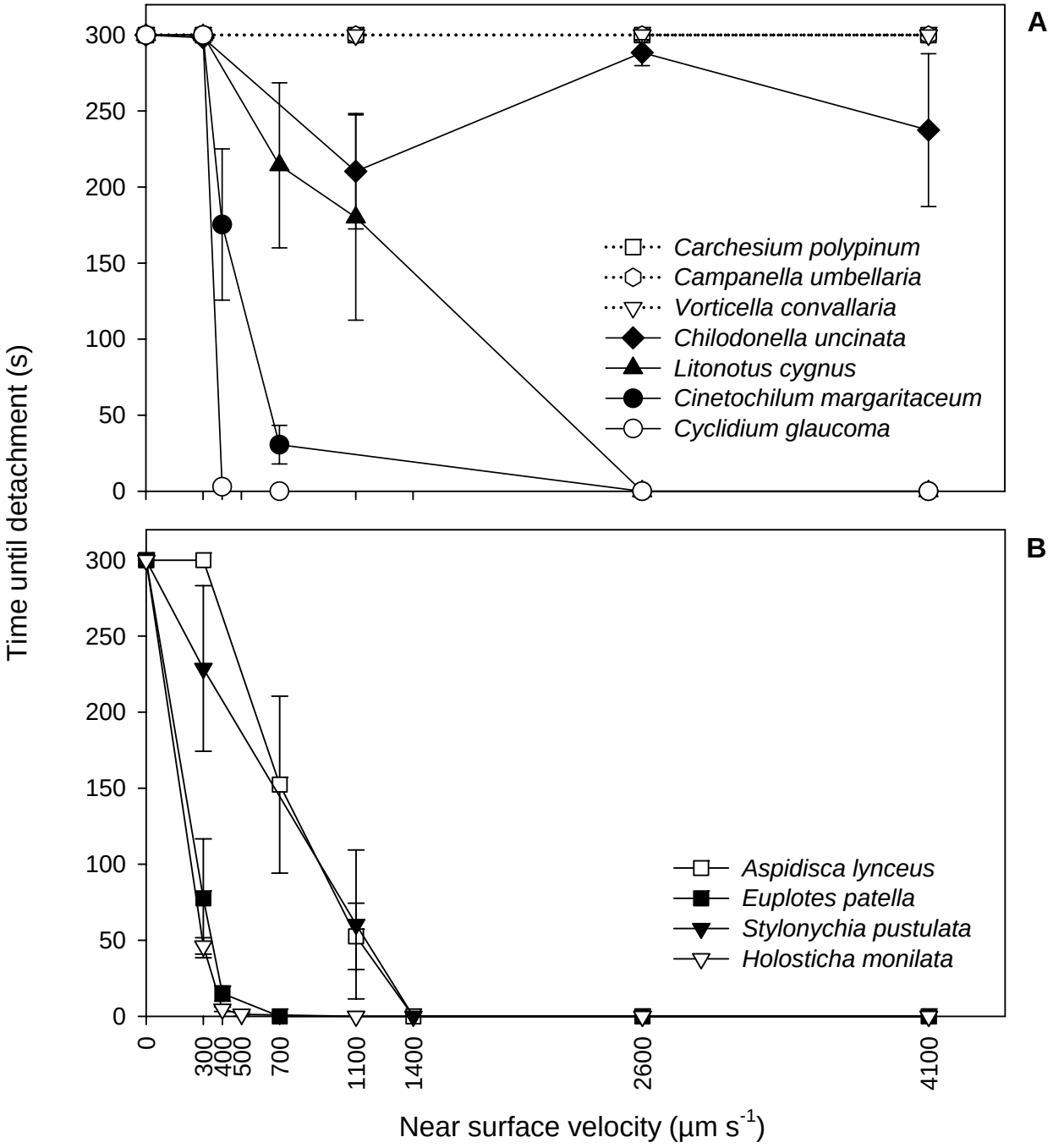

Fig. 1. (A) *Vorticella convallaria*, *Carchesium polypinum*, *Campanella umbellaria*, *Chilodonella uncinata*, *Litonotus cygnus*, *Cinetochilum margaritaceum*, and *Cyclidium glaucoma*, (B) *Aspidisca lynceus*, *Euplotes patella*, *Stylonychia pustulata*, and *Holosticha monilata*. Time (s) until detachment of investigated ciliate species at different flow velocities (µm s^{-1}) (mean ± SE, n = 3 - 8). The maximum investigation time was 5 min (vagile ciliates), or 10 min (sessile ciliates).

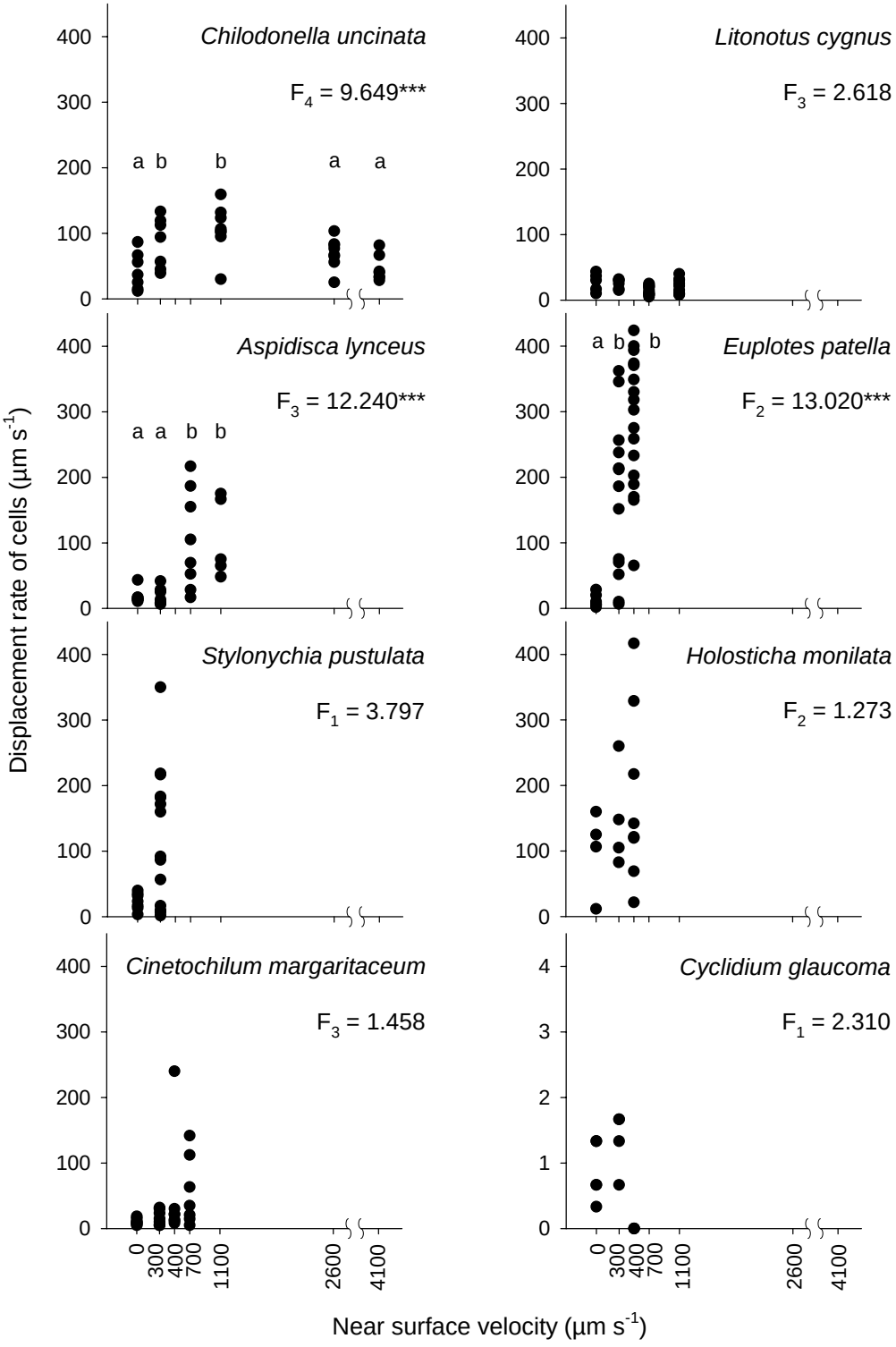

Fig. 2. *Chilodonella uncinata, Litonotus cygnus, Aspidisca lynceus, Euplotes patella, Stylonychia pustulata, Holosticha monilata, Cinetochilum margaritaceum,* and *Cyclidium glaucoma.* Displacement rate (µm s^{-1}) of ciliate cells at different near surface velocities. F-values represent results of a one-way ANOVA to test the impact of flow velocity on displacement rate (*** $p < 0.001$); [ab] Significant differences to control and between neighboring velocity treatments ($p < 0.05$), outliers were excluded from the analyses.

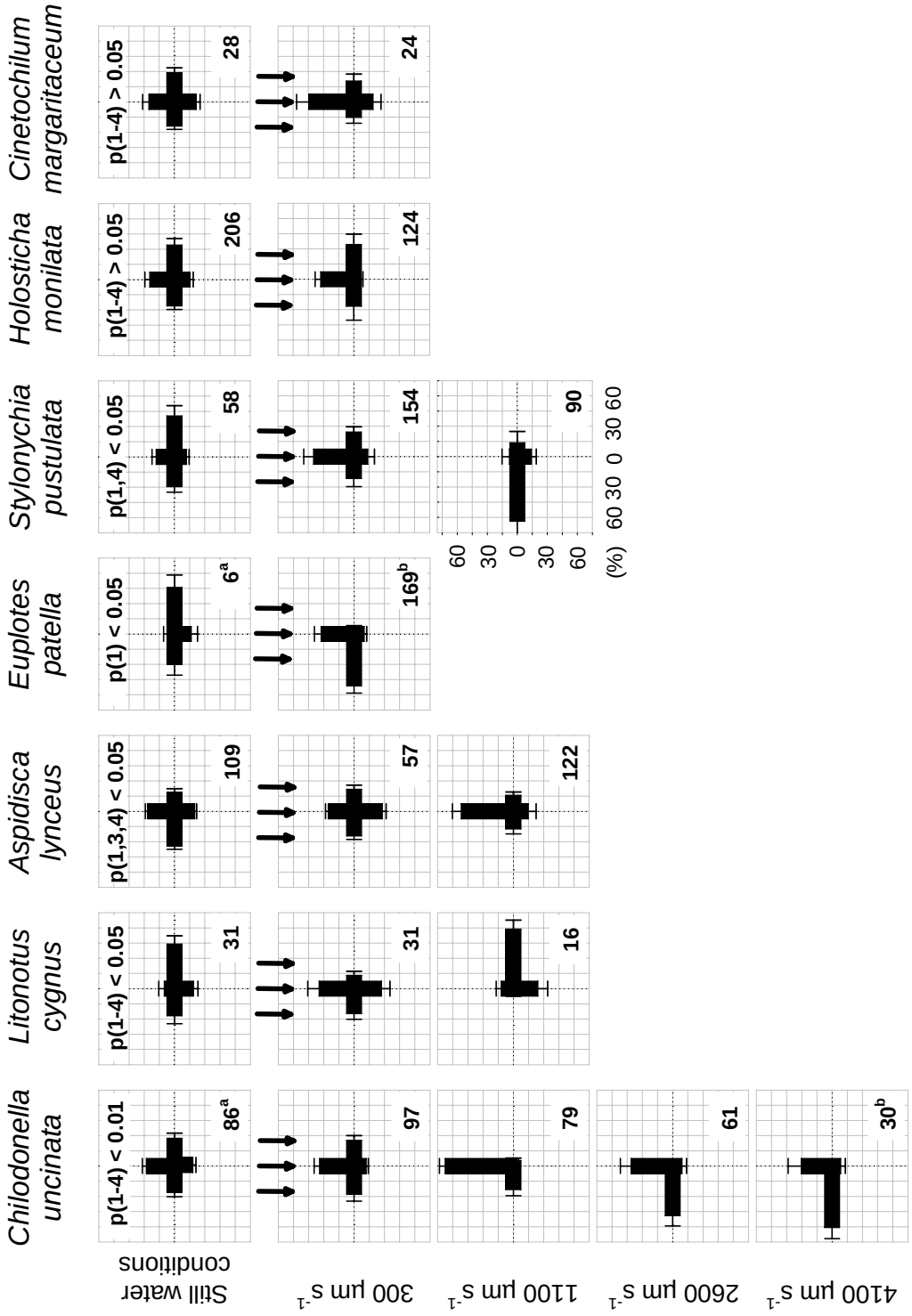

Fig. 3. Creeping direction of Chilodonella uncinata, Litonotus cygnus, Aspidisca lynceus, Euplotes patella, Stylonychia pustulata, Holosticha monilata, and Cinetochilum margaritaceum in response to different near surface velocities shown as percentage distribution when spots were changed (mean ± SE). Black arrows: flow direction; flow velocity: (a) at rotating disc (m s^{-1}) and (b) 20 µm above the surface (µm s^{-1}); median of spot changes (min^{-1}): lower right corner of graphs; ^{ab} significant differences of mean spot changes between treatments ($p > 0.05$); p-values in the upper graphs represent significant differences between control and flow velocity treatments of one creeping direction at different near surface velocities; creeping directions: 1 - against flow direction, 3 - with flow direction, 2 and 4 - 90° in relation to flow direction.

Table 2. Number of side stepping reactions per minute (min^{-1}) of four tested ciliate species shown as median (n = 2 - 17) and quartile range (25 - 75%) at different near surface velocities. nd: not determined; [abc] significant differences between velocity treatment and control, and between neighboring velocity treatments ($p < 0.05$), outliers were previously excluded from the analyses.

	Near surface velocity (µm s^{-1})					
	0	300	400	500	700	1100
Litontous cygnus	8.8	11.5	nd	nd	11.5	13.5
	6.6 - 9.8	7.9 - 14.0			6.8 - 14.5	9.0 - 15.8
Euplotes patella	2.0[a]	15.2[b]	23.1[b]	nd	nd	nd
	1.5 - 7.0	9.5 - 19.0	8.3 - 38.2			
Stylonychia pustulata	11.5[a]	37.0[b]	nd	nd	nd	102.5[c]
	4.8 - 13.0	21.5 - 49.0				101.9 - 130.9
Holosticha monilata	19.3	22.7	33.1	41.7	nd	nd
	14.5 - 24.4	18.8 - 27.5	0.0 - 46.9	20.8 - 62.5		

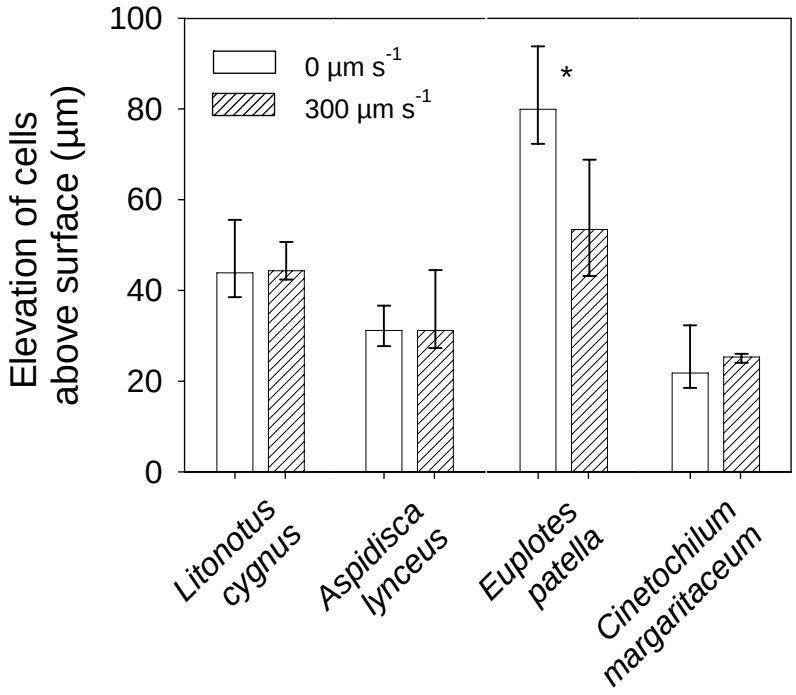

Fig. 4. *Litonotus cygnus*, *Aspidisca lynceus*, *Euplotes patella*, and *Cinetochilum margaritaceum*. Elevation (µm) of cells above the surface observed during still water and 300 µm s^{-1} (median with 25th and 75th percentile). * Significant differences between treatments, outliers were excluded from the analyses ($p < 0.05$).

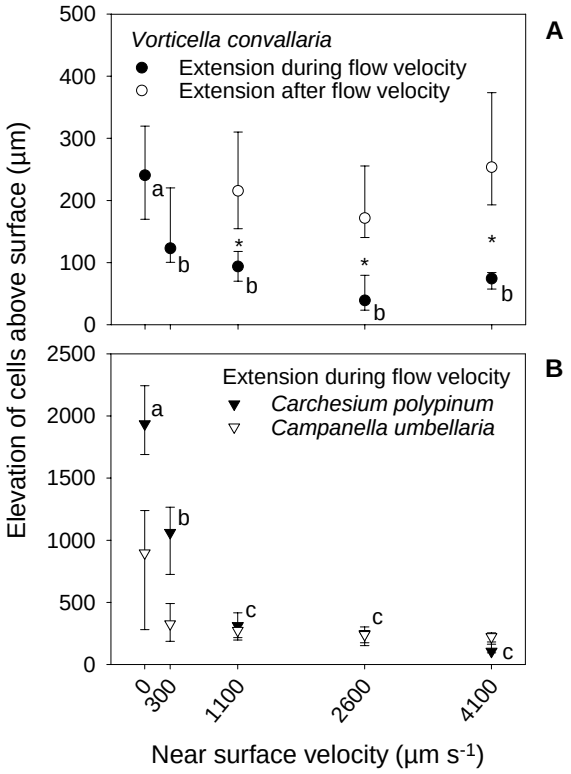

Fig. 5. (A) *Vorticella convallaria*, (B) *Carchesium polypinum,* and *Campanella umbellaria*. Elevation (µm) of cells above the surface observed during different flow velocity treatments (box: median with 25th and 75th percentile). [ab] Significant differences to control and between neighboring velocity treatments, outliers were excluded from the analyses ($p < 0.05$); * significant differences between flow velocity treatment and recovery ($p < 0.05$).

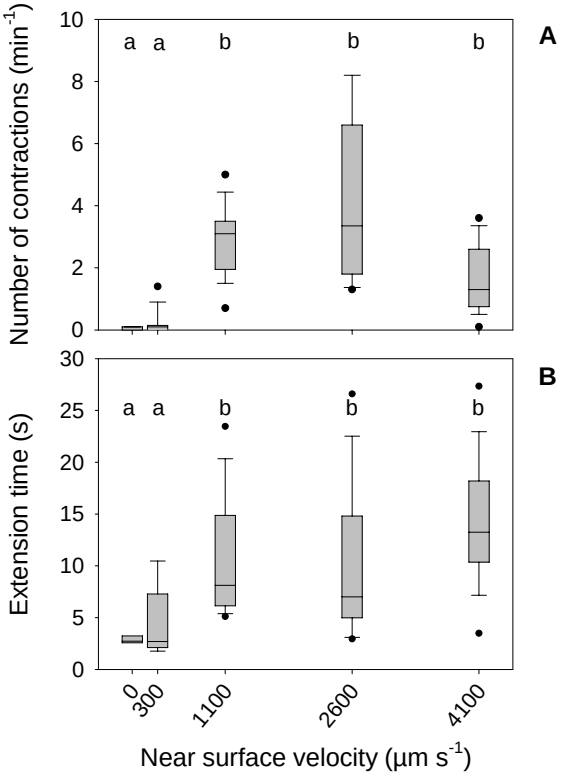

Fig. 6. *Vorticella convallaria.* (A) Number of contractions (min^{-1}) and (B) extension time (s) at different near surface velocities (box: median with 25th and 75th percentile, dots: 99th percentiles). [ab] Significant differences to control and between neighboring velocity treatments, outliers were excluded from the analyses ($p < 0.05$).

Detachment and contraction of sessile ciliates

The sessile *Carchesium polypinum, Campanella umbellaria,* and *Vorticella convallaria* (Peritrichia) stayed attached at all tested flow velocities during the 10 min observation (Fig. 1A). Even if the velocity was increased from 0 to 4100 µm s^{-1} within 30 sec, stalks did not detach from the surface. All studied Peritrichia species were lying on the surface resulting in a significant lower elevation above the surface during flow velocity treatments compared to still water conditions (Fig. 5). Cells of *V. convallaria* regained their former elevation after the flow was stopped. In still water conditions and at 300 µm s^{-1} *V. convallaria* contracted

0.05 min^{-1}, and the extension of the cell body and stalk lasted 2.7 s (Fig. 6). Higher flow velocities caused significantly longer extension times with a mean of 11.6 s ($F_4 = 11.755$, $p < 0.001$) and contractions occurred 60-times more often ($F_4 = 21.788$, $p < 0.001$) compared to still water conditions and 300 µm s^{-1}. Extension times at the beginning and end of the observation time were not significantly different.

Discussion

Flow velocity conditions and food supply at surfaces

In the 5 mm water column of the Petri dish, laminar flow occurs in the lower 700 µm layer from the dish surface at a disc flow velocity of 0.3 m s^{-1} (Willkomm et al. 2007). However, it is not trivial to define the exact flow velocity at the position of a single surface associated protist cell in rotating water. The presence of vagile ciliates with a maximum elevation of 140 µm increased the surface roughness and might have caused small turbulent eddies especially at faster flow velocities. Thus, we will not compare absolute values of flow velocities in the Petri dish with velocities in streams, but focus on the comparison of the behavior of the ciliate species tested. Species with a low elevation above the surface (<40 µm) like *Aspidisca lynceus* (Hypotrichia) had a higher resistance to faster flow velocities than species with a higher elevation into the water column like e.g. *Holosticha monilata* (Stichotrichia) (<140 µm). Thus, the elevation of cells above a surface was a critical factor to resist fast flow velocities. Additionally, small species like *A. lynceus* (Hypotrichia) and *Cyclidium glaucoma* (Hymenostomatia) (Table 1) withstood 3 and 10times faster flow velocities, respectively, when cells stayed behind or even within biofilm flocks. The flow velocity is highly reduced in the vicinity of bacterial microcolonies or behind snail shells (de Beer et al. 1994, Willkomm et al. 2007). A small cell size seemed to be advantageous in the exploitation of biofilm micro-niches.

Surface roughness of stones increases the retention of organic matter (Huettel et al. 1996), which can serve as a food source for biofilm associated ciliates. Fast flow velocities near biofilms and in biofilm voids minimize the thickness of the diffusion boundary layer and enhance nutrient and gas exchange (de Beer et al. 1994, 1996). It is advantageous for ciliates to colonize biofilms at faster flow velocities because prey advection is enhanced (Hunt &

Parry 1998, Shimeta et al. 2001), and bacteria are kept in a productive state due to enhanced nutrient transport from the water column into the biofilm (Kaplan & Newbold 2003).

Detachment of different ciliates' morphotypes at increased flow velocities

The three sessile Peritrichia stayed firmly attached to the surface at fast flow velocities. Peritrichia are known to withstand storm flows, although stalks show traces of abrasion after 12 h (Blenkinsopp & Lock 1994). However, the abundance of Peritrichia was negligible in initial biofilms grown at fast flow velocities in flow channel experiments and also in the stream Ilm (data not shown). Thus, attachment and stalk anchorage on virgin surfaces appears to be inhibited at fast flow velocities. In the present study, attached *Vorticella convallaria* (Peritrichia) remained about 45% of the observed time in a contracted state at >2600 µm s^{-1}. The cell and stalk contraction resulted in a lower filtration activity. Under conditions of inhibited food uptake swarmers are formed that can be dispersed to find more suitable habitats. Increased number of Peritrichia heads are found at the beginning of high water situations in the River Rhine, indicating disruption of sessile ciliates due to changing conditions of flow velocity (Scherwass & Arndt 2005). Thus, attached filter feeder might contribute only up to certain flow velocities to organic carbon channeling from the water column into stream biofilms.

Besides cell attachment by stalks, also the flattened cell shape of vagile gulper feeders facilitated a high resistance to withstand detachment at fast flow velocities. *Chilodonella uncinata* (Phyllopharyngia) dominates biofilms at faster flow velocities in the River Rhine (Schmitz 1985). Morphologically similar species i.e. *Trithigmostoma cucullulus* (Phyllopharyngia) and *Litonotus lamella* (Haptoria) tolerated faster flow velocities in biofilms of the stream Ilm (data not shown). The vagile flattened gulper feeder *C. uncinata* might avoid detachment either by creating a vacuum on the ventral side or by special cilia that produce a kind of adhesive substance. This strategy might enable vagile flattened species to survive in their preferred patch during the frequently occurring flood events in streams. Thus, they probably contribute to initial biofilm communities after flood events.

A lower resistance to faster flow velocities showed the vagile flattened and round filter feeders. Food limitation, which increases the propensity to leave the surface (Jonsson & Johansson 1997) can be neglected, since a bacterial biofilm was developed at the Petri dish

surface during the adaptation time (≥12 h). However, the movement of vagile flattened filter feeders with cirri enlarges the distance between cell and surface, which might cause cell detachment already at slow flow velocities.

Behavioral changes of vagile ciliates at increased flow velocities

The behavior of ciliates is not just cell motility but the adaptive behavior to the given environmental conditions (Fenchel 1987, Ricci 1989) to position themselves within the survival limits of their own biology (Meyer & Guillot 1990). The vagile flattened filter feeder *Euplotes* sp. (Hypotrichia) has a higher probability to feed in a inhabited food patch by lowering the walking speed and increasing the frequency of tumbling (Jonsson & Johansson 1997, Stock et al. 1997, Lawrence & Snyder 1998, Fenchel & Blackburn 1999). Actually, all studied vagile ciliates except the seldom creeping *Cyclidium glaucoma* (Hymenostomatia) showed distinct changes in their motility under the impact of flow velocity. The vagile flattened filter feeders *Euplotes patella* (Hypotrichia) and *Holosticha monilata* (Stichotrichia) repeatedly attached to the surface and resumed walking after detachment. *Euplotes* sp. efficiently uses tidal currents to disperse and to exploit patchily distributed food sources (Jonsson & Johansson 1997). This strategy might also be important in streams where flow velocity changes around stones cause a patchy distribution of food sources.

The straightened tracks and higher displacement rates at 300 - 1100 µm s^{-1} might enable ciliates to rapidly colonize adjacent patches protected against fast flow velocities, i.e. eddy waters behind invertebrate cages (Stiller 1957) and biofilm microstructures (Willkomm et al. 2007). These eddy water zones are accumulation zones (Silvester & Sleigh 1985) where also food sources for ciliates might accumulate. Thus, a high dispersion at increased flow velocity favors vagile flattened ciliates to find rapidly undepleted food patches.

A positive rheotactic response was observed for all ciliates between 300 and 1100 µm s^{-1}. In some ciliates, the movement towards the flow direction was assured with the help of numerous side stepping reactions (Stichotrichia, Hypotrichia). Due to the orientation of the cell's anterior-posterior axis in flow direction, the usual orientation of the cilia is maintained (Jennings 1906). Positive rheotaxis in *Uronychia setigera* (Hypotrichia) enables the cell to resist the tidal water currents and remain in their preferred patch (Ricci et al. 1999). Thus, losses due to downstream drift in streams might be balanced by a positive rheotaxis.

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PROTISTS WITH DIFFERENT FEEDING MODES INFLUENCE MORPHOLOGICAL BIOFILM CHARACTERISTICS

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Abstract

Grazing activity of protists contributes to morphological changes of biofilms. Since the lifestyle and food uptake of gulper feeders is strongly associated to surfaces, gulper feeders may influence biofilm morphology more than filter feeders. We investigated the impact of *Dexiostoma* (filter feeder), *Vannella, Chilodonella* (gulper feeder), *Spumella*, and *Neobodo* (interception feeders) on the morphology of multispecies bacterial biofilms. Vagile protists such as *Dexiostoma campylum* (Hymenostomatia, Ciliophora) and *Chilodonella uncinata* (Phyllopharyngia, Ciliophora) stimulated microcolony formation about 3.5 - 4times. Microcolony abundance was not altered by the sessile *Spumella* sp. (Chrysophyceae, Chrysophyta). Thus, the stimulation of microcolony formation seemed to be influenced by the mobility of grazing protists. *Vannella* sp. (Vannellida, Gymnamoeba) efficiently grazed bacteria from the biofilm surface leading to a lower microcolony size, maximal and basal layer thickness compared to ungrazed biofilms. Indicated by the high porosity within microcolonies, interception feeder utilized bacteria from the central part of microcolonies that were deeply embedded in exopolymer matrix. Biofilm volume was 2.5 - 6.3 times lower in the presence of the gulper and interception feeders. These protists caused a 1.5 - 3.7 times higher biofilm porosity and a 1.2 - 1.8 times higher biofilm surface area to biofilm volume ratio (BSA / BV). Both might improve exchange of nutrients and gases between the biofilm and its surrounding fluid, hence accelerating microbial growth.

Keywords: flagellates, ciliates, amoeba, biofilm morphology, bacterial biofilm

Introduction

The predominant microbial life modes in streams are biofilms, which are aggregations of bacteria and protists embedded in a matrix of exopolymeric substances (Geesey *et al.*, 1978, Lock *et al.*, 1984, Costerton *et al.*, 1994). In streams, biofilm bacterial activity can be higher than the activity of suspended bacteria (Fletcher, 1986, Romaní & Sabater, 1999). The morphology of biofilms either cultivated or in streams is complex and highly structured with tower- and mushroom shaped microcolonies and intersected open water channels (Lawrence *et al.*, 1991, Møller *et al.*, 1997, Stoodley *et al.*, 1999, Battin *et al.*, 2003). Liquid flow in biofilm channels maintains nutrient and gas exchange between the biofilm and its surrounding fluid (de Beer *et al.*, 1994, Stoodley *et al.*, 1994, de Beer *et al.*, 1996). Especially those channels that reach deep into the biofilm matrix maintain exchange processes also at the basal biofilm layer. The above mentioned exchange processes are enhanced at a high porosity (biofilm free area at a surface) and a high biofilm surface area (BSA) in relation to the biofilm volume (BV).

Biofilm structure and dynamics are controlled by physical and chemical conditions of the water column (Battin *et al.*, 2003, Costerton, 2007) but also by protists grazing activity (Pederson, 1990, Arndt *et al.*, 2003). Bacterial stasis is prevented by grazing protists and the bacterial community is kept in a productive state (Johannes, 1965). Protists can reduce the bacterial biomass in biofilms (Weitere *et al.*, 2005) as well as alter biofilm morphology due to grazing (Lawrence & Snyder, 1998, Matz *et al.*, 2004, Huws *et al.*, 2005, Weitere *et al.*, 2005, Queck *et al.*, 2006) and possibly motility (Jackson & Jones, 1991). Filter feeders of the Hymenostomatia that concentrate suspended and probably recently attached prey by producing strong feeding currents (Fenchel, 1986, Parry, 2004) frequently occur at high abundances in stream biofilms (Risse-Buhl & Küsel, subm.). Cells commonly leave the biofilm changing to a planktonic lifestyle. However, mainly the impact of the vagile filter feeding ciliate *Tetrahymena* sp. on biofilm morphology was studied (Weitere *et al.*, 2005, Parry *et al.*, 2007). Since the lifestyle and food uptake of gulper feeders is strongly associated to surfaces (Fenchel, 1986, Hausmann, 2002), gulper feeders may influence biofilm morphology more than the investigated filter feeders.

Similar to planktonic bacteria (Hahn *et al.*, 2000, Hahn & Höfle, 2001), also biofilm bacteria are known to develop defense strategies against grazing protists. Bacterial cell aggregations called microcolonies and quorum sensing mediated production of toxins are efficient grazing resistance mechanisms against flagellate and amoeba grazing, respectively (Matz *et al.*, 2004, Weitere *et al.*, 2005). Impact of grazing protists on biofilm bacteria was studied with single species bacterial biofilms (Matz *et al.*, 2002, Matz *et al.*, 2004, Weitere *et al.*, 2005, Queck *et al.*, 2006). In contrast, stream biofilms are usually composed of mixed bacterial communities. In our experiments, we studied the impact of filter, gulper and interception feeding protists on the three-dimensional morphology of multispecies bacterial biofilms. We hypothesized that gulper-feeding ciliates stimulate microcolony formation and increase the BSA / BV ratio of multispecies bacterial biofilms.

Materials and methods

Protists and bacterial cultures

Protists with different feeding modes that are typical biofilm colonizers of small streams (Foissner *et al.*, 1992, Schönborn, 1996, Franco *et al.*, 1998, Willkomm, 2007, Risse-Buhl & Küsel, subm.) were used in the experiments (Table 1). The amoeba *Vannella* sp. was isolated from biofilm samples of the 3rd order stream Ilm (50°44'58"N, 11°02'14"E). Since the isolation of the ciliates *Dexiostoma campylum* and *Chilodonella uncinata* from stream samples was not successful, K. Eisler (Institute of Zoology, University of Tuebingen) kindly provided a culture of *Dexiostoma campylum* that contained the vagile ciliate *Chilodonella uncinata* and the sessile flagellate *Spumella* sp. as well. *Dexiostoma campylum* and *Spumella* sp. were separated from the original culture. *Spumella* sp. was not successfully separated from *Chilodonella uncinata* and experiments were run as two (*Chilodonella uncinata* + *Spumella* sp.) and single (*Spumella* sp.) species treatments. Protists cultures were kept at 20 ± 2 °C in Volvic table water with 5 mg l^{-1} yeast extract (Fluka; DOC: 3.65 ± 0.1 mg l^{-1}) (VYE medium) and transferred into fresh medium every two weeks. Filtration (5 µm cellulose nitrate filter, Sartorius) of protists cultures was necessary one day before experiments started to enrich protists and to eliminate bacteria from the original culture. Over night, the filtrated protists could recover and further minimize the remaining bacteria by grazing. Fixed (Lugol's

solution) protists were enumerated in Sedgewick Rafter Chambers at 100x magnification (Axioplan, Zeiss, Jena, Germany).

The multispecies bacterial community that developed in VYE medium without protists after a three-day incubation period was used in the experiments (VYE-medium bacteria). Before inoculation, the bacteria were counted after staining with 4'6-diamidino-2-phenylindole (DAPI) (Porter & Feig, 1980) (samples fixed with Formaldehyde, final concentration: 2%) at 1000x magnification (Axioplan, Zeiss, Jena, Germany).

Table 1. Characteristics of the investigated protists species with different feeding modes.

Species	Length (µm)	Lifestyle	Feeding mode[3]	Food source[4]	Initial protists abundance (ml^{-1})	Source
Dexiostoma campylum Ciliophora (Hymenostomatia)	35 - 90[1]	Frequent change between plankton and biofilm	Filter feeder	Suspended and recently attached bacteria	505	Isolated from mixed culture
Vannella sp. Gymnamoeba (Vannellida)	25 - 70[2]	Biofilm	Gulper feeder	Recently attached and embedded bacteria	555	Stream Ilm
Chilodonella uncinata Ciliophora (Phyllopharyngia)	25 - 70[1]	Biofilm	Gulper feeder	Recently attached and embedded bacteria	510[5] 337[5]	Isolated from mixed culture
Spumella sp. Chrysophyta (Chrysophyceae)	5 - 15[2]	Biofilm	Direct interception feeder	Suspended, attached and embedded bacteria	4660[5] 2650[5]	Isolated from mixed culture
Neobodo designis Kinetoplastea (Neobodonida)	5 - 10[2]	Biofilm	Direct interception feeder	Suspended, attached and embedded bacteria	n.d.[6]	Stream Ilm

[1] Length of ciliates from Foissner et al. (1991, 1994)
[2] Length of flagellates and amoeba after own measurements
[3] From Hausmann (2002); Boenigk & Arndt (2002); Hausmann & Hülsmann (1996)
[4] Food source from Parry (2004)
[5] Upper and lower number indicate initial protists abundance for experiments with VYE-medium bacterial biofilm and stream bacterial biofilm, respectively.
[6] About 20% (Cynar *et al.*, 1985) of *Neobodo designis* in stream water samples passed the filters.

Impact of protists with different feeding modes on the morphology of bacterial biofilms

Biofilms were cultivated in three channel (40 x 4 x 4 mm) flow cells made of acryl glass (Møller *et al.*, 1998). A peristaltic pump (Ismatec Sa, Wertheim-Mondfeld, Germany) maintained a continuous discharge of 110 µl min^{-1}. The flow velocity between 50 and 500 µm above the cover slip ranged between 25.7 ± 4.2 and 182.3 ± 64.8 µm s^{-1} indicating laminar

flow conditions with Reynolds numbers of <1. The flow guaranteed a continuous supply of nutrients, organic carbon (yeast extract) and gases. Bacterial biofilms developed at the cover slip that sealed the flow cells. Bubble traps ahead of the flow cells entrapped destructive air bubbles. Flow channels were sterilized with a sodiumhypochloride solution (NaOCl, 0.5%) for 4 h and were washed with sterilized, distilled water over night. Sterilized VYE medium was pumped through the flow channels for 1 h before experiments started. Flow channels were inoculated with a bacterial suspension of the VYE-medium bacteria at concentrations of 1.1 - 3.0 x 10^7 cells ml^{-1}. Bacteria in flow channels were left for 2 h without flow to allow their settlement and attachment to the glass slide. Experiments were performed at a temperature of 23 ± 2 °C and a light regime of 15 h light / 9 h dark cycle. After 1 day of biofilm formation, protists were introduced into the flow channels by syringe (for abundances see Table 1) and were incubated without flow for 2 h. Since bacteria were introduced into the system with the introduced protists, a corresponding concentration of bacteria (0.1 - 20.7 x 10^6 bacteria ml^{-1}) was added to ungrazed biofilm treatments. Experiments run for a total of 5 days.

For every treatment, six separate flow channels served as independent replicates. The whole flow cell set-up was directly placed under an inverse microscope (Axiovert, Zeiss, Jena, Germany). With the calibrated fine drive of the microscope, the maximal biofilm thickness was measured daily. Concomitant, protists and microcolonies were enumerated. Microcolonies were defined as aggregations of bacterial cells with an approximately circular base and a diameter of >10 µm.

The bacterial biofilm was fixed with 4% formaldehyde solution that was slowly pumped through flow channels with the peristaltic pump to minimize alteration of the biofilm morphology. Six flow channels were fixed before the protists were inoculated to observe the initial biofilm structure. All other flow channels were fixed after four days of protists grazing activity. After fixation, the bacterial biofilm was stained with the nucleic acid marker propidium iodide (0.3 µM) (Sigma Aldrich) for 10 minutes in the dark. A washing step with PBS buffer removed excess of stain. Stained biofilms were observed with a confocal laser scanning microscope (LSM 510, Zeiss, Jena, Germany). Three-dimensional picture-stacks (z-stacks) were taken at three randomly chosen spots in each flow channel at a magnification of 400x (objective: Appochromat 1.2 W corr) using a helium neon laser with a vertical solution of 0.5 µm.

Impact of protists on the morphology of a stream bacterial biofilm

Stream bacteria were collected at the third order stream Ilm (50°44'58"N, 11°02'14"E) in August 2007 (abiotic parameters of the stream: temperature 16.2 °C, oxygen content 8.8 mg l^{-1}, pH 8.1, conductivity 0.23 mS cm^{-1}, PO_4^{3-} 0.5 mg l^{-1}, NO_3^- 11.2 mg l^{-1}, NH_4^+ <0.001 mg l^{-1}, DOC 5.49 mg l^{-1}). In the lab, stream water (400 ml) was filtrated through 0.45 µm cellulose acetate filter (Sartorius, Goettingen, Germany) to exclude protists and metazoans. Stream bacteria at an abundance of 5.8 x 10^5 cells ml^{-1} were introduced into the sterilized and washed flow channels as described above. Before bacteria were introduced, the flow channels were washed with sterilized and filtered (0.2 µm) stream water. To study the effect of different protists combinations on biofilm morphology, biofilms were co-cultivated with *Chilodonella uncinata, Spumella* sp. and *Neobodo designis* (for abundances see Table 1). *Chilodonella uncinata* and *Spumella* sp. were introduced into flow channels as described above. The flexible flagellate *Neobodo designis* (length x width: 6.2 x 3.6 µm) passed the filters probably through overlapping pores (Cynar *et al.*, 1985) and were introduced into the flow channels together with the bacterial filtrate. *Neobodo designis* is commonly found in stream biofilms and contributes with about 40% to the flagellate community (Willkomm, 2007).

Impact of protists abundance on biofilm morphology

The aim of this experiment was to evaluate the effect of the gulper feeder *Vannella* sp. at different abundances on the maximal biofilm thickness and microcolony abundance. A gulper feeding amoeba was chosen since they drastically reduce biofilm biomass and microcolony abundance (Weitere *et al.*, 2005). Bacteria that developed in VYE medium after three days were inoculated in sterilized and washed (procedure as described for flow cells) 24 well tissue culture plates at 1.7 x 10^7 cells ml^{-1}. After 1 day of biofilm formation, the gulper feeding amoeba *Vannella* sp. was added at concentrations of 10, 100, and 300 cells ml^{-1}. Per treatment, five wells served as independent replicates. Biofilm thickness, abundance of microcolonies and protists were observed daily for a period of 4 days with an inverse light microscope (Axiovert 25, Zeiss, Jena, Germany).

Image analysis

Three-dimensional image analyses were accomplished with the 'daime' software (Daims *et al.*, 2006). Images were edited before the analyses. Voxels with a brightness range between 0 and 12 and with less than five non-zero (not black) neighbor voxels were deleted before z-stacks were masked to give all non-zero voxels the maximum intensity. Images were segmented (2D or 3D) to define objects and to delete auto-fluorescent objects or protists. Analyzed parameters included the basal layer thickness (µm), the biofilm volume (BV) ($\mu m^3\ cm^{-2}$), and biofilm surface area (BSA) ($\mu m^2\ cm^{-2}$). The latter two parameters were used to calculate the biofilm surface area to biofilm volume (BSA / BV) ratio. From a two-dimensional image at the biofilm base, the porosity (biofilm free area at the surface of the cover slip) ($\mu m^2\ cm^{-2}$), microcolony area (μm^2) and porosity within microcolonies in relation to microcolony area (PM / MA) ($\mu m^2\ \mu m^{-2}$) were estimated. The basal biofilm layer was measured with the Zeiss LSM Image Browser (CZ Image Browser 4.0, offline version) after a new image stack displaying the biofilms side (xy) view was generated.

Statistical analysis

The time series of maximal biofilm thickness measurements and microcolony abundances were analyzed using a repeated measures analysis of variance (ANOVA) with the time as within subject factor and ungrazed and grazed biofilm treatments as between subject factor (SPSS 15.0). A one-way ANOVA with the Tukey's test for multiple comparisons was used to test for differences of biofilm characteristics between ungrazed (1 and 5 days) and grazed biofilms (5 days). Data were log10(x+1) transformed for the one-way ANOVA approach due to lack of homogeneity (Levene’s Test).

Results

Impact of protists with different feeding modes on the morphology of bacterial biofilms

Protists abundances increased with time (Fig. 1) indicating that all species were able to maintain growth in flow cells. After a lag-phase of three days, abundance of the filter feeder *Dexiostoma campylum* increased and reached $1.3 \pm 0.03 \times 10^4$ cells cm^{-2}. The abundance of *Vannella* sp. increased rapidly in the first days and reached the maximum of 5.1×10^4 cells cm^{-2} after three days. Cells that hatched out of cysts contributed only to a minor extent to the population. The grazing impact of *Dexiostoma campylum* (765 bacteria $protist^{-1}$ h^{-1}) (Parry, 2004) and *Vannella* sp. (230 bacteria $protist^{-1}$ h^{-1}) (Pickup *et al.*, 2007) on biofilm bacteria was calculated with protists abundances at day 5. *Dexiostoma campylum* and *Vannella* sp. grazed approximately $6.6 \pm 2.1 \times 10^5$ bacteria h^{-1} and $11.8 \pm 1.2 \times 10^5$ bacteria h^{-1}, respectively. The flagellate *Spumella* sp. in the single species treatment reached 98.9×10^4 cells cm^{-2}. In the two species treatment, abundance of *Spumella* sp. decreased to 33.5×10^4 cells cm^{-2} from day 4 to 5. *Spumella* sp. was about 42 times more abundant than *Chilodonella uncinata*, which made up 1 - 5% of total protists abundance in the two species treatment. Considering a mean grazing rate of 225 and 5 bacteria $protist^{-1}$ h^{-1} (Parry, 2004) for *Chilodonella uncinata* and *Spumella* sp., respectively, the calculated grazing impact on biofilm bacteria of *Chilodonella uncinata* ($4.2 \pm 0.6 \times 10^5$ bacteria h^{-1}) was 2.5 times higher compared to *Spumella* sp. ($1.7 \pm 0.6 \times 10^5$ bacteria h^{-1}) in the two species treatment at day 5.

The maximal biofilm thickness significantly increased during all experiments in both ungrazed and grazed biofilms (Fig. 1, Table 2). Grazing by *Dexiostoma campylum* and *Vannella* sp. significantly influenced the increase of maximal biofilm thickness (Table 2). The large filter feeder *Dexiostoma campylum* caused a 72% lower maximal biofilm thickness compared to ungrazed biofilms at the highest protists abundance. Feeding of the gulper *Vannella* sp. caused a 20 - 42% lower maximal biofilm thickness compared to ungrazed biofilms between day 3 and 5. In the *Chilodonella uncinata* experiment, maximal biofilm thickness of ungrazed and grazed biofilms of the two species treatment increased linearly and reached a maximum of 196.5 ± 21.4 µm. In the single species treatment with the flagellate *Spumella* sp., the maximal biofilm thickness reached a plateau after 2 days and remained at 140.0 ± 8.8 µm.

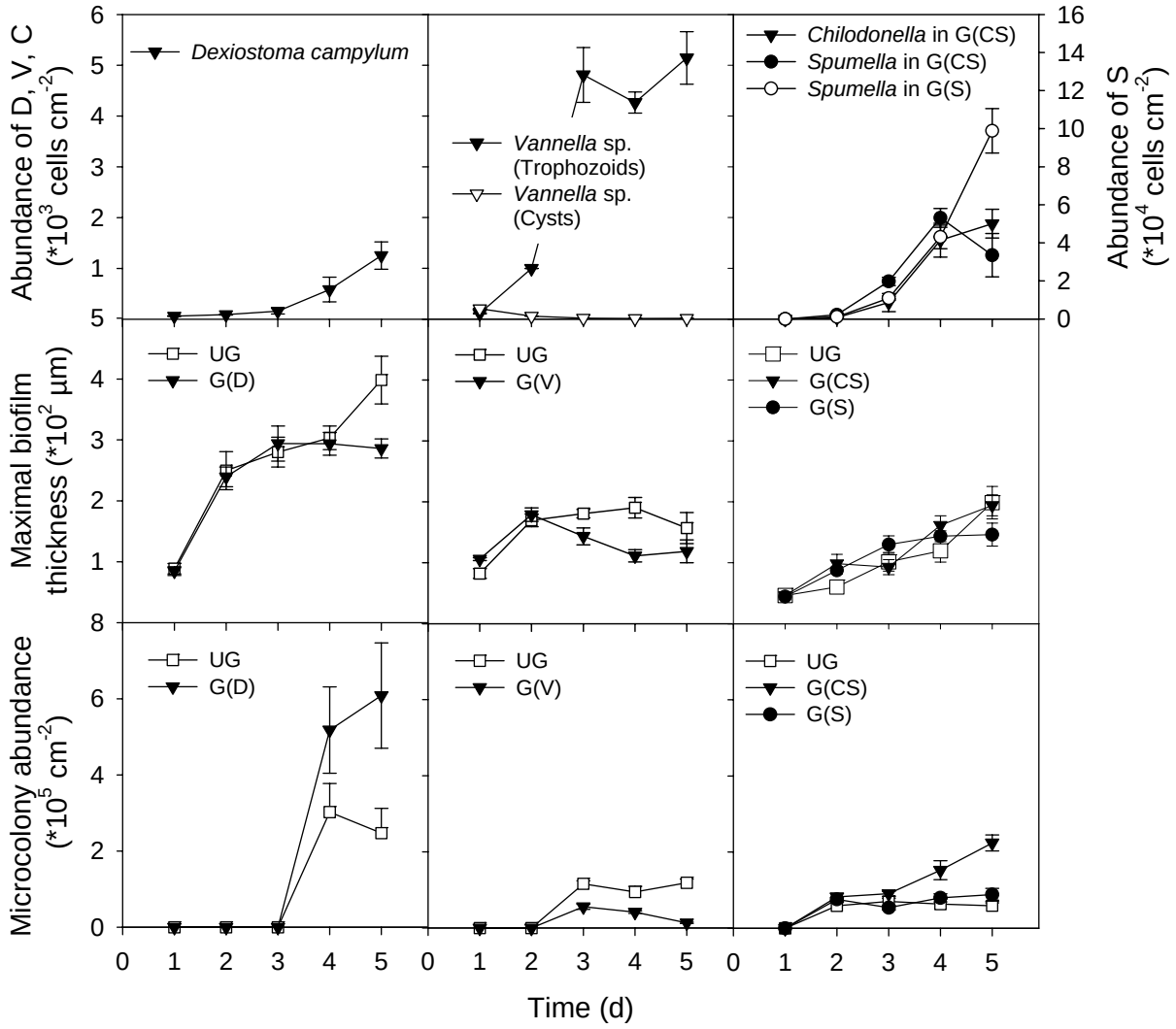

Fig. 1. Protists abundance (cells cm^{-2}) on biofilms and maximal biofilm thickness (µm) as well as abundance of microcolonies (cm^{-2}) of a VYE-medium bacterial biofilm. UG: ungrazed biofilms; G(D): biofilms grazed by *Dexiostoma campylum*; G(V): biofilms grazed by *Vannella* sp.; G(CS): biofilms grazed by *Chilodonella uncinata* and *Spumella* sp.; G(S): biofilms grazed by *Spumella* sp.

Table 2. Repeated measures ANOVA design for testing the effect of different protists (treatment: with and without protists) on biofilm thickness and microcolony abundance during 5 days of biofilm formation (time).

		Biofilm thickness			Microcolony abundance		
		df [1]	*F* [2]	*p*	*df*	*F*	*p*
Flow cell experiment: *Dexiostoma campylum*							
Within subject effects	Time	4, 40	50.803	0.001*	4, 40	29.570	0.001*
	Time *vs.* Treatment	4, 40	3.294	0.020*	4, 40	3.873	0.009*
Between subject effects	Treatment	1, 10	1.467	0.254	1, 10	4.990	0.050
Flow cell experiment: *Vannella* sp.							
Within subject effects	Time	4, 40	16.711	0.001*	4, 40	97.414	0.001*
	Time *vs.* Treatment	4, 40	7.440	0.001*	4, 40	28.992	0.001*
Between subject effects	Treatment	1, 10	2.831	0.123	1, 10	57.521	0.001*
Flow cell experiment: *Chilodonella uncinata, Spumella* sp.							
Within subject effects	Time	4, 60	54.660	0.001*	4, 60	56.980	0.001*
	Time *vs.* Treatment	8, 60	3.090	0.005*	8, 60	11.826	0.001*
Between subject effects	Treatment	2, 15	0.595	0.564	2, 15	21.796	0.001*
Batch experiment with different abundances: *Vannella* sp.							
Within subject effects	Time	3, 48	47.220	0.000*			
	Time *vs.* Treatment	3, 48	38.768	0.000*	No microcolonies formed.		
Between subject effects	Treatment	3, 16	99.930	0.000*			

[1] *df*: degree of freedom
[2] *F*-value of repeated measures ANOVA
Asterisks indicate significant differences ($p < 0.05$)

Microcolony formation started after a lag-phase of 1 to 3 days simultaneously in both grazed and ungrazed biofilms (Fig. 1). The increase of microcolony abundance was significantly influenced by some protists (Table 2). Both vagile ciliates *Dexiostoma campylum* and *Chilodonella uncinata* stimulated microcolony formation compared to ungrazed biofilms by up to 370 and 400%, respectively (Fig. 1). Microcolony size was significantly smaller in grazed biofilms by *Dexiostoma campylum* ($F_1 = 27.264$, $p < 0.001$) while grazing activity of *Chilodonella uncinata* accounted for significantly larger microcolonies ($F_2 = 4.836$, $p < 0.05$) (Fig. 2, Table S1). No stimulatory effect was found in the single species treatment with the sessile *Spumella* sp. Compared to 5-day-old ungrazed biofilms, significantly fewer microcolonies (50 - 88%) ($F_1 = 108.442$, $p < 0.001$) with a lower area ($F_2 = 18.489$, $p < 0.01$) were found in the presence of the amoeba *Vannella* sp. (Fig. 2).

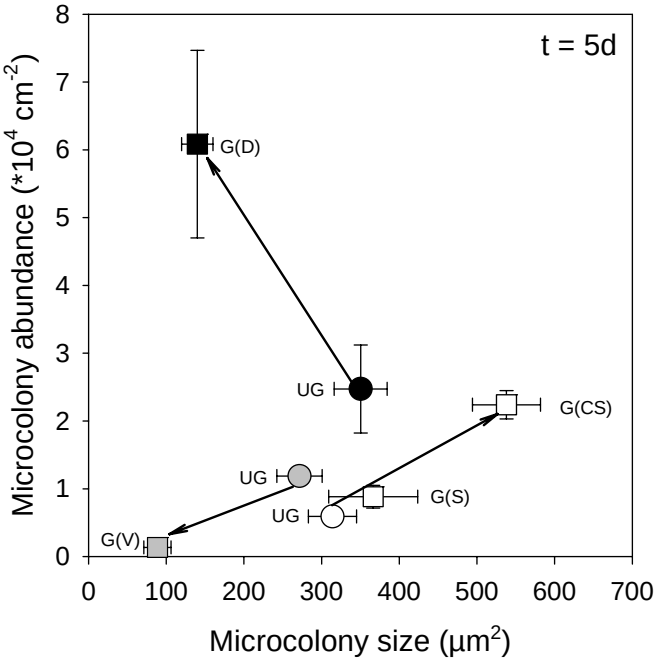

Fig. 2. Effect of protists grazing activity on abundance and size of microcolonies formed by VYE-medium bacterial biofilms. Circles: ungrazed biofilms (UG); Squares: grazed biofilms by *Dexiostoma campylum* (G(D), black symbols); *Vannella* sp. (G(V), grey symbols); *Chilodonella uncinata* (G(C), white symbols); *Spumella* sp. (G(S), white symbols). Arrows indicate changes from ungrazed to grazed biofilms. Letters (abc) display significant differences between treamtents ($p < 0.05$).

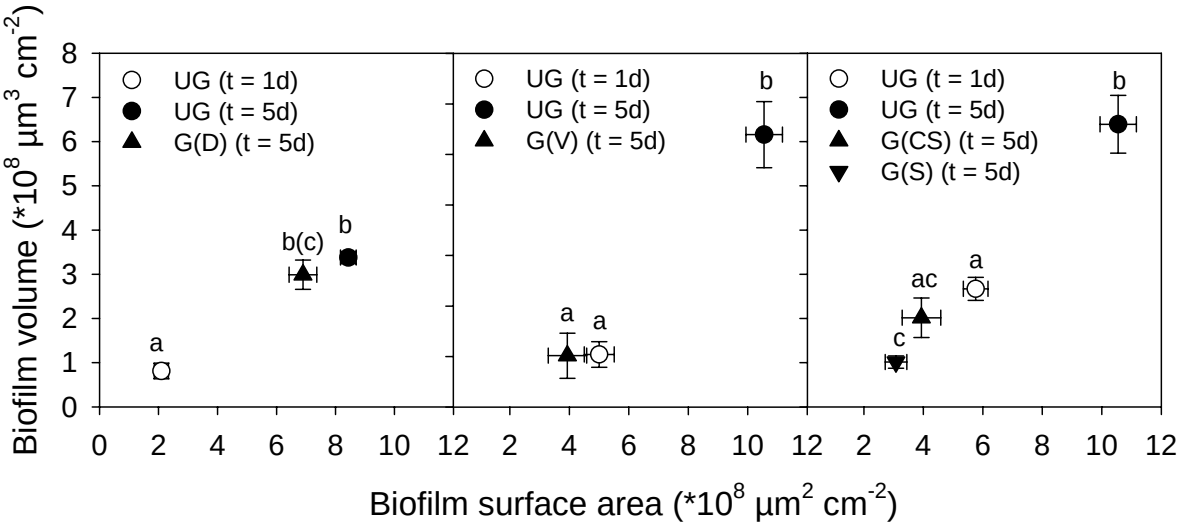

Fig. 3. Effect of protists grazing on biofilm volume and biofilm surface area of a VYE-medium bacterial biofilm. UG: ungrazed biofilms; G(D): biofilms grazed by *Dexiostoma campylum*; G(V): biofilms grazed by *Vannella* sp.; G(CS): biofilms grazed by *Chilodonella uncinata* and *Spumella* sp.; G(S): biofilms grazed by *Spumella* sp. Letters (abc) display significant differences between treamtents ($p < 0.05$).

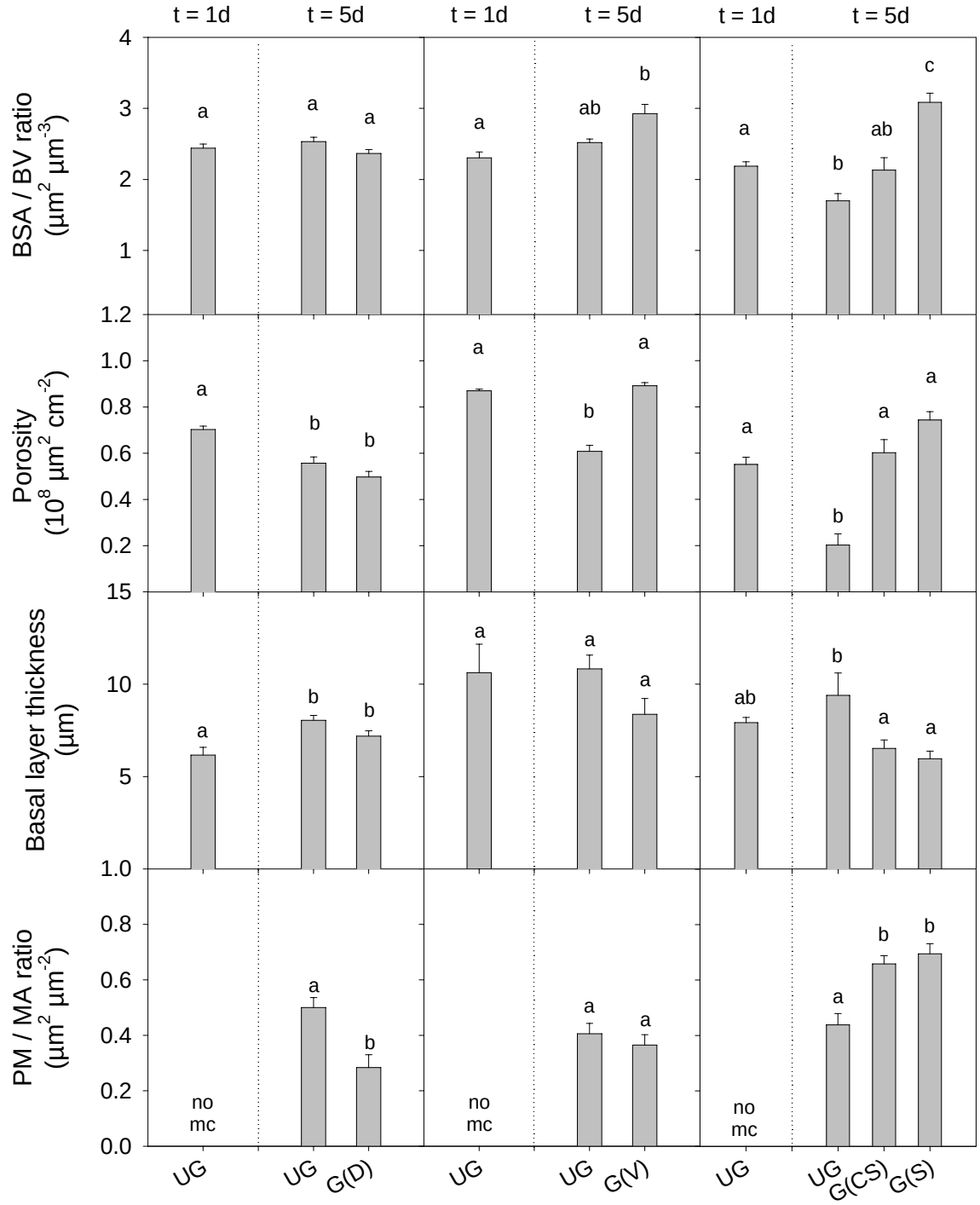

Fig. 4. Effect of protists grazing on biofilm surface area to biofilm volume ratio (BSA / BV ratio), porosity, basal layer thickness, and porosity of microcolonies to microcolony area (PM / MA ratio) of a VYE-medium bacterial biofilm. no mc: no microcolonies were observed in 1 day old ungrazed biofilms; UG: ungrazed biofilms; G(D): biofilms grazed by *Dexiostoma campylum*; G(V): biofilms grazed by *Vannella* sp.; G(CS): biofilms grazed by *Chilodonella uncinata* and *Spumella* sp.; G(S): biofilms grazed by *Spumella* sp. Letters (abc) display significant differences between treamtents ($p < 0.05$).

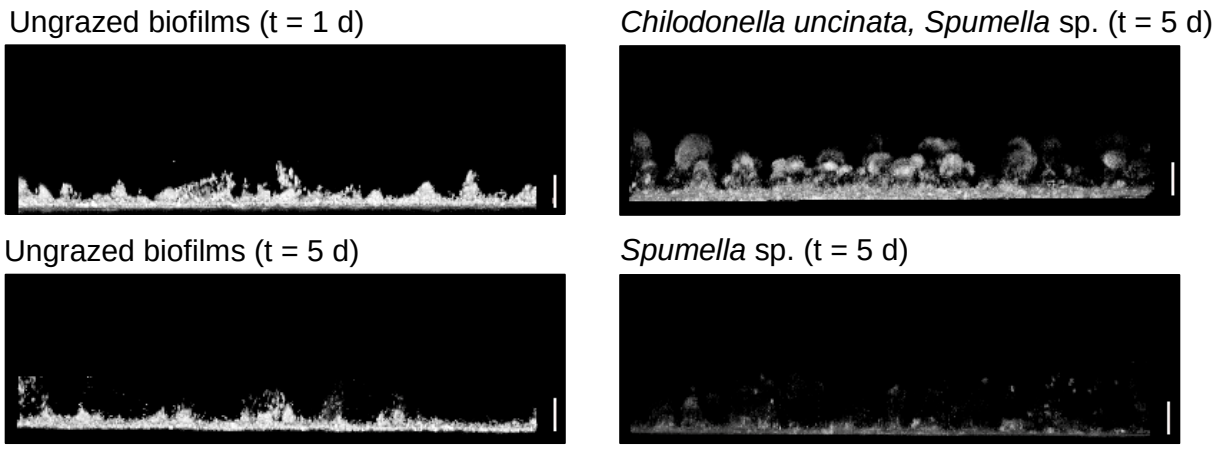

Fig. 5. CLSM images (xy) showing the morphology of VYE-medium bacterial biofilms grazed by gulper feeding ciliate *Chilodonella uncinata* and interception feeding flagellate *Spumella* sp. A: ungrazed biofilms at t = 1d, B: ungrazed biofilms at t = 5d, C: biofilms grazed by *Chilodonella uncinata* and *Spumella* sp., and D: biofilms grazed by *Spumella* sp. Scale bar represents 20 µm.

Examination of the CLSM images revealed that biofilm volume significantly increased from day 1 to 5 in ungrazed biofilms (Fig. 3) due to a higher maximal biofilm thickness, more microcolonies (Fig. 1), and a lower porosity (Fig. 4). A similar BSA / BV ratio was observed in 1- and 5-day-old ungrazed biofilms of the *Dexiostoma campylum* and *Vannella* sp. experiments ($p > 0.05$), while in the presence of *Chilodonella uncinata* and *Spumella* sp. the ratio was lower in 5- than in 1-day-old biofilms.

Vannella sp., *Chilodonella uncinata, Spumella* sp. but not *Dexiostoma campylum* significantly reduced the biofilm volume, biofilm surface area and caused the development of a less dense biofilm (Fig. 3, Fig. 4). *Dexiostoma campylum* was not grazing on the basal layer of the biofilms and porosity was similar to 5-day-old ungrazed biofilms. The biofilm volume was 2.5, 6.3, and 2.6times lower in biofilms co-cultivated with *Vannella* sp., *Spumella* sp., and *Chilodonella uncinata* compared to 5-day-old ungrazed biofilms, respectively, but similar to 1-day-old biofilms. The BSA / BV ratio was 1.2, 1.8, and 1.3times greater in biofilms grazed by *Vannella* sp., *Spumella* sp., and *Chilodonella uncinata* compared to 1- and 5-day-old ungrazed biofilms, respectively (Fig. 4). In addition, the porosity of grazed biofilms co-cultivated with *Vannella* sp. and *Chilodonella uncinata* (*Spumella* sp.) was similar to 1-day-old ungrazed biofilms, but significantly higher compared to 5-day-old ungrazed biofilms (Fig. 4). The thickness of the basal biofilm layer was reduced due to the grazing activity of *Vannella* sp., *Spumella* sp., and *Chilodonella uncinata* by 22.7, 36.5, and 30.5%, respectively.

The porosity within microcolonies was significantly lower in biofilms grazed by *Dexiostoma campylum* ($F_1 = 14.041$, $p < 0.001$) indicating that more bacteria made up the inner part of a microcolony (Fig. 4). *Vannella* sp. had no effect ($F_1 = 0.620$, $p < 0.449$) but especially *Spumella* sp. caused a higher porosity within microcolonies ($F_2 = 14.721$, $p < 0.001$) compared to ungrazed 5-day-old biofilms. Grazing activity of *Spumella* sp. caused translucent microcolonies with a high porosity within microcolonies (Fig. 5).

Impact of protists on the morphology of a stream bacterial biofilm

The abundance of *Neobodo designis* reached 14.9 ± 1.9 x 10^4 cm^{-2} in the single species treatment, but was lower in both other treatments (Table 3). *Spumella* sp. made up 14.9 and 1.5% of total protists abundance in the 2 and 3 species treatments, respectively. Abundance of *Chilodonella uncinata* reached 127.5 ± 45.3 cm^{-2} contributing only 0.2% to the community in the 3 species treatment. The grazing impact on biofilm bacteria was calculated assuming that the flagellates and ciliate ingest 5 and 225 bacteria $protist^{-1}$ h^{-1}, respectively (Parry, 2004). *Neobodo designis* had always the greatest grazing impact on biofilm bacteria with 1.2 - 7.3 x 10^5 bacteria h^{-1} followed by the ciliate *Chilodonella uncinata* with 0.3 x 10^5 bacteria h^{-1} and the flagellate *Spumella* sp. with 0.2 - 0.05 x 10^5 bacteria h^{-1}.

Table 3. Co-cultivation of a stream bacterial community with one, two and three protists for 3 days. G(N): biofilms grazed by *Neobodo designis*; G(NS): biofilms grazed by *Neobodo designis and Spumella* sp.; G(NSC): biofilms grazed by *Neobodo designis, Spumella* sp. and *Chilodonella uncinata*.

	Neobodo designis (cm^{-2})	*Spumella* sp. (cm^{-2})	*Chilodonella uncinata* (cm^{-2})	Biofilm volume (10^6 µm^3 mm^{-2})	Porosity (10^6 µm^2 mm^{-2})	Microcolony abundance (cm^{-2})	Maximal biofilm thickness (µm)	Basal layer thickness (µm)
G t = 1	n.d.[1]	0.0 (0.0)	0.0 (0.0)	0.42 (0.05)^a	0.92 (0.01)^a	0.0 (0.0)[2]	111.0 (3.0)^a	4.06 (0.30)^a
G(N) t = 4	14.6 x 10^4 (1.9 x 10^4)	0.0 (0.0)	0.0 (0.0)	1.18 (0.28)^b	0.81 (0.04)^{ab}	28.5 (9.1)^a	160.3 (10.2)^c	8.88 (039)^b
G(NS) t = 4	2.3 x 10^4 (0.6 x 10^4)	0.4 x 10^4 (0.3 10^3)	0.0 (0.0)	1.91 (0.33)^b	0.73 (0.05)^b	2.8 (0.8)^b	192.0 (14.3)^{bc}	8.29 (0.51)^b
G(NSC) t = 4	7.0 x 10^4 (1.7 x 10^4)	0.1 x 10^4 (0.3 x 10^3)	127.5 (45.4)	1.17 (0.09)^b	0.76 (0.02)^b	0.9 (0.5)^b	145.3 (14.0)^{ab}	6.49 (0.37)^c

[1] About 20% (Cynar *et al.*, 1985) of *Neobodo designis* in stream water samples passed the filters.
[2] No microcolonies were present in ungrazed biofilms, thus value was not included in the statistical analysis.
^{abc} display significant differences between treatments (ANOVA, $p < 0.05$)

Comparison of CLSM images of 1-day-old biofilms of VYE-medium bacteria, stream bacterial biofilms were 1.2 to 2.4 times thicker, with a 1.1 to 1.7 times higher porosity and a 1.6 to 6.3 times lower biofilm volume. The biofilm volume of grazed stream biofilms was 2.8 - 4.5 times higher in 4- compared to 1-day-old biofilms (Table 3).

Porosity was lowest in the two species treatments where biofilm volume and maximal biofilm thickness peaked and protists abundance was lowest (Table 3). Most microcolonies were found in the single species treatment with the highly abundant vagile *Neobodo designis*. 1- and 4-day-old biofilms grazed by three species had a similar maximal biofilm thickness. Despite the high abundance of *Neobodo designis* in the one species treatment, the maximal biofilm thickness was significantly higher in 5- than in 1-day-old biofilm. The basal layer thickness of grazed biofilms was significantly lower in the three species treatment compared to one and two species treatments.

Impact of protists abundance on biofilm morphology

Despite a lower abundance of *Vannella* sp. in the batch experiment (0.02 - 0.2 x 10^4 cells cm^{-2}) (Fig. 6) compared to the flow cell experiment (5.1 x 10^4 cells cm^{-2}), changes of the maximal biofilm thickness were significantly influenced during 4 days of grazing (Table 2). In ungrazed biofilms and biofilms with a low abundance, the maximal biofilm thickness increased over time, while *Vannella* sp. at intermediate and high abundances caused either no change or a reduction of maximal biofilm thickness with time (Fig. 6).

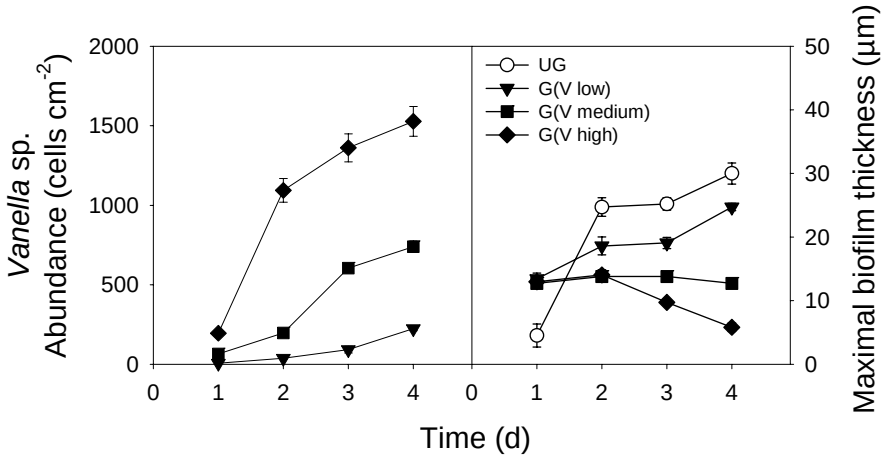

Fig. 6. Abundance of the amoeba *Vannella* sp. on biofilms and maximal biofilm thickness (µm) of the VYE-medium bacterial biofilm of the batch experiment. No microcolonies were observed in this experiment. UG: ungrazed biofilms, G(V): grazed biofilms by *Vannella* sp.

Discussion

Protists shape the biofilm morphology due to their grazing activity and mobility. Changes of morphological biofilm characteristics differed apparently due to the feeding mode of protists. In VYE-medium bacterial biofilms, the formation of microcolonies with a larger basal area was stimulated by the gulper feeder *Chilodonella uncinata*, while microcolonies in the presence of the filter feeder *Dexiostoma campylum* were smaller. Filter feeder which concentrate food particles due to strong feeding currents (Fenchel, 1986, Hausmann, 2002) preferably utilize suspended rather than attached bacteria (Eisenmann *et al.*, 1998). The filter feeder *Tetrahymena* sp. is able to reduce microcolony abundance and biofilm biomass indicating that biofilm bacteria were used as food source (Weitere *et al.*, 2005). However, bacterivorous protists can maintain growth by alternatively utilizing yeast extract of the medium or detritus (Broers *et al.*, 1991, Scherwass *et al.*, 2005). Thus in our experiment, growth of *Dexiostoma campylum* might depend on the utilization of yeast extract in the medium rather than on biofilm bacteria.

Filter feeders might slough biofilm fragments and utilize the sloughed biofilm bacteria (Huws *et al.*, 2005, Parry *et al.*, 2007) due to cell mobility or strong feeding currents of 500 $\mu m\ s^{-1}$ near the mouth (Fenchel, 1986). In our experiment, about 30% of *Dexiostoma campylum* cells were found within microcolonies or biofilm foldings where they might have caused sloughing of the upper biofilm layers or microcolony periphery. Both led to a lower maximal biofilm thickness and smaller microcolonies, respectively. The filter feeder *Euplotes* sp. with the adoral membranelles oriented towards the surface locally cleared patches on a bacterial biofilm and by that increased spatial heterogeneity (Lawrence & Snyder, 1998). The studied filter feeder *Dexiostoma campylum* could not confirm this phenomenon. Nevertheless, the low PM / MA ratio indicated that bacterial growth could be maintained also within microcolonies. Released nutrients (Zubkov & Sleigh, 1999) and the produced feeding currents of filter feeders might enhance O_2 and nutrient transport into biofilms (Glud and Fenchel 1999; Vopel et al. 2005). Thus, vagile filter feeders that can reach high abundances in biofilms at slow flowing stream sites (Risse-Buhl & Küsel, subm.) might promote nutrient transport into biofilms grown at slow flow velocities.

The gulper feeder *Vannella* sp. reduced microcolony abundance, microcolony size, but also maximal and basal biofilm layer thickness. Especially when *Vannella* sp. was highly

abundant, only a very thin bacterial biofilm remained. Similarly, the amoeba *Acanthamoeba castellanii* does not completely deplete biofilm bacteria (Huws *et al.*, 2005). Thus, biofilm morphology was altered both by feeding mode and by protists abundance. In natural biofilms, protists abundance is controlled by inter- and intraspecific competition for space and resources or by predation (Schönborn, 1998). Competition and predation on protists might contribute to a balancing between an effective population size of bacterivorous predators and bacteria.

Microcolony formation of the multispecies bacterial biofilm was not affected by the sessile interception feeder *Spumella* sp. In single species biofilms, microcolonies are efficient defense strategies against grazing by vagile flagellates (Matz *et al.*, 2004, Weitere *et al.*, 2005, Queck *et al.*, 2006). Microcolony formation in *Serratia marcescens* biofilms is not stimulated by chemical cues, but it is hypothesized that a mechanical process caused by the motion of flagella is involved (Queck *et al.*, 2006). However, the motion of flagella of *Spumella* sp. seemed not to be sufficient to stimulate microcolony formation. Thus, mobility of protists seemed to be another important factor to stimulate microcolony formation.

In the presence of the interception feeder *Spumella* sp. microcolonies were translucent with a high PM / MA ratio. The microcolony forming and EPS-producing morphotype of *Pseudomonas putida* was not grazed by the interception feeder *Ochromonas* sp. indicating that the production of exopolymeric substances is a grazing resistance strategy (Matz *et al.*, 2002). Deeply embedded bacteria (Parry, 2004) forming the central part of microcolonies are thus protected against protists grazing. *Spumella* sp. captures bacterial cells that are carried along the flow lines of its flagellum (Boenigk & Arndt, 2002). Our results indicated that *Spumella* sp. utilized even deeply embedded cells. We hypothesize that the movement of the flagellum of *Spumella* sp. might loosen bacterial cells from the inner parts of microcolonies.

Despite the stimulated formation of microcolonies, also *Chilodonella uncinata* (in the presence of *Spumella* sp.) reduced the biofilm volume. The gulper feeder *Chilodonella uncinata* actively searches for bacteria (and small flagellates) and takes up individual cells with its cyrtopharyngeal basket (a cylinder of microtubules) (Foissner *et al.*, 1991, Hausmann, 2002). Feeding *Chilodonella uncinata* seemed to rasp single bacterial cells from the biofilms surface by for- and backward movements, thus, causing the development of mushroom shaped microcolonies (Fig. 6). These shaped microcolonies are predicted to provide optimal diffusion paths between a biofilm and its surrounding fluid due to a high BSA / BV ratio

(Picioreanu *et al.*, 1998, Picioreanu *et al.*, 1998, Costerton, 2007). Hence, the transport of nutrients into deeper layers of biofilms as is possible by diffusion alone is promoted (Massoldeya *et al.*, 1995). Grazing gulper and interception feeders seemed to enhanced exchange of nutrients and gasses between the biofilm and its surrounding fluid due to a higher porosity and BSA / BV ratio. Thus, bacterial growth might be accelerated.

Biofilms formed by stream bacteria extended in maximal thickness over time rather than exploit the free surface area available (indicated by the high porosity) irrespective of the species combination grazing on these biofilms. Only minor differences were observed between treatments with one, two and three grazing species. *Neobodo designis* mainly contributed to the altered biofilm morphology due to its high abundance in all treatments. The observed changes of the biofilm morphology implicated that exchange of nutrients between the surrounding fluid and thicker biofilms might be enhanced when a network of channels and voids penetrated the biofilm.

The stimulated formation of microcolonies and the high biofilm surface area in grazed biofilms with gulper and interception feeder but also the channel network observed for stream bacterial biofilms indicated a rougher biofilm surface compared to ungrazed biofilms. Considering biofilms as microbial landscapes, the dispersal of drifting microorganisms is altered by changing surface characteristics (Battin *et al.*, 2007). More cells will stick to rough biofilms because of lower shear forces experienced by cells shielded from the main current and greater surface area for adsorption compared to smooth biofilms (Characklis, 1984). Dispersal distance and thus organism drift especially in flowing water might be reduced by rough biofilm surfaces.

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EFFECTS OF SMALL LOW-HEAD DAMS ON FEEDING GROUPS OF BIOFILM ASSOCIATED CILIATES

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(Manuscript in preparation to be submitted to *Limnologica*)

Abstract

The numerous small low-head dams in Central European streams alter local flow velocity patterns and sediment composition while little is known about their ecological effects. Since biofilms are hot spots of the microbial activity in streams, we studied the impact of a small low-head dam on biofilm associated ciliate communities in the 3rd order stream Ilm (Thuringia, Germany) at different seasons during April 2003 till September 2005. Biofilms on glass slides were observed after 2 and 6 weeks exposed at reservoir and outlet sites. The abundance and species number of biofilm associated ciliates was 1.1 to 4.6 times and 2.5 times higher at the outlet compared to the reservoir in both 2- and 6-weeks old biofilms, respectively. Major differences between dam sites were observed when ciliate abundances were highest in spring and autumn. The small low-head dams did not influence the relative contribution of feeding modes indicating that only the amount of channeled carbon by ciliates is lower in reservoirs. Further, three nearby located small low-head dams displayed similar changes with respect to the ciliate community in biofilms, indicating that the observed effects of one small low-head dam are transferable to other dams when located in a comparable stream reach.

Keywords: stream ecosystem, small low-head dam, flow velocity, biofilm, ciliates

Introduction

Most streams are regulated by dams, weirs, and straightening to control water discharge and to gain drinking water and electric power for human purposes. Physical, chemical as well as biological conditions are affected by stream regulation (Ward and Stanford 1979; 1983). Intensively studied large storage dams disrupt the river continuum (Vannote *et al.* 1980) by disconnecting upstream and downstream reaches and interrupt nutrient and carbon spiraling. In Central European stream systems, small low-head dams with a hydraulic head <5 meters and impounded areas of <20 hectare, are generally much more numerous than large storage dams (Poff and Hart 2002). However the ecological consequences of small low-head dam structures are poorly understood (Benstead et al. 1999; Hart et al. 2002; Poff and Hart 2002). Small low-head dams weaken flood peaks, change local flow velocity patterns, alter sediment composition as well as particulate organic matter storage (Magilligan and Nislow 2001; Stanley et al. 2002; Wagner 2003). Seasonally, increased discharges after snowmelt and during heavy rain fall enhance dissolved organic carbon entry into streams from adjacent terrestrial sites (Hornberger et al. 1994; Wilson and Xenopoulos 2008). Particulate organic matter standing stock (Arle 2005) and allochthonous matter input from the riparian vegetation occurs mainly during autumn. Thus, the effect of small low-head dams might differ in relation to season.

Allochthonous matter that enters the stream ecosystem is rapidly covered by microbial biofilms, which contribute to the degradation process (Golladay and Sinsabaugh 1991; Ribblett et al. 2005). Ciliates are an integral part of the microbial biofilm food web where they channel carbon and energy from bacteria and protists to the meio- and macrofauna (Bott and Borchard 1999; Schönborn 1987; Sleigh et al. 1992). Virgin surfaces at a slow flowing reservoir were rapidly colonized by ciliates while differences between reservoir and outlet were compensated in 2-weeks old biofilms (Risse-Buhl and Küsel 2008). The aims were to investigate, if (1) the effect of small low-head dams on biofilm associated ciliate communities varies with respect to season, and (2) the observed differences are transferable to other small low-head dams or they are restricted to one location.

Material & Methods

Characteristics and location of the small low-head dams. Field experiments were conducted at three small low-head dams (Fig. 1) located in the 3rd order stream Ilm (Thuringia, Germany) near the village Griesheim (50°44'58"N, 11°02'14"E). Most dams at the stream Ilm were created together with developing settlements and handcraft in the 11th century. Today the stream's continuum is disrupted by about 56 small low-head dams along the course of 137 km while the capacity and length of the reservoirs are small. The small low-head dam 1, with a width of 24.4 m and a height of 1.2 m, is located in the main channel of the stream Ilm downstream a morpho-dynamic active reach. The reservoir extends 125 m upstream of the dam with a mean slope of 4.3%. Upstream of the dam, a smaller side channel parts where both other small low-head dams are located. The third studied small low-head dam is located about 150 m downstream of the second dam. Channel width was lower than 5 m and reservoirs extend approximately 90 m. Riparian forest relicts surround the second dam, but bank vegetation at other sites is mainly a stripe of trees (*Salix* sp., *Alnus* sp., *Populus* sp.) and bushes or intensively used agricultural land and pasture.

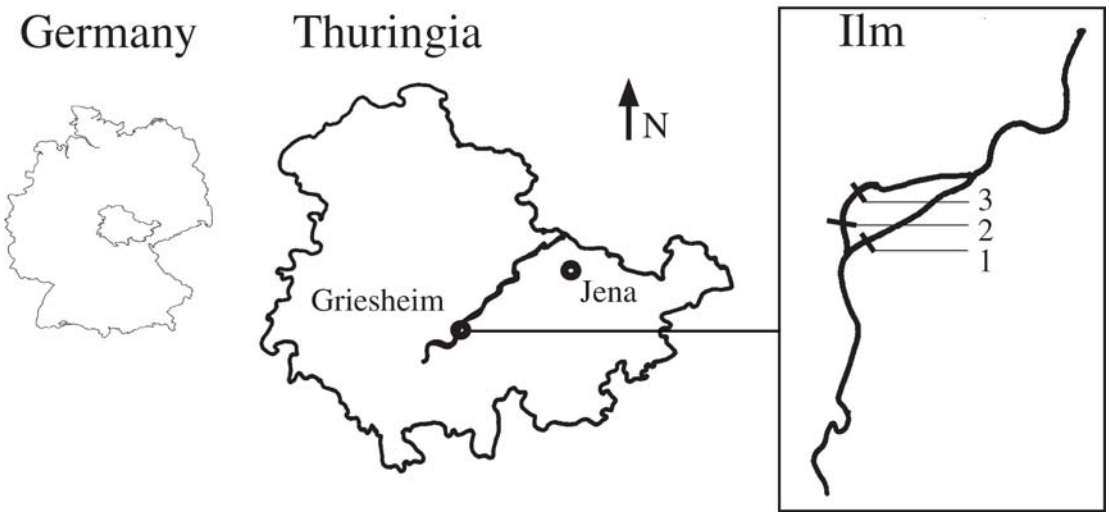

Fig. 1. Map of the 3rd order stream Ilm and location of the three studied small low-head dams (1 - 3).

Sampling. Biofilm samples were taken at reservoirs and downstream located outlets (Risse-Buhl and Küsel 2008). At the small low-head dam 2, ciliates in 2-weeks old biofilms were examined monthly from April 2003 to April 2004. In September 2005, 2- and 6-weeks old biofilms were compared. Due to several high waters, sampling was not possible between January and March 2004. Due to the high ciliate abundance and distinct differences between sites in April 2003, biofilm associated ciliates from three small low-head dams were investigated at one sampling in April 2004. Environmental variables (temperature, dissolved oxygen, conductivity, turbidity, and pH) were measured with a water quality checker U10 (Horiba; Kyoto). Periodically, nitrate NO_3^- and phosphate PO_4^{3-} were measured with a photometer (UVIKON 931; Kontron Instruments, Groß Zimmern). At 5-10 cm above ground similar to the exposition depth of glass slides the flow velocity (Flo-mate 2000; Marsh McBrunney Inc., Hyattsville) was measured.

Quantification of ciliates. Identification and enumeration of ciliates can be done either by live count (Dale and Burkhill 1982; Schönborn 1981; Sime-Ngando et al. 1990) or after fixation and staining (Montagnes and Lynn 1987; Skibbe 1994). The question arises which method is best qualified for handling the samples and enumerating biofilm associated ciliates. For the live count, the sampled glass slides were placed in Grainer tubes (50 ml), which were filled with filtered (0.2 µm) and sterilized stream water. Samples were stored at 10°C and processed within 24 h after sampling. Two to six transversal strips (0.143 cm^2) of the glass slides were scanned at an inverse light microscope at 400 times magnification (Axiovert; Zeiss, Jena). For the Quantitative Protargol Staining (QPS) method, biofilms were scraped off the glass slides and fixed with Bouin's solution (final concentration 5%) (see Risse-Buhl & Küsel (2008)). At least 50 individual cells or the whole filter was scanned. For ciliates identification guides from Foissner et al. (1995; 1992; 1994; 1991) and Kahl (1930-1935) were used. Ciliates were grouped according to the feeding mode in filter feeder (food source: mainly bacteria but also small algae), gulper feeder (food source: algae and bacteria or flagellates and other ciliates), and diffusion feeder (food source: flagellates and other ciliates).

Data analysis. A two-way analysis of variance was used to test for differences between time points, sites (reservoir and outlet), and small low-head dams (1, 2, and 3) (SPSS 15.0). Data were log10(x+1) transformed. The Shannon diversity index (Shannon and Weaver 1949) and the evenness (Koehler *et al.* 2002) were calculated.

Results

Seasonal variability of hydrologic and abiotic parameters

The small low-head dam 2 did not influence abiotic parameters such as temperature, oxygen content, pH, and conductivity (Fig. 2). During the lowest discharge period in August 2003 (Fig. 3), highest temperature and conductivity but lowest dissolved oxygen were measured in the water column of the stream Ilm. Discharge peaked in winter and spring as typical for the stream Ilm (Fig. 3). At low discharge periods, the slowest flow velocities were observed (Fig. 4). Despite these seasonal differences, flow velocity was always 1.3 - 2.8 times lower in the reservoir compared to the outlet of the small low-head dam 2 ($F_{1,36} = 14.804$, $p < 0.001$).

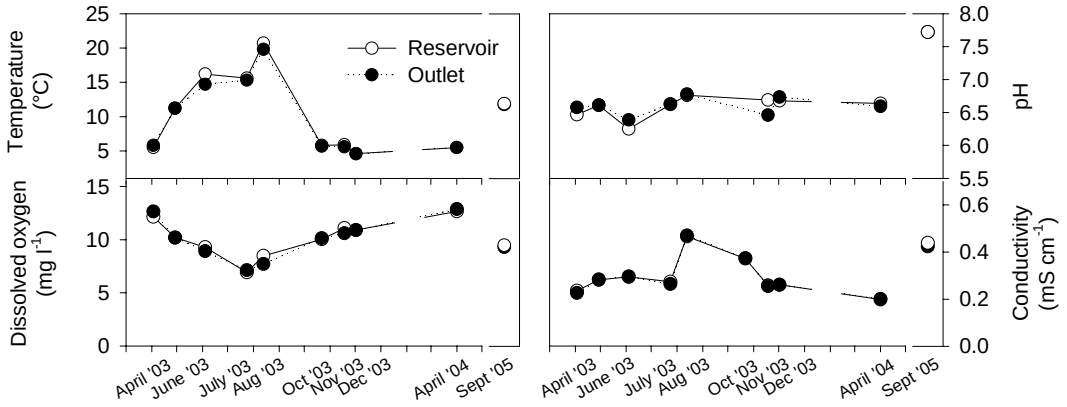

Fig. 2. Temperature, dissolved oxygen, pH, and conductivity at reservoir and outlet of the small low-head dam 2 from April 2003 to April 2004 and September 2005.

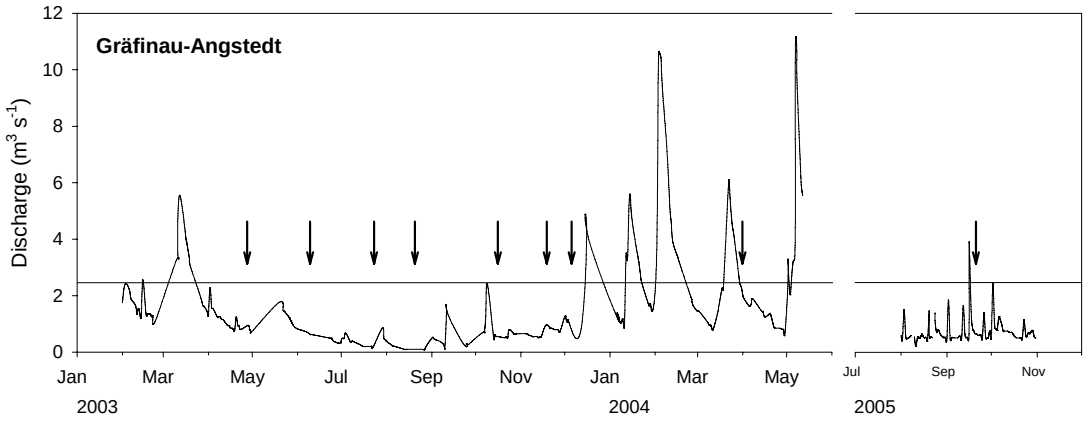

Fig. 3. Discharge at the water level gauge Gräfinau-Angstedt. Data were kindly provided by the 'Staatliches Umweltamt Erfurt' (DGJ 2003, 2005). Black arrows indicate sampling occasions.

Seasonal aspects of the biofilm associated ciliates at a small low-head dam

The application of the QPS method (maximal observed species 64) yielded a higher taxonomic resolution than the live count (maximal observed species 55) in 2- as well as in 6-weeks old biofilms and irrespective of the sampling site (Fig. 4). To avoid community changes, the live count needed to be done within a short time after sampling. The scanned area was always lower (0.05 - 0.33 cm^2, median = 0.28 cm^2) compared to the observed area when using the QPS method (0.25 - 9.88 cm^2, median = 2.00 cm^2). Due to the greater scanned area, a sufficient large number of cells (QPS: 60-728 cells $sample^{-1}$, median = 240 cells sample-1, live counting: 5-208 cells $sample^{-1}$, median = 107 cells $sample^{-1}$) and species (QPS: 27-76 species sample-1, live counting: 5-27 species $sample^{-1}$) were investigated with the QPS. Single and colony-forming Peritrichia that were highly abundant might be overestimated with live count due to a high cell number in the small, randomly chosen area. In addition, small species of the Hymenostomatia, Hypotrichia, Phyllopharyngia, and Stichotrichia might also be overestimated when counted alive due to their fast movement. Patchy distribution of ciliates in biofilms and coverage of cells by accumulated sediment particles might influence results when ciliates were counted alive. Statistically reliable results with a better taxonomical resolution can be expected when applying the QPS method since a greater area is scanned and biofilm material is homogenized. Washing steps reduce particle load of the sample and ciliates loss is less than 6% (Gücker and Fischer 2003). Thus, for ecological studies it seems more appropriate to enumerate fixed and stained ciliates.

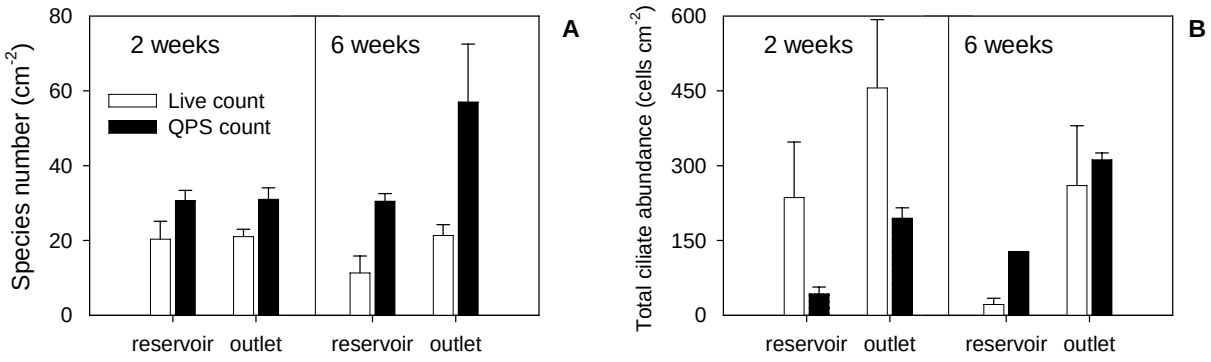

Fig. 4. Species number (A) and total ciliate abundance (B) determined in 2- and 6-weeks old stream biofilms at the small low-head dam 2 by live (left bar) and QPS (right bar) count (mean ± SE, n = 3).

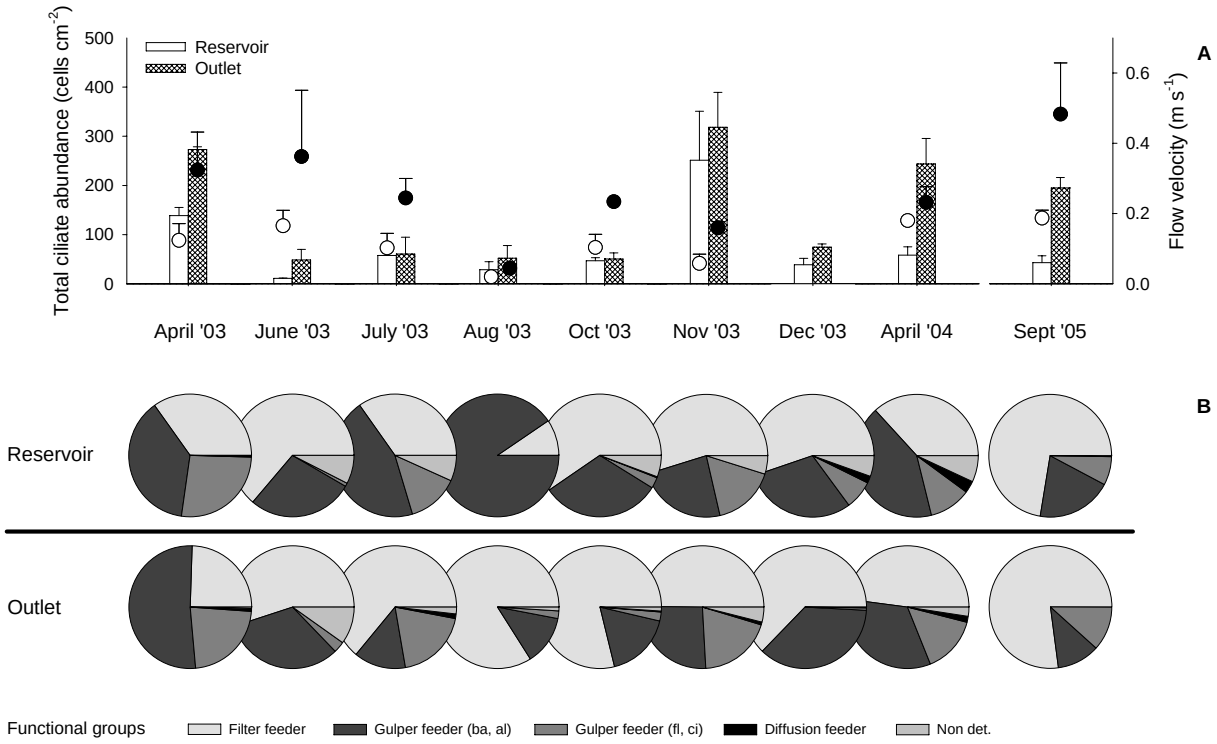

Fig. 5. Total ciliate abundance (bars) and flow velocity (circles: reservoir (white), outlet (black)) (A), and relative contribution of functional ciliate groups (B) in 2-weeks old biofilms at a small low-head dam. (mean ± SE, n = 3). Flow velocity measurement could not be done in December 2003 due to technical troubles. Abbreviations: ba - bacteria, al - algae, fl - heterotrophic flagellates, ci - ciliates. Data from April, July, and November 2003 derived from Risse-Buhl & Küsel (2008).

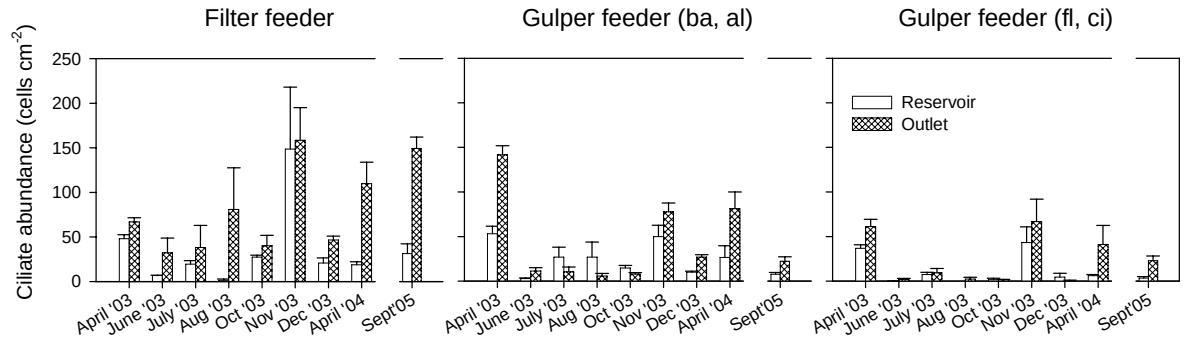

Fig. 6. Abundance of ciliates feeding groups in 2-weeks old biofilms at the reservoir and outlet of a small low-head dam (mean ± SE, n = 3). Abbreviations: ba - bacteria, al - algae, fl - heterotrophic flagellates, ci - ciliates. Data from April, July, and November 2003 derived from Risse-Buhl & Küsel (2008).

Table 4. Results of the two-way ANOVA's to test for effects of site (reservoir and outlet), time, and their interaction on biofilm associated ciliates. Abbreviations: ba - bacteria, al - algae, fl - heterotrophic flagellates, ci - ciliates.

Variable	Site 1, 38 d.f.	Time 8, 38 d.f.	Site * Time 8, 38 d.f.
Total ciliate abundance	13.359*	8.062*	1.474
Species number	7.582*	7.869*	2.206
Filter feeder	23.406*	7.149*	3.392*
Gulper feeder (ba, al)	0.335	16.040*	6.240*
Gulper feeder (fl, ci)	8.105*	25.075*	2.529*
Diffusion feeder	6.554*	6.237*	2.472*

Ciliate abundance in 2-weeks old biofilms at the small low-head dam 2 ranged between 10.5 and 317.8 cells cm^{-2} with the lowest abundances in late spring, summer and winter. Ciliate abundance was 2 to 3 times higher in 6- than in 2-weeks old biofilms (Fig. 2). The two-way ANOVAs for abundance and species number revealed significant site and time effects (Table 2). Differences of ciliate abundance between sites did not depend on sampling time (not significant interaction) and were similar or 1.3 to 4.6 times higher at the outlet compared to the reservoir (Fig. 5A). Greatest differences between sites were observed in spring and autumn when ciliate abundances were highest. Slightly more species were observed at the outlet than at the reservoir with 16 to 33 species cm^{-2} and 4 to 31 species cm^{-2}, respectively. The number of species common at both sites (5 - 28) was always lower than the maximal expected species number (6 - 38). The Shannon diversity index was similar or even higher at the outlet (2.31 - 3.39) compared to the reservoir (1.52 - 3.26) and ciliates were more evenly distributed in the reservoir (0.85 - 0.95) than in the outlet (0.68 - 0.93).

Considering functional feeding groups, filter feeder and gulper feeder that utilize bacteria and algae dominated the ciliate community (Fig. 5B). The significant interaction between sites and time indicated that differences between sites depended on sampling time (season) (Table 1). The small low-head dam did not affect the relative contribution of the different feeding groups (Fig. 5B). Only in July and August 2003, a higher proportion of gulper feeding ciliates that used algae as primary food source contributed to the ciliate community at the reservoir. Abundance of filter feeders was higher at the outlet (Fig. 6) due to highly abundant sessile Peritrichia. In spring and autumn, both gulper feeding groups (species of Phyllopharyngia and Haptoria) occurred more abundant at the outlet site.

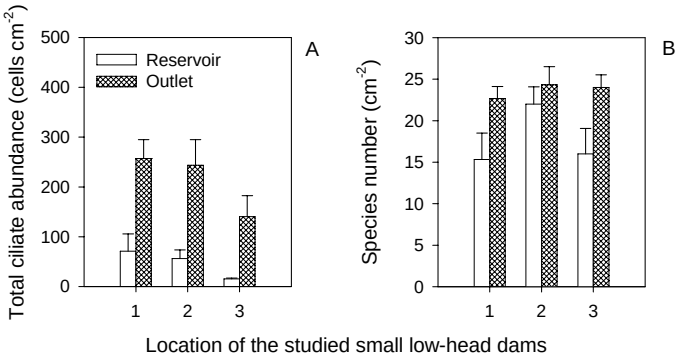

Fig. 7. Total ciliate abundance (A) and species number (B) of ciliates in 2-weeks old biofilms at reservoir and outlet of three small low-head dams in April 2004 (mean ± SE, n = 3).

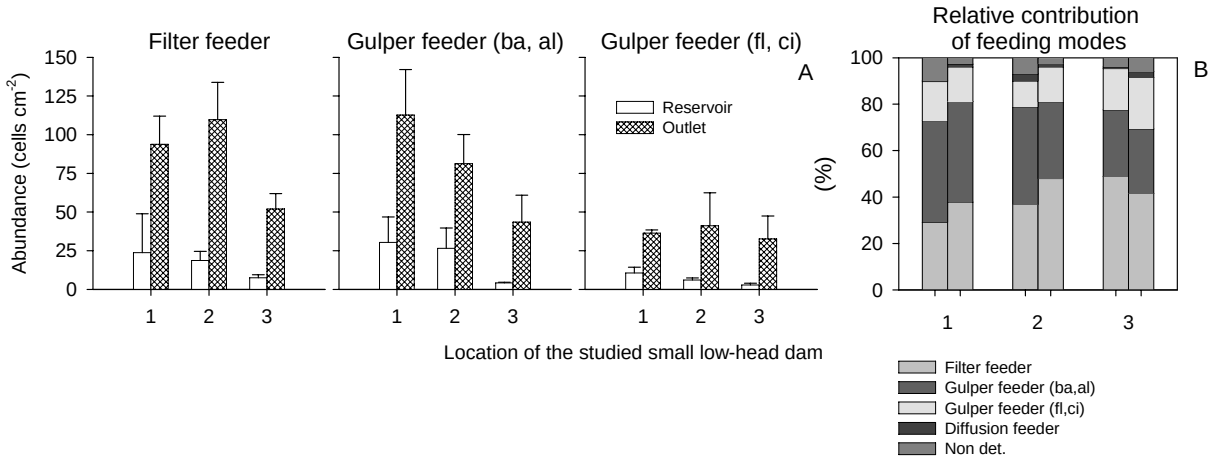

Fig. 8. Abundance (A) and relative contribution (B) of ciliates feeding groups in 2-weeks old biofilms at reservoir (left bar) and outlet (right bar) of three small low-head dams in April 2004 (mean ± SE, n = 3). Abbreviations: ba - bacteria, al - algae, fl - heterotrophic flagellates, ci - ciliates.

Biofilm associated ciliates at a three different small low-head dams

In April 2004, ciliates abundance ranged between 57.7 and 243.3 cells cm^{-2} similar to abundances observed in April 2003. The interaction effect between small low-head dams and sites was not significant for both ciliate abundance ($F_{2,12}$ = 1.274, p = 0.315) and species number ($F_{2,12}$ = 0.964, p = 0.409) indicating that similar differences between sites were found at all studied small low-head dams. Total ciliate abundance ($F_{1,12}$ = 17.514, p < 0.01) as well as species number ($F_{1,12}$ = 8.188, p < 0.05) differed significantly between reservoir and outlet.

At the outlet, ciliate abundance was 4 to 9 times higher and more biofilm associated ciliate species occurred than at reservoir sites (Fig.). A significant effect of small low-head dams was not observed for species number ($F_{2,12} = 1.525$, $p = 0.257$) but for total ciliate abundance ($F_{2,12} = 5.555$, $p < 0.05$) probably due to the low abundances at the small low-head dam 3. All three studied small low-head dams did not influence the relative contribution of feeding groups while the abundance of each groups was higher at the outlet compared to the reservoir.

Discussion

The effect of the small low-head dam on biofilm associated ciliate communities was greatest during spring and autumn

Temperature, dissolved oxygen, discharge, and flow velocity varied between seasons influencing ciliate abundance in stream biofilms. Lowest ciliate abundances were observed during high temperatures, low oxygen content and slow flow velocities in summer. A negative correlation of ciliate abundance with high temperatures is described for biofilms of the River Rhine (Norf et al. 2007). Seasonal variations of ciliate abundance correlate positively with the abundance of diatoms in biofilms (Schönborn 1981). Probably the low bacterial (E. Pohlon, pers. com.) as well as algal abundances (M. Willkomm, pers. com.) in biofilms of the stream Ilm might have caused the low ciliate abundance indicating that ciliates were limited by their food during summer.

In 2- and 6-weeks old biofilms, ciliate abundances were higher at the outlet of the small low-head dam 2. Since temperature, oxygen content, and nutrient concentrations (data not shown) were not altered by the small low-head dam, differences of ciliate abundance and species richness between sites were considered to be influenced by flow velocity and sediment composition. Heterotrophic flagellates in the stream Ilm are positively correlated with flow velocity (Willkomm 2007). At faster flow velocities, more organisms flowing past a biofilm (Hunt and Parry 1998) that might attach to the rough and sticky surface of an initial biofilm. In addition, the clearance rate of filter feeding ciliates is intensified (Shimeta *et al.* 2001). The turbulent motion of the flowing water causes a higher contact rate between suspended particles and biofilm surfaces (Kiørboe and Saiz 1995; Rothschild and Osborn

1988; Visser and MacKenzie 1998). Hence, advection of drifting ciliates and their prey seemed to be enhanced at faster flow velocities in the outlet of the small low-head dam.

Differences in ciliate abundance between reservoir and outlet were greater in spring and autumn. During both seasons, the input of allochthonous matter is enhanced either after snowmelt or during leave fall, respectively. A higher dissolved organic matter and nutrient content in the water caused higher bacterial productivity (Romaní and Sabater 1999) especially in biofilms at the outlet (E. Pohlon pers. com.). Due to the continuous nutrient transport by the flow, also diatoms have higher growth rates at faster flow velocities (Hunt and Parry 1998; Korte and Blinn 1983; Willkomm 2007). Reduced diffusional resistance at surfaces (de Beer et al. 1996) and increased uptake rates of easily degradable carbon sources into biofilms (Kaplan and Newbold 2003; C. Augspurger pers. com.) indicated that microbial biofilms rely more on dissolved organic carbon of the water column at a faster flowing outlet.

Fewer ciliate species were recorded in biofilms at the reservoir than at the outlet. At slow flow velocities in the reservoir, fine inorganic and organic matter accumulates (Arle 2005). In fine marine and freshwater sediments, similarly low species numbers are recorded (Hamels *et al.* 2005; S. Kröwer pers. com.). The small pore size (limited by the grain size of the sediment particles) might restrict the occurrence of large ciliates also in biofilms with high inorganic sediment content (Risse-Buhl and Küsel 2008). A layer of fine sediment particles that was easily resuspended when disturbed covered stones in the reservoir (own observations). Overall, the streambed of the upper Ilm reach is composed of large stones and cobble and according to the flow velocity differing amounts of sand and fine particulate matter (Arle 2005). At the outlet, flow velocity variations near the heterogeneous streambed surface created diverse micro-niches where a variety of ciliate species might find optimal growth conditions. Ciliates with specific morphological features (flattened profile, creeping without cirri) tolerated faster flow velocities (Risse-Buhl and Küsel 2008) and the high contribution to the ciliate community of this group at the outlet probably caused the lower evenness compared to the reservoir.

Especially in straightened streams where fallen trees are removed, the accumulation of fine particulate inorganic and organic matter implements that reservoir sites serve as retention structures and probably promote organic matter processing within the stream ecosystem (Arle 2005; Pohlon et al. 2007). Despite the observed differences of flow velocity and sediment composition, the investigated small low-head dam caused no changes in functional attributes

of biofilm associated ciliate communities in 2-weeks old biofilms. In contrast, the benthic invertebrate community of reservoirs is dominated by detritivorous collector gatherer like Chironomidae and Oligochaeta indicating that particulate organic matter including bacteria and protists is directly channeled to the invertebrate community (Arle 2005; Wood and Armitage 1997). Despite the numerous small low-head dams, the discharge regime of the stream Ilm is near natural with 4 to 5 major floods per year (Vetter 2001) that cause resuspension and sediment movement also in reservoirs (Arle 2005; Fjellheim et al. 1993). Due to abrasion during flood events, new surfaces for biofilm development are created. The available habitats are colonized more rapidly at slow flow velocities in reservoirs than at faster flowing outlet sites (Risse-Buhl and Küsel 2008).

Different small low-head dams had a similar effect on biofilm associated ciliates

The above discussed patterns regarding ciliates in stream biofilms might be related to the one small low-head dam that was extensively studied. For ecological theory and application in the field of restoration, it would be important to see whether the data are transferable to other, similar dam sites. The investigation of three small low-head dams revealed a similar effect with higher ciliate abundances and species numbers in the outlet. Thus, differences of dam and reservoir size seemed to be of minor importance. The three small low-head dams were chosen, since they are spatially restricted to a stream reach of <500 m. The effect of large storage dams on i.e. particulate matter, biotic diversity, and environmental heterogeneity differs with respect to location along the stream's course (Ward and Stanford 1983). Thus, the observed differences of biofilm associated ciliates at reservoir and outlet of small low-head dams might also change when different stream reaches are considered. However, at least within a stream reach similar variations caused by small low-head dams can be expected.

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QUERING THE OBVIOUS: LESSONS FROM A DEGRADED STREAM

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Abstract

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Keywords: stream ecosystems, disturbance, microorganisms, microbial activity, weirs, conceptual model

Querying the Obvious: Lessons from a Degraded Stream

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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 particulate organic matter 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: conceptual model, disturbance, microbial activity, biofilm, stream ecosystems, weirs.

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 also 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, that is, in a changing environment that is anything but easily assessable. Here, we present our experiences from studies in the Ilm, a third-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 (POM) 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

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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 (DOM) (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 DOM into 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 because 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 et al. 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*, 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 on both external and internal carbon supply because algae colonizing biofilms release extracellular organic carbon that can be rapidly used 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 et al. 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 the 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 toward 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 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, that is, 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. Because 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 2004). 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, for example, 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 not only 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, that is, 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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GENERAL DISCUSSION

As integrated in the Graduate Research School, the fieldwork of the study was conducted at the disturbed 3rd order stream Ilm. The stream's continuum (Vannote *et al.* 1980) is disrupted by numerous small low-head (height < 5 m) dams. In contrast to large dams (Ward & Stanford 1983), temperature, oxygen content, and nutrient concentrations were not influenced by the small low-head dams while flow velocity was always lower in the reservoir compared to outlet sites (chapter 1, 4 & 5). Flow velocity, which is the striking feature in lotic environments, influences nutrient transport processes, sedimentation of suspended matter, contact rate between organisms and surfaces, and detachment from surfaces. At slow flow velocities in reservoirs, fine particulate inorganic and organic matter accumulates implementing that reservoir sites serve as retention structures and promote organic matter processing in straightened streams (Arle 2005, chapter 5). Resuspension and movement of sediment is induced by major floods only, which reset the system also in reservoirs (Fjellheim *et al.* 1993). Despite the numerous small low-head dams, the discharge regime of the stream Ilm is relatively natural with 4 to 5 major floods per year (Vetter 2001). Abrasion caused by major flood events prevents biofilms to reach maturity and increase the importance of initial colonization processes in the stream ecosystem.

With respect to hypothesis 1 the main outcome of the thesis is:
Colonization of surfaces by ciliates is prolonged at faster flow velocities.
Small species are favored at a high content of fine particulate matter that accumulated at slow flow velocities.

As discussed in the first two chapters, flow velocity variations influenced colonization dynamics, community structure, and detachment of biofilm associated ciliates. Initially, ciliates colonization of virgin surfaces was prolonged at faster compared to slow flow velocities. Differences in colonization dynamics were compensated in later stages by entrapping more water column ciliates. The number of organisms flowing past a biofilm (Hunt & Parry 1998) and contact rate between suspended organisms and a surface (Kiørboe & Saiz 1995; Visser & MacKenzie 1998) is higher at a faster, turbulent flow. The probability

increases that these organisms attach and contribute to the biofilm community. The initially established biofilm layer with a sticky matrix of exopolymeric substances and a rough surface (Stoodley *et al.* 1999a; Stoodley *et al.* 1999b; Battin *et al.* 2003) may further contribute to an enhanced advection of particles and organisms. Irrespective of the higher risk of detachment (Characklis 1984), ciliates colonizing surfaces at faster flow velocities seemed to profit from the enhanced advection of prey and lower amount of fine sediment particles in biofilms. The clearance rate of biofilm associated filter feeders is 5 times higher when friction velocity increased from 0 to 0.9 cm s^{-1} (Shimeta *et al.* 2001). Higher abundance of bacteria and algae at faster flow velocities (Korte & Blinn 1983; Hunt & Parry 1998; Willkomm 2007) probably contributed to high numbers of gulper feeders at faster flowing sites. Nevertheless, ciliates of all functional groups contributed to initial biofilm communities implementing all trophic links within the microbial loop. However, the extent of the initial carbon turnover rates appeared to be lower at faster flow velocities.

At slow flowing stream sites, particle associated sedimentation of ciliates seemed to contribute to the initial colonization, since inorganic particles accumulated rapidly during biofilm development at slow flow velocities. Aggregates colonized by ciliates and a variety of other protists (Zimmermann-Timm 2002) might initiate biofilm development at slow flow velocities. A layer of fine sediment particles always covered stones at the slow flowing stream site that were easily re-suspended when disturbed (own observations). At slow flow velocities, an active ciliate community could rapidly establish and mediate the carbon flow from bacteria and protists to higher trophic levels especially in early stages of biofilm development.

The accumulation of sediment particles in biofilms at slow flow velocities seemed not to alter total abundance but species richness. Small sediment particles in the size range of ingestible bacteria might interfere with the feeding process (Pfandl & Boenigk 2006). However, higher bacterial (A. Huchel pers. com.) and algal (own observations) abundances were observed in biofilms with intermediate sediment contents probably due to a larger surface area. For ciliates, higher prey abundances might increase the probability to take up food rather than sediment particles. Thus, growth rate of ciliates was not affected. Especially small bacterivorous ciliates contributed to the ciliate community at high sediment content. Similarly, only few small sized ciliate species reached very high abundances in fine sediments of the lower River Elbe (mainly Scuticociliatia, S. Kröwer pers. com.) and in estuarine

sediments (Hamels *et al.* 2005). Due to the limited pore size, the movement of larger species might be inhibited also in biofilms with a high content of fine sediment particles.

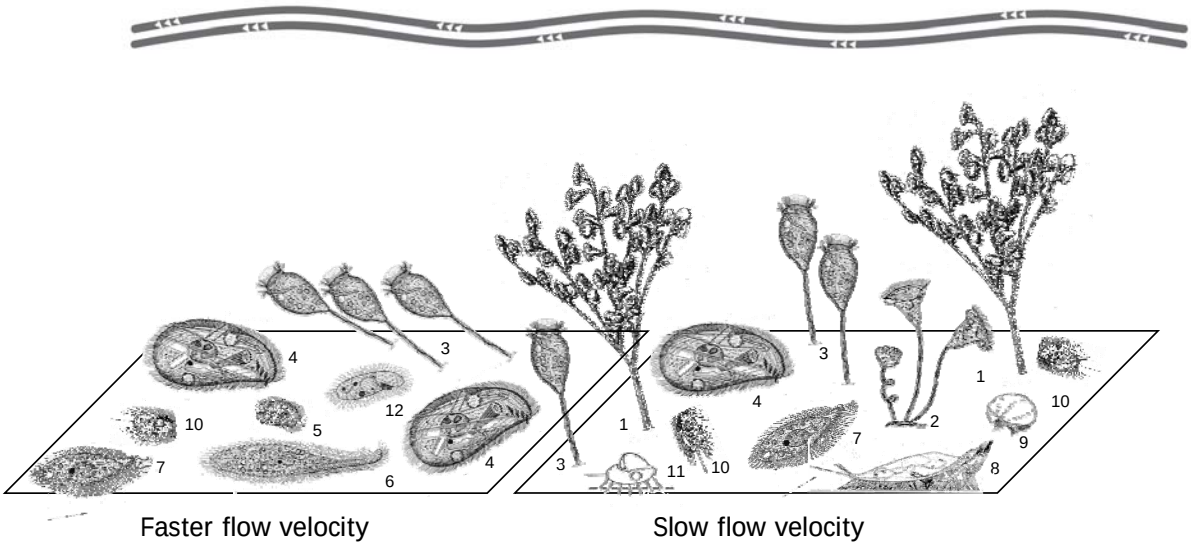
Differences in initial ciliate colonization dynamics might be not primarily correlated to flow velocity but rather to the dynamics of their prey. Within the microbial loop (Azam *et al.* 1983), ciliates utilize a great variety of food sources like bacteria, flagellates, algae, other ciliates, and even rotifers and serve themselves as food for the meio- and macrofauna. Initial colonization dynamics of the most important food sources such as bacteria (Pohlon pers. com.), heterotrophic nanoflagellates (Willkomm 2006; 2007), and diatoms (Willkomm pers. com.) were not influenced by contrasting flow velocities at the slow and faster flowing stream sites. Hence, a faster flow velocity caused the delayed ciliate attachment during the initial colonization processes. In later biofilms, the accumulation of fine sediment particles and competition for space due to high ciliate abundance at slow flow velocities might initiate detachment. These drifting ciliates might colonize biofilms further downstream of the slow flowing sites.

About 80% of the ciliate species observed in the water column occurred in biofilms. These drifting ciliates contributed to the initial biofilm colonization. In total, 108 species were found in stream biofilms of which 26 were sessile and 82 (41 round profile, 41 flattened profile) were vagile (Table A1, Appendix). Immigration of ciliate species was slower at faster flow velocities in both stream and flow channel biofilms. Similarly, in a small mountain stream the maximum species number is reached later with fewer species at a faster flow velocity (Primc & Habdija 1987). The observed species richness in the stream Ilm might be an artifact of the sampling method. Glass slides were chosen as artificial surfaces to standardize the sampling method and to compare biofilm formation at different stream sites. Since more species will be found at a high biotope diversity (Schwerdtfeger 1975), artificial surface samplings underestimate the natural species richness (Foissner 1992). Nevertheless, comparisons between glass slides and natural surfaces of the stream Ilm displayed a similarity of 74% (Soerensen Quotient). The most important ciliate species were observed on both artificial and natural surfaces and only species that occurred sporadic were missed when using artificial surfaces. In 14 days old stream biofilms, 10 to 27 species out of all functional groups occurred in one sample. The coexistence of 30 to 50 ciliate species is typical for small streams (Foissner *et al.* 1992) and might be supported by food niche partitioning. Either different food sources or different sized food items are ingested due to morphological properties of the

ciliates oral apparatus (Fenchel 1986; Finlay *et al.* 1993). In addition, short reproduction times of ciliates prey guarantee sufficient food to maintain the species rich ciliate community. Spatial niche separation might be important as shown for *Oxytrichia bifaria* and *Euplotes crassus* that are adapted to flat and three-dimensional surface, respectively (Ricci 1989).

With respect to hypothesis 2 the main outcome of the thesis is:
Vagile flattened gulper feeder dominated and stayed longer attached at faster flow velocities in stream as well as in flow channel biofilms.

At contrasting flow velocities, different ciliate morphotypes (vagile flattened and round profile, sessile) contributed to functional attributes of the biofilm ciliate community (chapter 1 & 2). Vagile flattened ciliate morphotypes (Phyllopharyngia, Haptoria) colonizing stream and flow channel biofilms tolerated faster flow velocities (Fig. 1). Especially species of the Phyllopharyngia detached only at very fast flow velocities (> 2600 µm s^{-1} near the surface). Both gulpers with a flattened profile live in close contact to the surface and utilize attached biofilm bacteria and protists (Foissner *et al.* 1991; Hausmann 2002). The mechanism of how the cells can stand faster flow velocities is completely unknown. Hypothetically, the excretion of glue like substances at the tip of ventral cilia might enable the cell to stick to the surface (Foissner pers. com.). On the other hand, the oval cell shape in combination with the dorso-ventral flattening might enable the cell to generate a low pressure between cell and surface. Vagile dorso-ventrally flattened Phyllopharyngia dominated the benthic ciliate community in the River Elbe after flood events (S. Kröwer pers. com.). They seemed to tightly attach to grains that sediment down to the streambed at decreased flow velocities after flood events. In contrast, larger sized thigmotactic invertebrate larvae are more vulnerable to abrasion (Elser 2001). At high discharge periods, invertebrates try to escape into deeper sediment layers or actively detach from surfaces and passively drift in the water column (Statzner *et al.* 1984). The smaller sized ciliates might avoid abrasion by living in close contact to the surface. In addition, they might use the fractal structure of stones as refuge (Schönborn 1998). Thus, they probably contribute to initial biofilm communities. Broader food spectra of *Trithigmostoma* spp. (Phyllopharyngia) and *Litonotus* spp. (Haptoria) might enable them to compete for the limited food resources in initial biofilms.



1 *Carchesium polypinum* (Peritrichia)
2 *Vorticella convallaria* (Peritrichia)
3 *Vorticella aquadulcis* (Peritrichia)
4 *Trithigmostoma cucullulus* (Phyllopharyngia)
5 *Thigmogaster potamophilus* (Phyllopharyngia)
6 *Litonotus lamella* (Haptoria)
7 *Litonotus alpestris* (Haptoria)
8 *Stylonychia* sp. (Stichotrichia)
9 *Aspidisca lynceus* (Hypotrichia)
10 *Uronema nigricans* (Hymenostomatia)
11 *Cyclidium glaucoma* (Hymenostomatia)
12 *Dexiostoma campylum* (Hymenostomatia)

Fig. 1. Distribution of ciliate morphotypes in biofilms at contrasting flow velocities. Ciliate sketches are from Foissner et al. (1991, 1992, 1994, 1995). White triangles indicate flow direction.

Ciliates that directly attach to surfaces by means of a stalk could stand near surface flow velocities of up to 4100 μm s^{-1} when attached. In river biofilms, Peritrichia are found to withstand storm flows with flow velocities of up to 2.36 m s^{-1} in the water column while stalks show traces of abrasion (Blenkinsopp & Lock 1994). As pointed out in chapter 2, stalked ciliates were pressed towards the surface by flow velocity but extended more into the water column than the vagile flattened gulpers extended. Peritrichia did not contribute to the benthic ciliate community after a flood event (S. Kröwer pers. com.). Thus, sessile ciliates seemed to be more vulnerable to abrasion. The low abundance of sessile species in initial biofilms at faster flow velocities indicated that attachment and stalk anchorage was inhibited (Table A1, Appendix).

Vagile flat and round filter feeders preferred biofilms at slow flow velocities (Fig. 1) where they reached high abundances. Filter feeder concentrate food particles from their near vicinity by strong feeding currents (Fenchel 1986; Hausmann 2002). The movement of vagile flattened filter feeders with cirri enlarges the distance between cell and surface, which might cause cell detachment already at slow flow velocities. The vagile flattened *Aspidisca lynceus* increased the frequency of tumbles and the vagile round *Cyclidium glaucoma* could stand 7 times faster flow velocities when observed behind or within biofilm flocks, respectively. Flagellates actively search and settle in zones of slowest flow velocity created by shells of *Ancylus fluviatilis* (Willkomm et al. 2007). Thus, microniches where flow velocity is slowed down, i.e. behind microstructures of biofilms or macroinvertebrate houses (de Beer *et al.* 1994; Willkomm *et al.* 2007) might serve as refuge from faster flow velocities.

With respect to hypothesis 3 the main outcome of the thesis is:
Ciliates displacement rate was increased and positive rheotaxis was induced already at slow flow velocities. Grazing activity of sessile filter feeders was inhibited at fast flow velocities.

Live observations revealed that flow velocity changes were sensed by ciliates (chapter 2). Already a small increase of near surface flow velocity altered the ciliate's motility. Ciliate tracks were straightened, displacement rate increased, and movement was oriented towards the flow direction (positive rheotaxis) at 300 to 1100 $\mu m\ s^{-1}$. Sensing the hydro-mechanical signal of flow velocity fluctuations is important for protists to escape predators (Jakobsen 2001; Jakobson 2002) or to recognize prey organisms (postitive rheotaxis towards moving prey) (Jakobsen et al. 2006). It has been suggested that certain ciliary organelles, such as the dorsal bristle complex of hypotrich ciliates, and dorsal brushes with their clavate cilia of certain ciliates, are involved in mechanosensory transduction (Machemer 2003). The combined motility changes might enable the ciliates to rapidly colonize more favorable flow conditions, i. e. eddy water zones behind biofilm microstructures. These eddy water zones are accumulation zones (Silvester & Sleigh 1985) where also food sources for ciliates might aggregate. Thus, food patches are rapidly exploited due to the high dispersion at increased

flow velocities. A positive rheotaxis is the fundamental reaction of macroinvertebrates to compensate drift (Schönborn 1992). Due to the smaller cell size and shorter traveled distance of ciliates, the positive rheotaxis might enable ciliates to stay close to the already successful exploited habitat patch.

With respect to hypothesis 4 the main outcome of the thesis is:
Grazed biofilms by gulper and interception feeders displayed a higher porosity and biofilm surface area to biofilm volume ratio. Deeply embedded bacteria seemed to be vulnerable to grazing by interception feeding flagellates.

At faster flow velocities, biofilm processes are enhanced since the diffusive layer around biofilms is reduced (Oertel *et al.* 2001) and uptake of dissolved nutrients and carbon is improved (Battin *et al.* 2003). At slow flow velocities, internal processes might me more important to promote biofilm function. With the help of confocal laser scanning images, spatial biofilm morphology can be quantified (chapter 3). Grazing gulper feeders raised the porosity and the biofilm surface area to biofilm volume ratio of the bacterial biofilm more than filter feeders. Exchange processes of carbon and nutrients are enhanced at a higher biofilm surface area in relation to the biofilm volume. Further, the high porosity indicated a network of channels and voids through the biofilm. Channels extended down to the biofilm's base where bacteria profit from nutrient and gas transport. Thus, bacterial production and growth is enhanced due to grazing gulper and interception feeder.

Highly abundant vagile filter feeder at slow flow velocities (chapter 1) might enhance the nutrient and gas transport into the biofilm due to the production of feeding currents. Microcolonies in biofilms grazed by a filter feeder were densely packed with bacterial cells supporting the stated assumption. On the other hand, porosity within microcolonies was higher when an interception feeding flagellate was grazing the biofilm. Thus, bacterial cells from the central part of microcolonies might be detached by the flagellum's motility of the sessile interception feeder.

With respect to restoration ecology the main outcome of the thesis is: Small low head dams increase the structural diversity in straightened streams. Flow velocity differences increase the diversity of biofilm associated ciliates that might contribute to enhanced ecosystem resilience. Thus, removal of small low head dams should be substituted by natural retention structures.

Irrespective of season, lower total ciliate abundances and species numbers were observed in reservoirs. Greater differences were found during times of increased nutrient load from allochthonous sources (chapter 4). The accumulation of detritus increased the role of particulate matter in reservoirs indicated in the dominance of detritivorous collector-gatherer (Chironomidae, Oligochaeta) (Wood & Armitage 1997; Arle 2005; Pohlon *et al.* 2007), which contributes to the direct channeling of particulate organic matter (detritus and bacteria) to the invertebrate community. In contrast, easily degradable carbon sources are rapidly taken up into biofilms (Kaplan & Newbold 2003; C. Augspurger, pers. com.) and the microbial community in biofilms seemed to utilize rather dissolved organic carbon at a faster flowing outlet. Thus, all available carbon sources are utilized at sites with contrasting flow velocities created by the small low-head dams. For ecological theory and application in the field of restoration, it would be important to extrapolate data from one case study and observe patterns at different locations. The investigation of three small low-head dams revealed similar effects, with higher ciliate abundances and species numbers in the outlets than in reservoirs.

Beside dams, a natural streambed composition is crucial for POM storage (Wagner 2003). The streambed of the studied Ilm region was near natural with mainly larger stones and cobble, and in relation to the flow velocity varying contents of sand and fine material (own observations). Especially larger stones create diverse small-scale flow velocity patterns. Eddy water zones that establish behind stones are characterized by a slower flow velocity and turbulent backward motion (Oertel *et al.* 2001). Different flow velocities at the streambed surface increase habitat diversity and create optimal conditions for a diverse biofilm associated ciliate community. The maintenance of a rich species pool will enhance the resilience (Steiner et al. 2006) of stream ecosystems to recover after perturbations. Thus, if dam removal is the best and least expensive option (Santucci *et al.* 2005), it should be

combined with the recreation of a heterogeneous channel structure with distinct retention zones (Pohlon et al. 2007) to restore the structural diversity of streams or rivers.

Chapter 5 is a part of a special issue of the journal 'Restoration Ecology' considering the present state and future prospectives of restoration ecology (Halle 2007). Extensive straightening and canalization of streams cause a loss in stream length, streambed area and habitat diversity. In the past, organic matter processing was more efficient in streams where natural structures like fallen trees and backwaters created retention zones (Webster *et al.* 1983). The removal of natural retention structures resulted in a continuous advective transport of particulate organic matter. However, small dams might compensate the loss of retentive structures caused by straightening and reservoirs might act as hot spots for the turnover of organic matter (Arle 2005; Pohlon *et al.* 2007).

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SUMMARY

In small streams that are characterized by large discharge fluctuations, and consequently variable flow velocities, the majority of ciliates occurs associated with stationary surfaces in biofilms. Ciliates, that channel carbon and energy from bacteria and protists to the meio- and macrofauna, maintain the stream's self-purification activity. Frequently occurring flood events initiate abrasion of biofilm communities due to sediment movement. Hence, a fast colonization of surfaces by ciliates is crucial for ecosystem functioning. Besides biofilm abrasion, eddies, and microcurrents in biofilm channels might cause detachment of ciliates. Detachment of ciliates with certain feeding modes characterized by specific morphological features might be delayed or prevented. The present thesis focuses on the impact of flow velocity on colonization dynamics, community structure, and detachment of biofilm associated ciliates and their morphotypes.

Colonization dynamics of biofilm associated ciliates were investigated in the 3rd order stream Ilm (Thuringia, Germany) at a slow flowing site (0.09 m s^{-1}) and two faster flowing sites (0.31 m s^{-1}). These studies were supplemented with biofilms grown in flow channels at flow velocities of 0.05, 0.4, and 0.8 m s^{-1}. At slow flow velocities, surfaces were rapidly colonized by ciliates. In slow flowing flow channels, the majority of ciliates together with the suspended, particulate inorganic matter entered the biofilm within 4.5 h under slow flow velocities. Thus, initial carbon turnover rates appeared to be higher. Differences in colonization dynamics were compensated in later stages by entrapping more water column ciliates in biofilms at faster flow velocities. Since sedimentation of particles seemed to contribute to ciliate community structure at slow flow velocities, flow channel biofilms were grown in the presence of different sediment concentrations (0, 0.6 and 7.3 mg cm^{-2}). Irrespective of the feeding mode, the occurrence of larger species seemed to be inhibited at high sediment content.

Initial stream biofilms inhabited all functional groups of ciliates irrespective of flow velocity implementing all trophic links within the microbial loop. The initial ciliate community structure differed at slow and faster flowing sites, but became more similar after 14 days. According to ciliate morphotypes, differences suggested that vagile flattened gulper feeder, including bacterio-algivorous species (*Trithigmostoma cucullulus, Thigmogaster potamophilus*) and carnivorous species (*Litonotus* spp.) and the bacterivorous sessile filter

feeders tolerated faster flow velocities. The low abundance of sessile filter feeders at faster flow velocities during the initial colonization process suggested that the attachment of these cells was inhibited. Small vagile round ciliates, like the bacterivorous *Uronema nigricans* were more abundant at slow flow velocities, but occurred at faster flow velocities in later stages of biofilm development. The small size of this species might be advantageous to exploit small micro-niches where flow velocities are close to zero. Feeding currents produced by filter feeders might contribute to transport processes of nutrients and gases into biofilms at slow flow velocities.

Live observations of detachment at increased flow velocities confirmed observations from stream and flow channel biofilms. The vagile gulper feeders with a flattened cell profile delayed or avoided detachment probably by a low pressure on the ventral side, or by special cilia that produce an adhesive substance. Thus, vagile flattened species survive in their preferred patch and probably contribute to initial biofilm communities after flood events. The distance between cell and surface of vagile flattened filter feeders walking with cirri is enlarged, which might cause cell detachment already at slow flow velocities. The repeated attachment after detachment of some vagile flattened filter feeders might be important in streams where flow velocity differences around stones cause a patchy distribution of food sources. Attached *Vorticella convallaria* (Peritrichia) remained about 45% of the observed time in a contracted state at >2600 μm s^{-1} indicating an inhibited filtration activity.

Ciliates behavior at increased flow velocities was studied by video analysis. The vagile round filter feeder rarely moved along the surface but cells oriented their anterior-posterior axis in flow direction. Tracks of vagile flattened filter and gulper feeders were straightened, with a higher displacement rate at near surface velocities of 300 to 1100 μm s^{-1}. The observed behavior might enable ciliates to rapidly colonize adjacent patches protected against faster flow velocities, i.e. eddy water zones behind stones and invertebrate cages. These ciliates behaved positively rheotactic already at a slight increase of flow velocity.

At slow flow velocities, ciliates interaction with biofilm morphology was studied in small flow cells and spatial biofilm morphology was observed at a confocal laser scanning microscope. Vagile filter (*Dexiostoma campylum*) and gulper feeding (*Chilodonella uncinata*) ciliates stimulated microcolony formation of a bacterial biofilm. The question remains whether microcolonies served as effective grazing defense strategy in the multispecies bacterial biofilms since abundance of both ciliates increased. The filter feeder probably

sloughed the outer biofilm layers due to the ciliates motility resulting in a reduced maximal biofilm thickness and microcolony area. Enhanced nutrient transport towards the biofilm by the generated feeding currents of the filter feeder guaranteed a high bacterial density also within microcolonies. In contrast, the highly abundant interception feeding flagellate *Spumella* sp. seemed to utilize deeply embedded bacteria from the central part of microcolonies with its motile flagellum. Gulper and interception feeder caused a higher porosity and biofilm surface area to biofilm volume ratio indicating that nutrient exchange between biofilm and the surrounding fluid was improved and bacterial growth was accelerated.

Most of the European streams are straightened and numerous small low-head dams disrupt their continuum. The small low-head dams altered the stream's flow velocity and sediment characteristics. Reduced ciliate abundance and species number were observed at reservoir sites at all samplings during a seasonal cycle. However, the observed differences were higher during periods of high nutrient load in spring and autumn. Examination of ciliates and their feeding groups at three different small low-head dams indicated that differences from one site can be extrapolated to other sites, thus indicating that patterns from one site can be generalized for ecological theory. Due to the reduced flow velocity, particulate organic matter seemed to be the main carbon source for stream biofilms in reservoirs. Contrastingly, biofilm communities at the faster flowing outlet seemed to rely more on dissolved organic matter uptake. To enhance resilience of stream ecosystems, a diverse ciliate community utilizing various organic matter sources is maintained where a natural streambed, heterogeneous channel morphology, and retention structures create different micro-niches. Thus, the studied small low-head dams with small reservoirs might compensate the loss of natural retention structures and increase structural diversity within the straightened stream.

ZUSAMMENFASSUNG

In hydrodynamisch fluktuierenden Ökosystemen, wie kleinen Fließgewässern, ist ein Großteil der mikrobiellen Prozesse an stationären Oberflächen in Biofilmen gebunden. Ciliaten, welche den Kohlenstoff- und Energiefluss von Bakterien und Protisten zur Meio- und Makrofauna gewährleisten, erhalten die Selbstreinigungskraft von Fließgewässern. Regelmäßig auftretende Hochwasserereignisse verursachen die Umlagerung des Sediments und den Abrieb der Biofilmgemeinschaft. Für den Erhalt der Ökosystemfunktion ist eine schnelle Besiedlung von freien Oberflächen entscheidend. Auch durch Turbulenzen und Mikroströmungen in Biofilmkanälen könnten Ciliaten verdriftet werden. Spezifische morphologische Merkmale, die die verschiedenen Ernährungstypen kennzeichnen, könnten das Ablösen der Zellen beeinflussen. Die vorliegende Dissertation verfolgte das Ziel, den Einfluss der Fließgeschwindigkeit auf Besiedlungsdynamik und Gemeinschaftsstruktur biofilm-assoziierter Ciliaten und deren Morpho- und Ernährungstypen zu untersuchen.

Die Besiedlungsdynamik biofilm-assoziierter Ciliaten wurde in der Ilm (Fluss 3. Ordnung, Thüringen, Deutschland) an einer langsam (0,09 $m\ s^{-1}$) und zwei schneller fließenden Stellen (0,31 $m\ s^{-1}$) untersucht. Die Freilanduntersuchungen wurden durch Fließrinnenexperimente vervollständigt, in denen Biofilme bei 0,05, 0,4, und 0,8 $m\ s^{-1}$ wuchsen. Ein bis drei Tage alte Biofilme wurden bei langsamer Fließgeschwindigkeit schneller besiedelt. In Biofilmen der langsam fließenden Fließrinnen, akkumulierte sich innerhalb von 4,5 h der Großteil der Ciliaten zusammen mit dem suspendierten partikulären anorganischen Material. Mit fortschreitender Besiedlung wurden bei höheren Fließgeschwindigkeiten die vorgefundenen Unterschiede kompenisert. Da die Sedimentation suspendierter Partikel die Struktur der Ciliatengemeinschaft beeinflusste, wurden Fließrinnenbiofilme mit unterschiedlich hohen Sedimentkonzentrationen (0, 0,6 und 7,3 $mg\ cm^{-2}$) untersucht. Ungeachtet der Nahrungsaufnahmestrategien wurde das Vorkommen größerer Arten in Biofilmen mit feinem Sediment behindert.

Unabhängig von der Fließgeschwindigkeit waren bereits in initialen Biofilmen alle Ernährungstypen der Ciliaten vertreten. Die Struktur der Ciliatengemeinschaft variierte in initialen Biofilmen unterschiedlicher Fließgeschwindigkeit. Die Verteilung der Ciliaten-morphotypen deutete darauf hin, dass vagil abgeflachte Gulper, sowohl bakteri-algivore (*Trithigmostoma cucullulus*) als auch carnivore Arten (*Litonotus* spp.), und sessile,

bakterivore Filtrierer schnellere Fließgeschwindigkeiten tolerierten. Die niedrige Abundanz der sessilen Filtrierer bei schnelleren Fließgeschwindigkeiten in initialen Biofilmen lässt darauf schließen, dass die Anheftung an Oberflächen gehemmt wurde. Kleine, vagil runde Filtrierer, wie *Uronema nigricans,* waren häufiger bei langsamen Fließgeschwindigkeiten, kamen aber in späteren Phasen der Biofilmentwicklung auch bei schnelleren Fließgeschwindigkeiten vor. Kleine Arten können wahrscheinlich Mikronischen nutzen, in denen die Fließgeschwindigkeit reduziert ist. Die erzeugten Filtrierströme scheinen Transportprozesse von Nährstoffen und Gasen bei langsamen Fließgeschwindigkeiten zu begünstigen.

Die Beobachtungen in Freiland- und Fließrinnenbiofilmen konnten mit Lebendbeobachtungen der Ciliaten bei erhöhten Fließgeschwindigkeiten bestätigt werden. Abgeflachte Gulper vermieden bzw. verzögerten das Verdriften bei schnelleren Fließgeschwindigkeiten, da die Zellen sehr eng an den Oberflächen anliegen und vermutlich klebrige Substanzen ausscheiden. Dadurch sind Gulper befähigt, in dem bereits erfolgreich besiedelten Patch zu überleben, so dass sie vermutlich zur initialen Biofilmgemeinschaft nach Hochwasserereignissen beitragen. Das Verdriften vagil abgeflachter Filtrierer bereits bei geringen Fließgeschwindigkeiten ist auf den größeren Abstand zwischen Zelle und Oberfläche zurückzuführen. Um die durch Fließgeschwindigkeitsunterschiede an Steinoberflächen hervorgerufene patchartige Verteilung von Ressourcen zu erschließen, erscheint die Strategie einiger vagil abgeflachter Filtrierer nützlich, welche sich kurzzeitig verdriften lassen, um sich danach wieder an der Oberfläche anzusiedeln. Die Filtrationsaktivität angehefteter *Vorticella convallaria* (Peritrichia) war vermutliche dadurch gehemmt, da die Zellen bei >2600 μm s^{-1} ca. 45% der Untersuchungszeit kontrahiert blieben.

Mit Hilfe von Videoaufnahmen wurden Verhaltensmuster der Ciliaten bei erhöhten Fließgeschwindigkeiten analysiert. Vagile Filtrierer mit rundem Zellprofil bewegten sich selten an Oberflächen, wobei die anterior-posterior Achse der Zellen in Fließrichtung orientiert wurde. Tracks der vagil abgeflachten Filtrierer und Gulper waren gestreckt und die zurückgelegte Netto-Distanz zwischen zwei Punkten war höher bei Fließgeschwindigkeiten von 300 bis 1100 μm s^{-1}. Dieses Verhaltensmuster scheint Ciliaten zu befähigen, benachbarte, vor hohen Fließgeschwindigkeiten geschützte Patches schnell zu besiedeln. Bereits bei einer geringen Erhöhung der Fließgeschwindigkeit reagierten alle untersuchten Ciliaten positiv rheotaktisch.

Im Rahmen von Fließzellenexperimenten wurde die Interaktion zwischen Ciliaten und räumlicher Biofilmmorphologie bei langsamen Fließgeschwindigkeiten mit Hilfe der confocalen Laser Scanning Mikroskopie untersucht. Vagile Filtrierer (*Dexiostoma campylum*) und Gulper (*Chilodonella uncinata*) stimulierten die Bildung von Mikrokolonien eines bakteriellen Biofilms. Die Frage, ob Mikrokolonien als effiziente Grazingabwehrstrategie eingesetzt werden, konnte nicht vollständig geklärt werden. Filtrierer reduzierten die maximale Biofilmdicke und Mikrokoloniegröße vermutlich durch ihre Bewegungsweise. Dicht besiedelte Mikrokolonien deuteten vermutlich darauf hin, dass der erzeugte Filtrierstrom einen erhöhten Nährstofftransport zum Biofilm gewährleistet. Die abundante *Spumella* sp. nutzte, vermutlich mit Hilfe des Flagellums, auch die in Matrix eingebetteten Bakterien aus dem Zentrum der Mikrokolonien. Gulper und Interceptor verursachten eine höhere Porösität und ein höheres Biofilmoberfläche / Biofilmvolumen Verhältnis. Die Änderungen der räumlichen Biofilmmorphologie verdeutlichten, dass Austauschprozesse zwischen Biofilm und Freiwasser durch Protisten begünstigt und somit das Bakterienwachstum beschleunigt wurde.

Ein Großteil der europäischen Flüsse ist begradigt und Staunanlagen stören das Fließkontinuum. Kleine Wehre reduzieren in den oberhalb gelegenen Abschnitten die Fließgeschwindigkeit und Sedimentzusammensetzung. Unabhängig von der Jahreszeit waren Ciliatenabundanz und Artenzahl im Staubereich niedriger. Deutlichere Unterschiede wurden im Frühjahr und Herbst bei erhöhten Nährstoffkonzentrationen des Wassers beobachtet. Sowohl Ciliatenabundanzen als auch Ernährungstypen belegten, dass Muster die an einem Wehr beobachtet wurden auf andere Wehre übertragbar waren. Durch die reduzierte Fließgeschwindigkeit akkumulierte partikuläres Material, welches die Hauptkohlenstoffquelle im Staubereich zu sein scheint. Im Gegensatzt dazu sind die Biofilmgemeinschaften im schneller fließenden Abfluss eher auf gelöste Kohlenstoffverbindungen angewiesen. Eine diverse Ciliatengemeinschaft, welche heterogene Sohlen- sowie Retentionsstrukturen besiedeln, tragen vermutlich zu einer erhöhten Resilienz des Ökosystems bei. In einem ansonsten begradigten Fließgewässer könnten die untersuchten Wehre mit den kleinräumigen Staubereichen die Strukturvielfalt erhöhen und den Verlust von natürlichen Retentionsstrukturen kompensieren.

APPENDIX

Table A1. Species list of all ciliates found in stream as well as in flow channel biofilms (chapter 1 & 4) and ciliates that where studied in Petri dish flow channels (chapter 2) and small flow cells (chapter 3). Ciliates are grouped according to their morphology. Food sources derived from Foisser et al. (1991, 1992, 1994, 1995). Abbreviations: b - bacterivorous, h - herbivorous, c - carnivorous, o - omnivorous, ff - filter feeder, gf - gulper feeder, df - diffusion feeder, R - Reservoir, O - Outlet, N - Natural site, - species not found.

	Taxon	Feeding mode	Food	Stream biofilms: Fow velocity ($m\ s^{-1}$) 0.09 R	0.32 O	0.30 N	Flow channel biofilms: Fow velocity ($m\ s^{-1}$) 0.05	0.40	0.80	Sediment content ($mg\ cm^{-2}$) 0.0	0.6	7.3	Petri dish flow channel	Flow cells
	HETEROTRICHIA													
	Stentor muelleri Ehrenberg 1831	ff	bh	1			-	-	-	-	-	-		
	Stentor sp.	ff	b	1	1			1		1				
	PERITRICHIA													
	Campanella umbellaria Goldfuss 1820	ff	b	-	-	-	-	-	-	-	-	-	1	
	Carchesium polypinum Ehrenberg 1830	ff	b	1	1	1	1	1	1	1	1	1	1	
	Epistylis coronata Nusch 1970	ff	b	-	-	-		1	1	-	-	-		
	Epistylis galea Ehrenberg 1831	ff	b		1		-	-	-	-	-	-		
	Epistylis sp.	ff	b	1	1	1	1	1	1	1	1			
	Opercularia sp.	ff	b		1	1	-	-	-	-	-	-		
	Platycola decumbens Kent 1882	ff	bh	1	1	1	1	1		-	-	-		
	Pseudovorticella chlamydophora Jankowski 1976	ff	b	1			1			-	-	-		
	Pseudovorticella monilata Foissner & Schiffmann 1974	ff	b	1			1			-	-	-		
	Thuricola kellicottiana Kahl 1935	ff	b	1	1		-	-	-	-	-	-		
	Vaginicola ingenita Kent 1881	ff	b		1	1	-	-	-	-	-	-		
	Vorticella aquadulcis - Komplex	ff	b	1	1	1	1	1	1	1	1	1		
	Vorticella campanula Ehrenberg 1831	ff	b	-	-	-	1	1	1	-	-	-		
	Vorticella convallaria - Komplex	ff	b	1	1	1	1	1	1	1	1	1	1	
	Vorticella infusionum - Komplex	ff	b	1	1	1	1	1	1	1				
	Vorticella microstoma - Komplex	ff	b	-	-	-	1			-	-	-		
	Vorticella octava - Komplex	ff	b	-	-	-	1			-	-	-		
	Zoothamnium sp.	ff	b	1	1	1	1		1	-	-	-		
	SUCTORIA													
	Acineta tuberosa Ehrenberg 1833	df	c	1	1	1		1	1	1	1			
	Heliophrya minima Foissner 1988	df	c	1			-	-	-	-	-	-		
	Metacineta sp.	df	c	1	1		-	-	-	-	-	-		
	Podophrya sp.	df	c	1	1	1	1	1	1	1	1			
	Sphaerophrya magna Maupas 1881	df	c	1		1	-	-	-	-	-	-		
	Staurophrya sp.	df	c	1	1		-	-	-	-	-	-		
	Tocophrya infusionum Buetschli 1889	df	c			1	-	-	-	-	-	-		
	Tocophrya lemnarum Entz 1903	df	c		1	1	1	1	1	-	-	-		
	COLPODEA													
	Colpoda cucullulus Mueller 1773	ff	b	1		1	-	-	-	-	-	-		
	HAPTORIA													
	Acinaria incurvata Dujardin 1841	gf	c		1	1	-	-	-	-	-	-		
	Amphileptus pleurosigma Foissner 1984	gf	c	1	1		1	1		-	-	-		
	Litonotus alpestris Foissner 1978	gf	c	1	1	1	1	1	1	1	1	1		
	Litonotus cygnus nov. comb.	gf	c	1	1	1	1	1	1	1	1	1	1	
	Litonotus crystallinus nov. comb.	gf	c	1	1	1	-	-	-	-	-	-		
	Litonotus fusidens nov.comb.	gf	c	1	1	1	-	-	-	-	-	-		
	Litonotus lamella nov. comb.	gf	c	1	1	1	1	1	1	1	1	1		
	Litonotus varsaviensis Wrześniowski 1870	gf	c	1	1	1	1	1		1	1	1		
	Loxophyllum meleagris Dujardin 1841	gf	c	1	1		-	-	-	-	-	-		
	Spathidium sp.	gf	o		1	1	1			-	-	-		
	Trachelophyllum apiculatum Claparède & Lachmann, 1859	gf	o			1	-	-	-	-	-	-		

Sessile species

Vagile and flattened cell profile

Table A1 continued.

	Taxon	Feeding mode	Food	Stream biofilms: Fow velocity (m s^{-1}) 0.09 R	0.32 O	0.30 N	Flow channel biofilms: Fow velocity (m s^{-1}) 0.05	0.40	0.80	Sediment content (mg cm^{-2}) 0.0	0.6	7.3	Petri dish flow channel	Flow cells
	HYMENOSTOMATIA													
	Cinetochilum margaritaceum Ehrenberg 1831	ff	bh	1	1	1	1	1	1	1		1	1	
	Frontonia angusta Kahl 1931	ff	o	1	1	1	1	1	1	1				
	Frontonia leucas Ehrenberg 1833	ff	o	1	1		-	-	-	-	-	-		
	Lembadion lucens Kahl 1931	ff	o	1	1	1	1	1	1	-	-	-		
	Paramecium caudatum Mueller 1773	ff	b	1	1		-	-	-	-	-	-		
	Paramecium putrinum Claparède & Lachmann 1859	ff	b	1	1		1			-	-	-		
	Pleuronema sp.	ff	b		1		-	-	-	-	-	-		
	Satrophilus muscorum Corliss 1960	ff	b	1	1	1	1	1	1	-	-	-		
	HYPOTRICHIA													
	Aspidisca cicada Mueller 1786	ff	b	1	1	1	1	1	1	1	1			
	Aspidisca lynceus Mueller 1773	ff	b	1	1	1	1	1	1	1	1		1	
	Euplotes aediculatus Pierson 1943	ff	o	1	1	1	-	-	-	-	-	-		
	Euplotes affinis Kahl 1932	ff	bh	1	1		-	-	-	-	-	-		
	Euplotes moebiusi Kahl 1932	ff	bh	-	-	-	1			-	-	-		
	Euplotes patella Mueller 1773	ff	bh	1	1	1	-	-	-	-	-	-	1	
	Euplotes sp.	ff	bh	1	1	1	1			1	1			
	NASSOPHOREA													
	Chilodontopsis depressa Perty 1852	gf	bh	1	1	1	1	1	1	1	1			
	Microthorax sp.	gf	b	-	-	-	1			-	-	-		
	Leptopharynx costatus Mermod 1914	gf	b		1		-	-	-	-	-	-		
	Pseudomicrothorax agilis Mermod 1914	gf	b	1	1	1	-	-	-	-	-	-		
	Zoosterdasys transversa nov. comb.	gf	h	1	1	1	1			-	-	-		
	PHYLLOPHARYNGIA													
	Chilodonella uncinata Strand 1928	gf	b	1	1	1	1	1	1	1			1	1
	Chlamydonella alpestris Pätsch 1974	gf	bh	1	1	1	1	1	1	1	1	1		
	Dysteria fluviatilis Blochmann 1895	gf	b	1	1	1	-	-	-	-	-	-		
	Gastronauta glatratus Deroux 1976	gf	h	1	1	1	1	1		-	-	-		
	Pseudochilodonopsis fluviatile Foissner 1988	gf	h	1	1	1	1	1	1	1	1	1		
	Thigmogaster potamophilus Foissner 1988	gf	h	1	1	1	1	1	1	1	1	1		
	Trithigmostoma cucullus Jankowski 1967	gf	bh	1	1	1	1	1	1	1	1	1		
	Trithigmostoma srameki Foissner 1988	gf	h		1		1	1	1	-	-	-		
	Trithigmostoma steini Foissner 1988	gf	bh	1			-	-	-	-	-	-		
	Trochilia minuta Kahl 1931	gf	b	1	1	1	1	1	1	1	1	1		
	Trochilioides recta Kahl 1931	gf	b	1	1	1	-	-	-	-	-	-		
	STICHOTRICHIA													
	Holosticha monilata Kahl 1928	ff	bh	1	1	1	1	1	1	1	1	1	1	
	Holosticha multistilata Kahl 1928	ff	o	1	1	1	1	1	1	1	1			
	Stichotrichia spp.*	ff	o	1	1	1	1	1	1	1	1	1		
	Stylonychia mytilus Mueller 1773	ff	o	1	1		-	-	-	-	-	-		
	Stylonychia pustulata Ehrenberg 1838	ff	o	1	1	1	-	-	-	-	-	-	1	
	Stylonychia sp.	ff	o		1	1	1			1				

Vagile and flattened cell profile

* Species from different genera are grouped together that could not be distinguished with the QPS. Typical species observed alive were *Holosticha pullaster* Mueller 1773, *Oxytricha setigera* Bore de St. Vincent 1824, *Tachysoma pellionellum* Borror 1972, *Urostyla grandis* Ehrenberg 1830

Table A1 continued.

	Taxon	Feeding mode	Food	Stream biofilms: Fow velocity (m s^{-1}) 0.09 R	0.32 O	0.30 N	Flow channel biofilms: Fow velocity (m s^{-1}) 0.05	0.40	0.80	Sediment content (mg cm^{-2}) 0.0	0.6	7.3	Petri dish flow channel	Flow cells
	COLPODEA													
	Cyrtolophosis mucicola Stokes 1885	ff	b	1			-	-	-	-	-	-		
	Platyophrya vorax Kahl 1926	gf	b	1	1		-	-	-	-	-	-		
	HAPTORIA													
	Dileptus margaritifer Dujardin 1841	gf	o	1	1	1	-	-	-	-	-	-		
	Enchelyodon sp.	gf	o	-	-	-			1	-	-	-		
	Enchelys gasterosteus Kahl 1926	gf	o	1	1	1	1	1		-	-	-		
	Homalozoon vermiculare Stokes 1890	gf	o		1	1	-	-	-	-	-	-		
	Lacrymaria olor Bory de Saint-Vincent 1824	gf	c	1	1	1	1	1	1		1			
	Mesodinium sp.	gf	o	1			1	1	1	-	-	-		
	Monilicaryon monilatus Jankowski 1967	gf	o	1	1		1			-	-	-		
	HETEROTRICHIA													
	Bryophyllum sp.	ff	b		1	1	-	-	-	-	-	-		
	Spirstomum minus Roux 1901	ff	b	1	1		-	-	-	-	-	-		
	Spirostomum teres Claparède & Lachmann 1858	ff	bh	1		1	1			-	-	-		
	HYMENOSTOMATIA													
	Calyptotricha languinosa Wilbert & Foissner 1980	ff	b	1	1	1	1	1		1				
	Colpidium colpoda Stein 1860	ff	bh		1	1	-	-	-	-	-	-		
	Ctedoctema acanthocryptum Stokes 1884	ff	b	1	1	1	1	1	1	-	-	-		
	Cyclidium glaucoma Mueller 1773	ff	b	1	1	1	1	1	1	1		1	1	1
	Dexiostoma campylum Stokes 1886	ff	bh	1	1	1	1	1	1		1	1		1
	Dexiotrichides centrali Kahl 1931	ff	b	1	1		1			-	-	-		
	Glaucoma scintillans Ehrenberg, 1830	ff	b	1	1	1	1	1	1	-	-	-		
	Kahlilembus attenuatus nov.comb.	ff	b	1	1	1	-	-	-	-	-	-		
	Paramecium bursaria Focke 1836	ff	b		1		-	-	-	-	-	-		
	Philasterides armatus Kahl 1931	ff	h		1	1	-	-	-	-	-	-		
	Pleuronema sp.	ff	bh	1	1	1	-	-	-	-	-	-		
	Pseudocohnilembus pusillus Foissner & Wilbert 1981	ff	b	1	1	1	1			-	-	-		
	Tetrahymena pyriformis Ehrenberg 1830	ff	b	1	1		1	1		-	-	-		
	Uronema nigricans Mueller 1786	ff	b	1	1	1	1	1	1	1	1	1		
	OLIGOTRICHIA													
	Codonella cratera Imhof 1885	ff	h		1			1		1				
	Halteria grandinella Mueller 1773	ff	bh			1	-	-	-	-	-	-		
	Rimostrobidium humile Petz & Foissner 1992	ff	h		1		1	1	1	1	1	1		
	Strobilidium caudatum Fromel 1876	ff	bh	-	-	-	1	1	1	1	1	1		
	Tintinnidium pusillum Entz 1909	ff	bh	1	1	1	1			-	-	-		
	PROSTOMATEA													
	Coleps nolandi Kahl 1930	gf	o		1	1	-	-	-	-	-	-		
	Coleps sp.	gf	o		1	1	-	-	-	-	-	-		
	Holophrya discolor Ehrenberg 1833	gf	o	1	1	1	1	1	1	1				
	Placus luciae Kahl 1930	gf	o	1	1		-	-	-	-	-	-		
	Prorodon ellipticus (Kahl 1930)	gf	o	-	-	-	1	1	1	-	-	-		
	Prorodon niveus Ehrenberg 1833	gf	o	1	1	1	1	1	1	-	-	-		
	Urotricha agilis Kahl 1930	gf	bh	1	1	1				1				
	Urotricha armata Kahl 1927	gf	c	1	1	1	-	-	-	-	-	-		
	Urotricha furcata Schewiakoff 1892	gf	bh		1	1	1			-	-	-		
	Urotricha globosa Schewiakoff 1892	gf	bh	1	1	1	-	-	-	-	-	-		
	Urotricha ovata Kahl 1926	gf	h	1	1	1	1	1		-	-	-		
	STICHOTRICHIA													
	Stichotricha secunda Perty 1849	ff	bh		1		-	-	-	-	-	-		

Vagile and round cell profile

DANKSAGUNG

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EIGENSTÄNDIGKEITSERKLÄRUNG

Ich versichere an Eides statt, dass ich die von mir vorgelegte Dissertation selbständig angefertigt und nur die von mir angegebenen Quellen und Hilfsmittel verwendet habe.

Die Bestimmungen der Promotionsordnung der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität Jena sind mir bekannt.

Die Hilfe eines Promotionsberaters wurde nicht in Anspruch genommen und Dritte erhielten weder unmittelbar noch mittelbar geldwerte Leistungen, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen.

Die Dissertation oder Teile davon wurde noch nicht als Prüfungsarbeit an der FSU oder an einer anderen Einrichtung für eine staatliche oder andere wissenschaftliche Prüfung eingereicht.

Jena, den

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Ute Risse-Buhl

NACHWEIS DES EIGENANTEILS AN DEN MANUSKRIPTEN

Teile der Dissertation sind (Kapitel 1 & 2) bzw. werden (Kapitel 3 & 4) als Publikationen bei internationalen Fachzeitschriften eingereicht. Ein anderer Teil (Kapitel 5) ist bereits in der genannten Fachzeitschrift erschienen. Mein Beitrag an der Erstellung der Manuskripte gestaltete sich folgendermaßen:

Risse-Buhl, U. & Küsel, K. Colonization dynamics of biofilm associated ciliate morphotypes at different flow velocities.

Submitted to the *European Journal of Protistology* (15.04.2008)

Konzept der Freiland- und Fließrinnenexperimente wurde mit K. Küsel diskutiert, Planung und Durchführung der Experimente, sowie Zählung der Ciliaten und Auswertung der Daten erfolgten durch U. Risse-Buhl, Ergebnisse wurden mit K. Küsel diskutiert, Erstellung des Manuskripts durch U. Risse-Buhl, Überarbeitung des Manuskripts durch K. Küsel

Risse-Buhl, U., Scherwass, A., Arndt, H., Kröwer, S. & Küsel, K. Detachment and motility of biofilm associated ciliates at increased flow velocities.

Submitted to *Aquatic Microbial Ecology* (20.06.2008)

Konzept des Experiments wurde mit K. Küsel, H. Arndt und A. Scherwass diskutiert, Planung und Durchführung des Experiments sowie Auswertung der Daten durch U. Risse-Buhl, Durchführung des praktischen Teils der Experimente an der Universität zu Köln, Isolierung bzw. Anreicherung und Kultivierung der Ciliaten durch U. Risse-Buhl und S. Kröwer, Erstellung des Manuskripts durch U. Risse-Buhl, Überarbeitung des Manuskripts durch K. Küsel, Korrektur des Manuskripts durch H. Arndt, A. Scherwass und S. Kröwer

Böhme, A., **Risse-Buhl, U.** & Küsel, K. Protists with different feeding modes influence morphological biofilm characteristics.

Manuscript will be submitted to *FEMS Microbiology Ecology* in July 2008

Konzept der Fließzellenexperimente von U. Risse-Buhl aufgestellt, Isolierung und Kultivierung der Ciliaten sowie Aufbau und Durchführung der Experimente durch A. Böhme, Auswertung der Daten durch A. Böhme, alle Autorinnen beteiligten sich an der Diskussion der Ergebnisse, Erstellung des Manuskripts durch A. Böhme (Material & Methoden sowie Ergebnisse) und U. Risse-Buhl (Einleitung und Diskussion), Überarbeitung des Manuskripts durch K. Küsel

Risse-Buhl, U. & Küsel, K. The effect of small low-head dams on biofilm associated ciliates.

Manuscript in preparation to be submitted to *Limnologica*

Konzept der Freilandexperimente entwickelt im Graduiertenkolleg geleitet durch S. Halle, Planung und Durchführung der Experimente, sowie Auswertung der Daten durch U. Risse-Buhl, Erstellung des Manuskripts durch U. Risse-Buhl, Überarbeitung des Manuskripts durch K. Küsel

Pohlon, E., Augspurger, C., **Risse-Buhl, U.**, Arle, J., Willkomm, M., Halle, S. & Küsel, K. Quering the obvious: Lessons from a degraded stream.

Published in *Restoration Ecology* 15(2): 312-316 in June 2007

Initiierung des Beitrags von S. Halle, Zielstellung des Konzeptes gemeinsam von allen AutorInnen entwickelt, Beitrag von U. Risse-Buhl umfasst einen Beitrag zur Datengrundlage der Ciliaten im Biofilm, sowie Korrektur des Manuskripts

CURRICULUM VITAE

Persönliche Daten

Ute Risse-Buhl
geboren am 07.02.1977 in Arnstadt
verheiratet, eine Tochter (geboren am 31.03.2004)

Bildungsweg

Seit 06/2003	Doktorandin im Rahmen des Graduiertenkollegs "Funktions- und Regenerationsanalyse belasteter Ökosysteme" an der Friedrich-Schiller-Universität Jena, Thema: *Colonization dynamics and grazing activity of ciliates in stream biofilms*
07/2006	Forschungsaufenthalt an der Universität zu Köln bei Prof. Dr. H. Arndt
02/2004 – 03/2005	Babypause
09/2001 – 04/2003	Anfertigung der Diplomarbeit an der Friedrich-Schiller-Universität Jena mit dem Thema: *Longitudinal distribution of pelagic ciliates in the River Elbe – considering turbulence as a stress factor* (1,1)
09/1998 – 07/1999	Auslandsstudium am Department of Biological Sciences, University of Birmingham, Great Britain
07/1999	Praktikum ‚Physiological Ecology of Algae' unter Leitung von Prof. Dr. B. S. C. Leadbeater im Laboratory of Marine Biology Concarneau, Frankreich, Thema: *Movement of blue-green algae in salt marsh mud*
10/1995 – 04/2003	Studium der Biologie mit Schwerpunkten Ökologie, Zoologie, Botanik und Umweltrecht an der Friedrich-Schiller-Universität Jena (Diplom, Gesamtnote 1,4)
09/1993 – 08/1995	Albert Schweizer Gymnasium, Erfurt Abschluß: Allgemeine Hochschulreife (Note: 2,2)

Studienbegleitende Tätigkeiten

10/2005	Advanced Biofilm Course unter Leitung von Dr. T. Neu am Umweltforschungszentrum, Magdeburg; Einführung in Methodik und Anwendung von Mikrosensoren, Laser Scanning Mikroskopie und Biofilmmodellierung
01/2002	Einarbeitung in die Taxonomie von Ciliaten bei Prof. Dr. W. Foissner, Universität Salzburg, Österreich
2001 - 2002	Beprobung des Benthals der Elbe im Verbundprojekt Elbe-Ökologie
2000 - 2001	Qualitative und quantitative Erfassung der Crustaceae im Verbundprojekt Elbe-Ökologie
09/2000 – 10/2000	Präparationstechniken und Einarbeitung in die Taxonomie der Diatomeae bei Dr. R. M. Crawford, Alfred-Wegener-Institut, Bremerhaven

Lehre

2007 - 2008	Anleitung von A. Böhme im Labor, Diplomarbeit Titel: *Protists with different feeding modes influence morphological biofilm characteristics*
2008	Vorlesung Angewandte Limnologie (1,5 h)
2008	Betreuung eines zweiwöchigen Einzelpraktikum gemeinsam mit A. Böhme Titel: *Impact of protozoa on the three dimensional structure of a bacterial biofilm*
2007	Betreuung eines zweiwöchigen Einzelpraktikum gemeinsam mit A. Böhme Titel: *Impact of protozoan grazing on the spatial structure of a stream bacterial biofilm*
2007	Betreuung eines zweiwöchigen Einzelpraktikum Titel: *Impact of sediment particles on the colonization of surfaces by algae*
2007	Betreuung eines zweiwöchigen Einzelpraktikum Titel: *Isolation and cultivation methods of protists with different feeding modes*
2006 - 2008	Betreuung verschiedener Praktika zum Thema: *Methoden zur Untersuchung von Fließgewässern und stehenden Gewässern*
2006	Betreuung von vier einwöchigen Praktika Titel: *Einfluss der Fließgeschwindigkeit auf die Artendynamik der biofilm-assoziierten Algen*
2005	Betreuung eines zweiwöchigen Praktikum Titel: *Impact of small low-head dams on the structure of the biofilm community in the stream Ilm*
2003 - 2008	Geländepraktikum zu dem Thema: *Wozu sind Protisten nütze? – Gewässergütebestimmungen anhand von einzelligen Organismen*
2003 - 2006	Ringvorlesung im Hauptfach Ökologie (3 x 1,5 h), Präsentation des Dissertationsthemas

Tagungen und Präsentationen

Risse-Buhl, U., Scherwass, A., Arndt, H., Kröwer, S. and Küsel, K. (2008) Vortrag: *Flow velocity at the liquid-solid interface influences detachment and creeping behavior of biofilm associated ciliates,*
27. Jahrestagung der Deutschen Gesellschaft für Protozoologie, Rostock/Warnemünde

Böhme, A., **Risse-Buhl, U.** and Küsel, K. (2008) Poster: Changes in the three-dimensional structure of a multispecies bacterial biofilm due to grazing of *Chilodonella uncinata* and *Spumella* sp.,
27. Jahrestagung der Deutschen Gesellschaft für Protozoologie, Rostock/Warnemünde

Risse-Buhl, U. & Küsel, K. (2007) Vortrag: *Besiedlungsdynamik von biofilmassoziierten Ciliaten unter dem Einfluss der Fließgeschwindigkeit,*
Jahrestagung der Deutschen Gesellschaft für Limnologie e.V., Münster

Risse-Buhl, U. & Küsel, K. (2007) Poster: *Colonization dynamics of ciliates on artificial surface in the field and flow channels,*
26. Jahrestagung der Deutschen Gesellschaft für Protozoologie, Salzburg

Risse-Buhl, U., Scherwass, A., Arndt, H. & Küsel, K. (2006) Poster: *Impact of flow velocities on biofilm associated ciliates,*
10. Meeting of the International Society of Microbial Ecology, Vienna; p. A68

Küsel, K., Augspurger, C., Pohlon, E. & **Risse-Buhl, U.** (2006) Poster: *Dynamics of biofilm formation and biofilm turnover activities in small streams,*
10. Meeting of the International Society of Microbial Ecology, Vienna; p. A69

Risse-Buhl, U. & Küsel, K. (2006) Vortrag: *Einfluss der Fließgeschwindigkeit auf biofilmassoziierte Ciliaten,*
25. Jahrestagung der Deutschen Gesellschaft für Protozoologie, Berlin

Risse-Buhl, U. & Zimmermann-Timm, H. (2003) Posterpräsentation: *Longitudinale Verteilung pelagischer Ciliaten in der Elbe – Auswirkung anthropogener Verbauun,*
Jahrestagung der Deutschen Gesellschaft für Limnologie e.V., Köln
Erweiterte Zusammenfassung der Jahrestagung in Köln (2004) pp. 177-182.

Risse-Buhl, U. & Zimmermann-Timm, H. (2002) Posterpräsentation: *Turbulenz und Stauung – Einfluss von Querverbauungen auf die Ciliatenverteilung im Rhithral der Elbe*
Jahrestagung der Deutschen Gesellschaft für Limnologie e.V., Braunschweig
Erweiterte Zusammenfassung der Jahrestagung in Braunschweig (2003) pp. 449-454.

Eingeladene Vorträge

Risse-Buhl, U. (2008) *Ciliaten in Fließgewässerbiofilmen: Fließgeschwindigkeit und Grazing beeinflussen die Biofilmdynamik*, Universität Salzburg, Österreich (Prof. Dr. U. Berninger)

Jena, den