„Long-term dynamics of microbial biofilm communities of the river Rhine“

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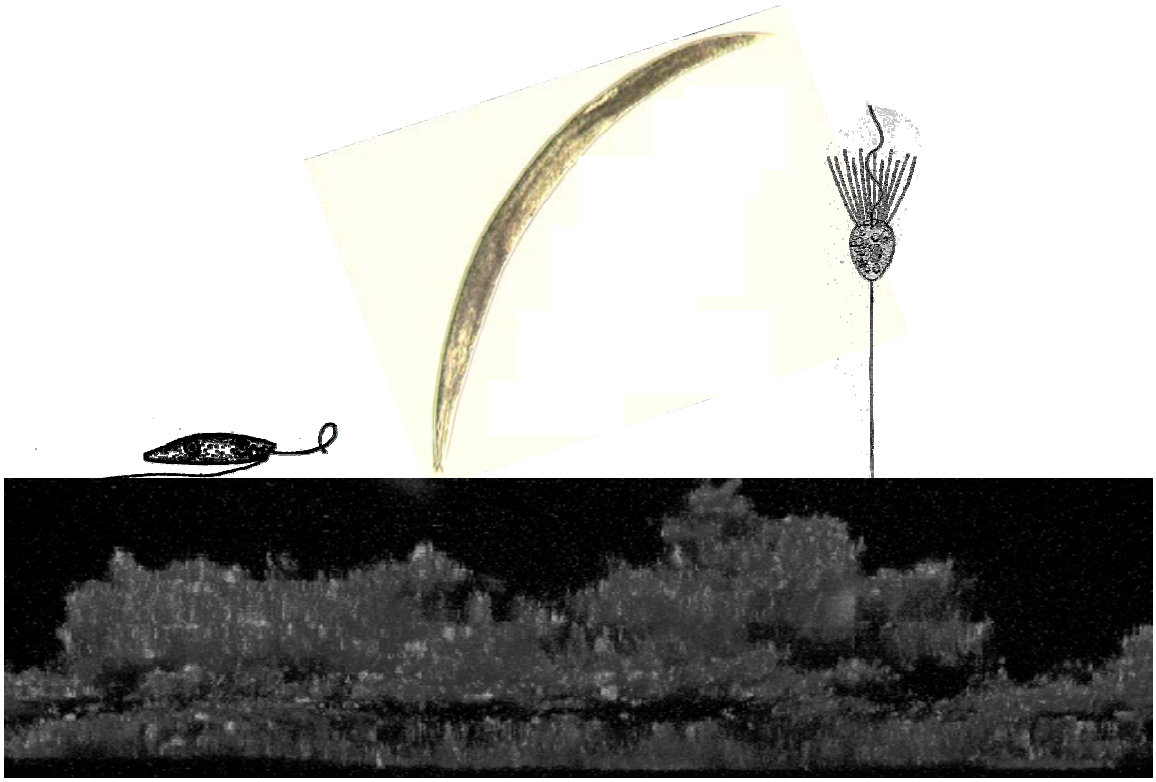

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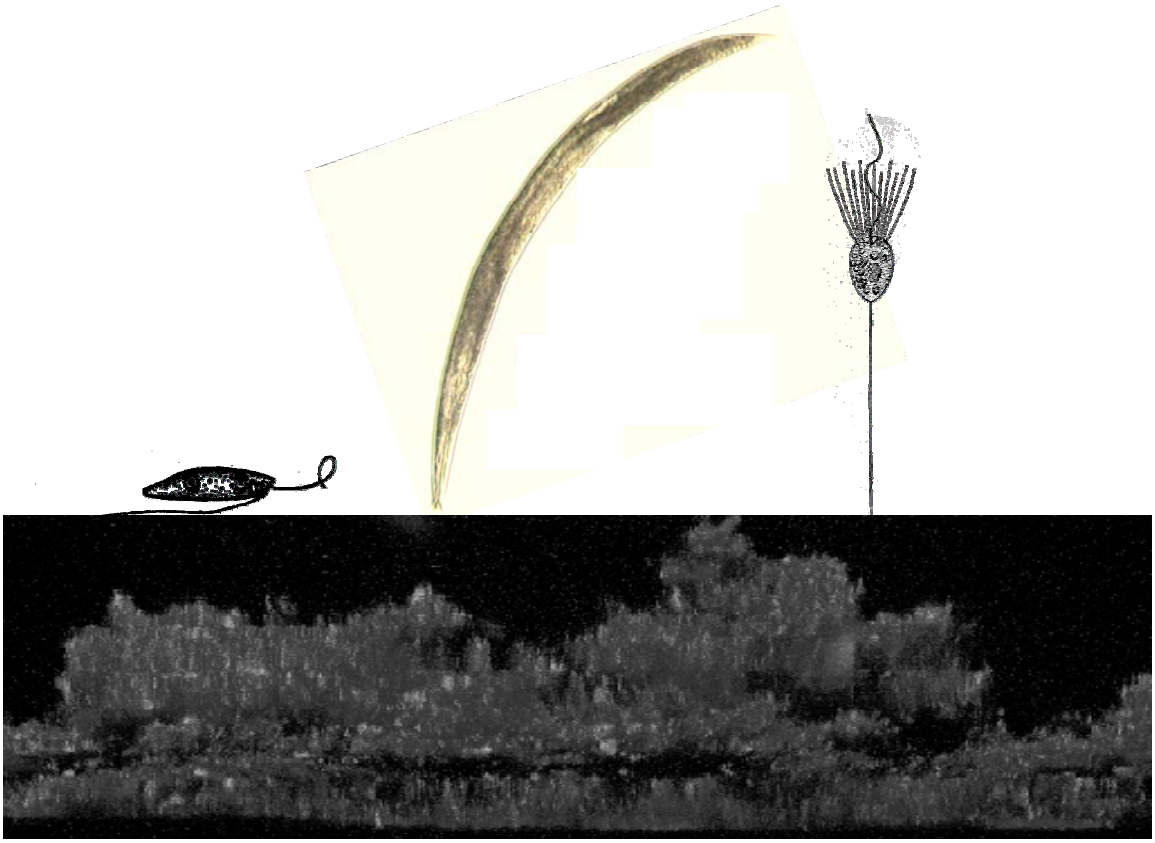

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Einleitung

In Fließgewässern spielen Biofilmgemeinschaften eine entscheidende Rolle im Stofftransfer (Pusch et al., 1998; Weitere et al., 2003). Das Interstitial eines Flussbettes stellt eine große besiedelbare Oberfläche dar. Bisherige Studien, die einzelne Komponenten benthischer Gemeinschaften untersucht haben, richteten den Fokus ihrer Untersuchungen exklusiv auf Bakterien, Algen und Ciliaten (Riedel-Lorje, 1980; Foissner et al., 1992; Berger et al., 1997; Hillebrand und Sommer, 2000; Hillebrand, 2002; Kahlert et al., 2002).

Frühere Studien postulierten, dass in Flüssen mit einem hohen Verhältnis von Gewässergrundfläche zu Wasservolumen und einer hohen Durchmischungsrate eine top-down Kontrolle des Planktons durch benthische Gemeinschaften möglich ist (Welker und Walz, 1998). Bisherige Arbeiten haben hierbei jedoch vornehmlich die benthische Makrofauna und ihre Bedeutung für den Stofftransport untersucht (Alpine und Cloern, 1992; Köhler, 1995; Basu und Pick, 1997; Caraco et al., 1997; Pace et al., 1998; Welker und Walz, 1998; Schöl et al., 1999; Weitere und Arndt, 2002). Jüngere Untersuchungen zeigen, dass die Umsätze und damit der Abbau von organischer Substanz in einem Fließgewässer vermutlich nur zu einem geringen Teil von Makrozoobenthos bestimmt werden (Monaghan et al., 2001; Weitere et al., 2005). Es gibt Anhaltspunkte, dass insbesondere die auf großen Oberflächen am Gewässerboden und im angeschlossenen Interstitialraum angesiedelten Biofilme diesen Stofftransfer erheblich mit bestimmen (Schmidt-Denter, 1999; Reiss, 2002; Arndt et al., 2003; Weitere et al., 2003). Von Biofilmen weiß man heute, dass die Struktur sehr vielfältig sein kann und maßgeblich die Funktion des Biofilms beeinflusst (Costerton et al., 1995). Die Bakterien sind auf dem Biofilm in eine Matrix aus extrazellulären Polysachariden eingelagert (Blenkinsopp und Costerton, 1991). Diese Matrix stellt ein Reservoir für Wasser (Lawrence et al., 1991) und Nährstoffe dar (Freeman et al., 1995) und bietet zudem Schutz vor verschiedenenartigen biotischen und abiotischen Faktoren (Costerton et al., 1995).

Wegen der hohen Komplexität des Systems Biofilm und seiner schweren methodischen Zugänglichkeit liegen bisher kaum Arbeiten vor, die die langfristige Sukzession von kompletten Biofilmgemeinschaften untersucht haben. Im Rahmen dieser Langzeitstudie sollten die Hauptkomponenten langfristig wachsender Biofilmgemeinschaften nachgewiesen werden. Es sollte erstmalig eine Modellvorstellung für den Stoffffluss in einem Biofilm in einem großen Fluss entwickelt werden und geklärt werden, welches die wichtigsten Interaktionen innerhalb des Systems Biofilm sind. Eine Besonderheit der vorliegenden Studie besteht darin, dass versucht werden sollte, alle wichtigen Organismengruppen im Biofilm parallel auf dem Modellsubstrat zu untersuchen. Die Kooperation mit Frau Bärbel Ackermann, für die ich mich in diesem Rahmen ausdrücklich bedanken möchte, sollte es ermöglichen, diesen holistischen Fokus auf das System Biofilm zu richten. Frau Ackermann sollte während der Langzeituntersuchung die Algen, Ciliaten und Makrofauna bearbeiten, während die Bakterien, Flagellaten und Meiofauna von mir bearbeitet werden sollten. Im Rahmen dieser Studie wurden Objektträger als Modellsubstrat für die Untersuchung der Biofilmgemeinschaften gewählt. Frühere Untersuchungen belegen, dass das Modellsystem Objektträger eine gute Annäherung an Biofilme auf natürlichen Oberflächen darstellt (Schönborn, 1981, 1998; Schmidt-Denter, 1999). Um die Relevanz der ermittelten Daten darzustellen, sollten im Rahmen dieser Arbeit zusätzlich Vergleichsuntersuchungen von Biofilmen auf künstlichen Substraten (Objektträgern) und Biofilmen auf natürlichen Substraten (Steinen) vom Uferbereich und von der Gewässersohle des Rheins angestellt werden. Bisherige Sukzessionsuntersuchungen zu Biofilmen im Rhein waren auf kurzfristige Exposition von Modellsubstraten ausgerichtet (Schmidt-Denter, 1999). In dieser Arbeit wurden Objektträger über einen Zeitraum von 14 Monaten in einer Fließrinne an der Ökologischen Rheinstation der Universität zu Köln direkt im Rhein exponiert. Alle 3 Wochen wurden 16 Objektträger zufällig ausgewählt, aus dem Trägersystem entnommen und die

Biofilme mittels direkter Lebendzählung (Arndt et al., 2000) auf ihre taxonomische Zusammensetzung und die Abundanz der nachgewiesenen Arten untersucht.

Das erste Kapitel ist primär auf die Rolle der heterotrophen Flagellaten (HF) im System Biofilm konzentriert. Erstmalig sollte die Sukzession der HF auf einem Biofilm über einen Zeitraum von 14 Monaten dargestellt werden. Zahlreiche Untersuchungen haben gezeigt, dass Protozoen eine bedeutende Position im Stoffumsatz aquatischer Systeme einnehmen (Pomeroy, 1974; Azam et al., 1983; Güde, 1989; Weisse et al., 1990). Nach dem Konzept des „microbial loop“ (Azam et al., 1983) sind die HF die wichtigsten Prädatoren der Bakterien im Pelagial. Die vorliegende Untersuchung sollte Hinweise erbringen, ob das modellhafte Schema von Azam et al. (1983) auf den Biofilm übertragen werden kann. Bakterien als Konsumenten der von den Algen gelösten organischen Substanz transferieren organische Substanz in Biomasse (Marxsen, 1988). Der Abbau und die Respiration der Algen- und Bakterienbiomasse ist somit ein Schlüsselweg zum Verständnis der Funktion und Leistungsfähigkeit von Biofilmgemeinschaften. Bisher haben jedoch nur wenige Untersuchungen HF als Komponente der Biofilmgemeinschaft untersucht (Railkin et al., 1990; Zolotarev, 1995; Widera, 1997). Im Rahmen dieser Arbeit sollten Sukzessionsmuster der HF auf Art- und Gruppenebene beschrieben werden und wesentliche Hinweise zur Beantwortung der Frage nach der Bedeutung der HF für das System Biofilm gegeben werden. Anhand der gewonnenen Ergebnisse sollte die Frage beantwortet werden, wodurch die Abundanz und Sukzession der HF im System gesteuert wird und ob die HF top-down oder bottom-up kontrolliert werden.

Im zweiten Kapitel wird das Hauptaugenmerk der Studie auf die Meiofauna und ihre Bedeutung für das System Biofilm sowie die Anbindung an weitere trophische Ebenen im Nahrungsgewebe des Rheins gerichtet. Bisherige Studien haben sich meist auf die methodisch

leichter zu erfassende Makrofauna gestützt. Das bisherige Verständnis von ökologischen Prozessen im Benthos basiert daher vor allem auf Untersuchungen, die auf Bakterien und Makrofauna fokussiert waren (Allan, 1995; Schmid-Araya und Schmid, 2000; Reiss, 2002; Bergtold und Traunspurger, 2004). Die Bedeutung der Meiofauna in benthischen Nahrungsgeweben wurde bisher nur wenig bearbeitet (Borchard und Bott, 1995; Schmid-Araya, 1994; Traunspurger, 1991; Bergtold und Traunspurger, 2004). Die Meiofauna stellt auf Abundanz bezogen jedoch mehr als 95% aller Metazoen in den meisten Flüssen, hierbei sind die häufigsten Gruppen Nematoden und Rotatorien (Duft et al., 2002). In Biofilmen ist die Meiofauna ebenfalls eine abundante Komponente und es wird angenommen, dass die Meiofauna einen starken Effekt auf die Mikrofauna und die Mikroalgen in Biofilmen besitzt (Schmid-Araya und Schmid, 2000). Dieser Effekt könnte sowohl aus direkten Einflüssen wie „Fraßdruck“ als auch aus indirekten Einflüssen durch Bewegung und Exkretion bestehen (Abrams und Mitchell, 1980; Alkemade et al., 1992b; Aller und Aller, 1992; Traunspurger et al., 1997; De Mesel et al., 2004). Der Meiofauna in lotischen Fließgewässerhabitaten dienen Bakterien, Algen und Detritus als Nahrungsgrundlage (z.B. Perlmutter und Meyer, 1991; Borchard und Bott, 1995). Aufgrund ihrer Größenklasse erscheinen Meiofaunaorganismen geeignet, eine Verbindung zwischen mikrobiellem Nahrungsnetz und dem Nahrungsgewebe der Makrofauna darzustellen. Daten über Meiofaunagemeinschaften sind deshalb nötig, um ihre tatsächliche Bedeutung innerhalb des benthischen Nahrungsgewebes abschätzen zu können. In der vorliegenden Studie sollte die taxonomische Zusammensetzung der Meiofauna in Biofilmen des Rheins über einen Zeitraum von 14 Monaten beschrieben werden. Darüber hinaus sollten mit Vertretern der beiden im Rahmen dieser Untersuchung dominierenden Meiofaunataxa (Nematoden und Rotatorien) Fraßexperimente in Miniaturfließkammern durchgeführt werden, um den Effekt dieser beiden dominanten Meiofaunagruppen auf das System Biofilm beschreiben zu können.

Innerhalb des dritten Kapitels sollte erstmalig eine Modellvorstellung für den Stoffffluss in einem Biofilm eines großen Fließgewässers entwickelt werden. Die Annahme eines „microbial loop“ durch Azam et al. (1983) hat zu einem steigenden Interesse an Interaktionen in Nahrungsnetzen und der Rolle von Protozoen in pelagischen und benthischen Nahrungskreisläufen geführt (Alongi, 1991; Sherr and Sherr, 2000). Während Informationen über Organismen des mikrobiellen Nahrungsgewebes für das Pelagial existieren, ist wenig bekannt über die Organismen, die die großen Oberflächen der Gewässer besiedeln (Sherr und Sherr, 1994). Die Entwicklung von Modellen für das Benthal ist bisher durch die geringe Anzahl von Daten über benthische Organismen und die Zusammensetzung von benthischen Lebensgemeinschaften beschränkt (Silvert, 1991; Weitere et al., 2003; Junk, 2005). Durch die Ergebnisse der quantitativen und qualitativen Erhebung der Biofilmflora und -fauna im Rhein über einen Zeitraum von 14 Monaten sollte eine Abschätzung der Bedeutung der verschiedenen Komponenten im System Biofilm vorgenommen werden. Die Produktion und Biomasse der verschiedenen Gruppen im Biofilm sollten untereinander verglichen und der mögliche Stoffffluss zwischen den Organismengruppen des Biofilms abgeschätzt werden, um den prinzipiellen Verlauf des Kohlenstoffes durch den Biofilm im Rhein charakterisieren zu können. Bei der Kalkulation des Stoffflusses sollte auch die offenbar wichtige Kopplung zwischen pelagischem und benthischem Nahrungsgewebe in Betracht gezogen werden, die bis zum heutigen Tage in der Literatur nicht vollständig verstanden ist (Hershey et al., 2005). Hierbei konnte auf die von Weitere et al. (2005) publizierten Ergebnisse für den Stoffffluss im Pelagial des Rheins bei Köln zurückgegriffen werden. Die in dieser Studie vorgenommene Abschätzung sollte eine wichtige Grundlage für das Verständnis von Stoffflüssen in großen Fließgewässern liefern. Die immer tiefgreifendere Kenntnis von den Stoffkreisläufen in den verschiedenen Ökosystemen ist von immenser Bedeutung, dieses Verständnis könnte u.a. zu einer Optimierung des Selbstreinigungsvermögens der Gewässer beitragen, indem die

ohnehin vom Menschen stark überprägten Fließgewässer beispielsweise durch Umstrukturierung der Gewässersohle gestaltet werden.

Im vierten Kapitel dieser Arbeit werden Ergebnisse vergleichender Untersuchungen von Biofilmen in der Antarktis diskutiert. Einige der größten einheitlichen Oberflächen auf der Erde, die von Biofilmen besiedelt werden, sind die verschiedenen Eishabitate der Antarktis. Aufgrund der räumlichen Ausdehnung dieses Lebensraumes stellen diese Biofilme in Eishabitaten der Antarktis ein interessantes Objekt für Vergleichsuntersuchungen dar. Im Rahmen von ISPOL, einem dreimonatigen Driftexperiment im nördlichen Weddellmeer, bestand die Möglichkeit, im Frühjahr Flagellatengemeinschaften in Eishabitaten in der Antarktis zu untersuchen. Hierdurch sollte ermöglicht werden, einen Vergleich der heterotrophen Flagellatengemeinschaft in Biofilmen des Rheins mit heterotrophen Flagellatengemeinschaften in Eishabitaten der Antarktis anzustellen. Heterotrophe Flagellaten sind als typische Komponente der Eisfauna bekannt (Garrison et al., 2005), bisher wurde ihre Zusammensetzung in den Eishabitaten jedoch nur wenig untersucht. An den Rändern von Eisschollen existiert ein Eis-Wasser-Gemisch (slush). Dieser Bereich, der sich insbesondere im Frühling/Sommer in der Antarktis stark vergrößert, stellt eine gewaltige besiedelbare Oberfläche für mikrobielle Biofilme dar. Es sollte untersucht werden, ob sich in diesem Lebensraum eine typische eigene Nanofauna entwickelt hat. Bisherige Untersuchungen beschreiben, dass die Zusammensetzung des „microbial food webs“ in den Eishabitaten vergleichbar mit der Zusammensetzung im Pelagial ist (Legendre et al., 1992; Garrison und Mathot, 1996). Weiterhin sollte untersucht werden, ob sich die Slush-Flagellatengemeinschaft von der Zusammensetzung der Flagellatengemeinschaft in den anderen Lebensräumen im Eis unterscheidet (Eis, Porenwasser).

Die folgende Arbeit ist in mehrere selbständige Kapitel unterteilt. Der Aufbau der Arbeit ist als kumulative Arbeit angelegt, bestehend aus vier Manuskripten, die zukünftig in internationalen Zeitschriften publiziert werden sollen. Durch diesen Aufbau war es nicht vermeidbar, dass sich einzelne Passagen oder Abschnitte (insbesondere in den Teilen zu Material und Methoden) innerhalb dieser Dissertation wiederholen.

Kooperationspartner

-Die Daten der Langzeitbeprobung zur Algen, Ciliaten– und Makrofaunazusammensetzung in Biofilmen des Rheins wurden mir von Bärbel Ackermann zur Verfügung gestellt (Kapitel 1, 2 und 3)

-Die Daten zur Zusammensetzung der Flagellatengemeinschaft im Pelagial des Rheins wurden mir von Brigitte Gräfe und Prof. Dr. Hartmut Arndt zur Verfügung gestellt (Kapitel 1).

-Die molekularbiologischen Untersuchungen der antarktischen Choanoflagellaten wurden von Frank Nitsche durchgeführt (Kapitel 4).

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Kapitel 1

Long-term dynamics of microbial biofilm communities of the river Rhine with special reference to heterotrophic flagellates

Abstract

The major biotic components biofilms of the river Rhine (bacteria, algae, heterotrophic flagellates, ciliates, meiofauna, macrofauna) were analyzed with respect to their taxonomic composition, seasonal dynamics, succession and biotic interactions. This publication focuses on the relative importance of heterotrophic nanoflagellates (HNF) in the biofilm food web structure. Glass slides were used as artificial substrates. Colonization on the glass slides was similar to that of natural substrates. Seasonal changes in abundance and biomass of different organisms were investigated by use of a live-counting technique. During the annual cycle, the relative contribution of several protozoan groups changed significantly. Ciliates became the dominating factor in biomass throughout the year, followed by macrofauna, bacteria and meiofauna. HNF achieved their maximum abundance during winter. Their abundance ranged from only about 150 to 1.1 x 10^5 cells/cm². Taxonomic classification of the HNF revealed Choanoflagellida, Bodonea and Ancyromonadida to be the most important groups in terms of abundance and biomass. Filter-feeding HNF (Choanoflagellaida and Chrysomonodida) dominated the community. Abundance of Bodonea exhibited a strong positive correlation with that of bacteria $\leq$ 2µm, and a negative correlation with the abundance of ciliates and water discharge. Due to the high abundance of ciliates, meio- and macrofauna, a high grazing pressure was exerted on bacteria and HNF. The ratio of microphagous ciliates to HNF was 1 to 6.2, indicating a strong top down control by predation. A potential bottom-up control of HNF due to strong competition for the resource bacteria seems to be overcompensated by top-down control due to the high grazing pressure by ciliates and meio- and macrofauna.

Introduction

Intensive research has demonstrated that in particular protozoans play a major role in food webs and therefore also in the metabolic turnover in aquatic systems (Pomeroy, 1974; Azam et al., 1983, Güde, 1989; Weisse et al., 1990). Since the microbial food web was postulated (Azam et al., 1983), food web interactions and the role of protozoans in both pelagic and benthic food webs had received increasing attention by scientists within the last two decades (Alongi, 1991; Sherr and Sherr, 2000). Being main consumers of bacterial production, productive protozoans play an important role among the plankton. They are an essential component of the pelagic food web and thus of pivotal importance in the degradation of organic matter in aquatic ecosystems. In addition, several ciliates and flagellate species are able to consume algae and other protozoans and could perform similar functions in the food web as the metazoans (Sanders, 1991; Sherr and Sherr, 1994; Arndt et al., 2000). Weitere et al. (2005) examined the planktonic web structure in the lower Rhine river. In the pelagic zone, the zooplankton was dominated by HNF, contributing more than 65% of the overall zooplankton biomass in all seasons. In accordance with the dominance of the HNF and their high growth rates, this group contributed the largest part of the planktonic matter turnover within the zooplankton. The calculation suggests that HNF were able to consume between 11 and 65% of the seasonal mean bacterial production and that they consumed a larger amount of phytoplankton than both ciliates and metazooplankton. While basic information on organisms of the microbial food web is available for the pelagial (Sherr and Sherr, 1994), little is known about the organisms, in particular about nano-, micro- and meiofauna, colonizing the large surfaces of water bottoms. Only few studies investigated the benthic zone, and they focused exclusively on bacteria, algae and ciliates (Riedel-Lorje, 1980; Foissner et al., 1992; Berger et al., 1997). Top-down control due to benthic-pelagic coupling can reduce planktonic biomass in rivers, where high water mixing rates enhance the exploitation of pelagic resources by

benthic consumers. Recent studies demonstrate that turnover and decomposition of organic matter in running waters is only little controlled by macrozoobenthos (e.g. Monaghan et al., 2001; Weitere et al., 2005). This turnover is expected to be controlled to a large extent by biofilms growing in particular on the large surfaces of water bottoms and the adjacent interstitial biotope (Arndt et al., 2003; Weitere et al., 2003). Biofilm communities play a decisive role in the transportation of matter in rivers (Pusch et al, 1998). In water management, biofilm-dwelling protozoans are of interest due to their impact on water quality (e.g. Sibille et al., 1998; Fried et al., 2000). However, with the exception of algae, bacteria and some ciliates it is still unknown which organism communities appear in such biofilms. Only few studies (e.g. Railkin et al., 1990; Zolotarev, 1995; Widera, 1997) examined heterotrophic flagellates of the biofilm community. The present study investigates the composition of biofilm communities of the river Rhine on slides as a model substrate directly in the Rhine in a flow channel over a period of 14 months. The author analysed the benthic food web structure of one of the largest Central European rivers, the river Rhine. All components of the biofilm community were considered including algae, bacteria, heterotrophic flagellates, ciliates, meiofauna and macrofauna. Major focus was given to the relative role of protozoans.

Material and methods

Study site.

The river Rhine is one of the largest rivers in Europe with a total length of about 1320 km and a catchment area of approximately 224.000 km². The mean discharge into the Lower Rhine is about 2000 m^3sec^{-1} at Cologne (Tittitzer and Krebs, 1996) at a mean flow velocity of about 1.5 m sec^{-1}. Near Cologne, the bed of the Rhine is strongly solidified, and it contains large stones forming a bottom typical for this part of the river. The research platform of Ecological Rhinestation (University of Cologne) at Rhine-km 685 on the river Rhine allows conducting experiments within the original water flow. Biofilms colonizing glass sides were used as a model system to study the biofilm community in the river Rhine at Cologne. Slides were placed in vertical position to obtain an optimum fouling colonization (Railkin et.al., 1990). For experimental purposes, the slides were exposed in a permanent flow-through system at the research platform on the river Rhine (Fig.1). The glass slides were exposed at a depth of 10 cm for a period of fourteen months. Experiments were run from April 2003 to June 2004. Eight slides were removed from the flow channel every three weeks, and the periphyton communities were investigated.

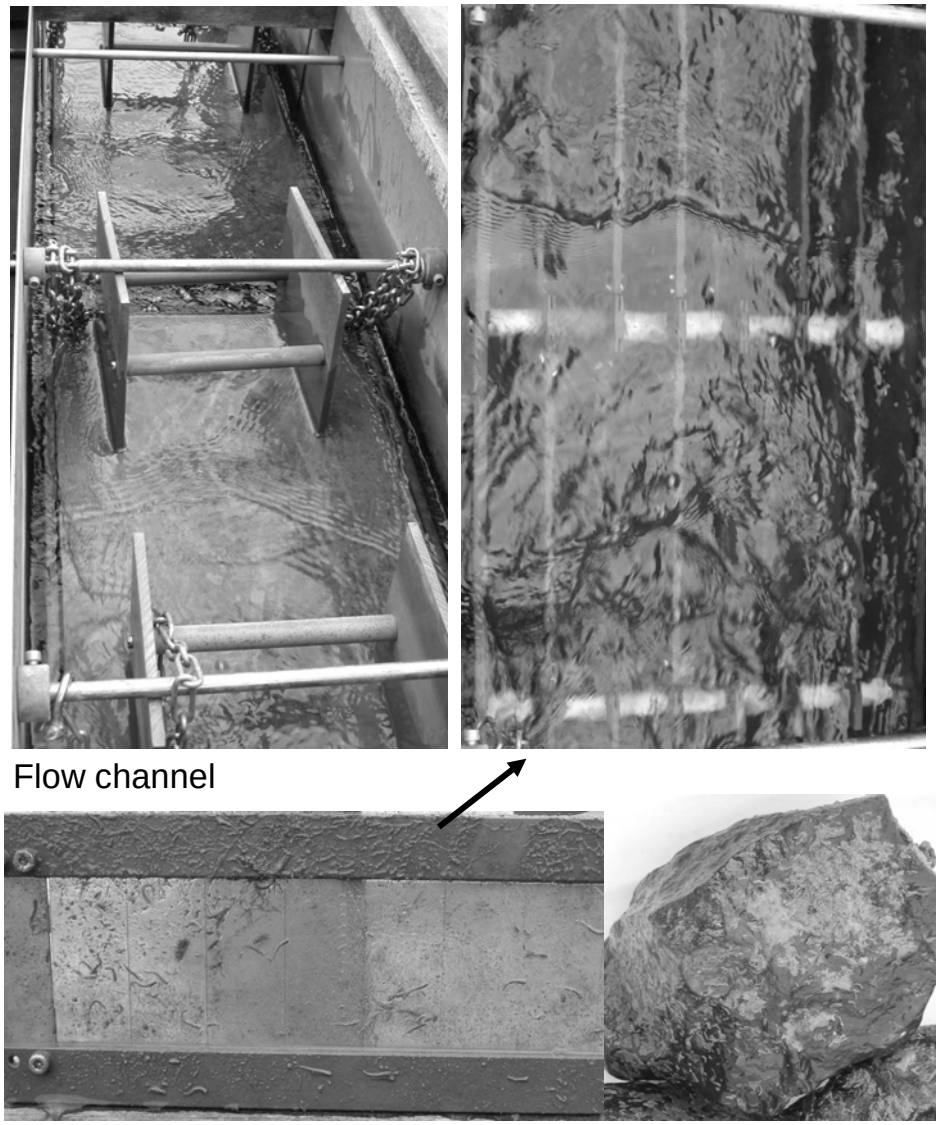

Figure 1 Experimental design. From April 2003 to June 2004, glass slides in frames were vertically exposed in a flow channel directly in the Rhine.

Sampling of the biofilm community.

For the determination of **bacterial abundance** and size class distribution, the biofilm was scraped off from the slides and fixed in a 4% ice-cold glutaraldehyde solution (final concentration 2%). 500 µl aliquots of the fixed samples were stained with DAPI (4'6-diamino-2-phenylindol, Porter and Feig, 1980), filtered to membrane filters (Nuclepore, 0.2 µm pore size) and counted with an epifluorescence microscope (Zeiss Axioskop, 1000x

magnification). 200 bacteria per aliquot were counted and arranged by size classes. For the analysis of **heterotrophic flagellates**, the biofilm was scraped off from one side of the slides. The residual biofilm on the opposite side of the glass slide was investigated for species composition and abundance in the Ecological Rhinestation by direct live count (Arndt et. al., 2000) with a microscope (Zeiss Axiostar, 400x magnification, phase contrast, ocular micrometer, video recording) immediately after sampling. At least 30 flagellates were counted per slide (n=3). **Meiofauna** was investigated in biofilms scraped off from each slide using a cover glass and transferred into a petri dish. A subsample of 50% of one slide side was resuspended in 5 ml filtered (0.2 µm) river water, transferred into a counting chamber (Bogorov tray, Hydrobios, Kiel-Holtenau) and analyzed by live count under a binocular microscope (Olympus S Z X9, 12.6x-114x magnification) (n=3).

Data of **ciliates, algae** and **macrofauna** were taken from Ackermann (in preparation). In principle, sampling and counting of ciliates and algae followed the methods for heterotrophic flagellates. Macrofauna was collected from the whole glass slide holder.

Reference samples. Natural substrates were examined in addition to slides as artificial substrates. For this purpose, stones were collected by scuba diving from the bottom, close to the research platform of the Ecological Rhinestation (water depth 3-4m depending on the water level). Sample collection was performed on one day each in August 2003, December 2003, March 2004 and July 2004. The biofilm was removed from the stones (n=3) with a biofilm sampler specially designed for this application (Fig. 2). The biofilm was resuspended in filtered (0.2 µm) river water, and aliquots were processed as described above. However, the abundance of the flagellates was determined in 20 µl aliquots each of the biofilm suspension (n=3) by live count.

For comparative studies, additional live count data of the abundance of heterotrophic flagellates in **plankton samples** from the Ecological Rhine Station during the study period were provided by Arndt and Gräfe, unpubl.

Biovolumes of organisms were calculated by measuring the dimensions of living cells and an approximation to simple geometrical forms.

Figure 2 Biofilm sampler: Plunger with integrated brush for sample scraping-off. The biofilm is rinsed from one syringe to the other with filtered water.

Calculation of potential production and grazing.

Based on the mean biomass of the biofilm organisms, calculations of the potential production of the respective groups and their grazing impact were conducted. For this purpose, mean growth rates were assumed (Tab.1) (for details see Weitere et al., 2005). For the HNF, gross growth rates based on biomass were taken from size fractionation experiments (Weitere and Arndt, 2002a, b). The individual growth rates of ciliates were calculated using the regression established by Müller and Geller (1993). For the estimates of meiofauna growth rates, temperature and food concentrations were taken into account (cf. Stemberger and Gilbert, 1985; Stelzer, 1998). The growth rate of the larger metazoans was assumed to be half the rotifer growth rate (cf. Gillooly, 2000). Algal growth rates were assumed to be 0.7 d^{-1} (cf. Schöl et al., 2002). Potential production was estimated involving the factors biomass and growth rates of the respective groups for calculation. Potential consumption was calculated assuming growth efficiency values of 33% (Straile, 1997).

Abiotic parameters

In addition to the biotic data, data on seston content (organic and inorganic), temperature and water discharge were collected. Seston was sampled in parallel to the plankton samples. For the measurement, 2000 to 500 ml of water was filtered through pre-weighed GF/F filters (Whatman). Total seston dry weight was determined after drying at 100°C (24h), and inorganic seston content was determined after combustion at 500°C. Immediately after sample collection, temperature, pH-value and conductivity were measured with multi-probes (WTW, Germany, and Yellow Spring Instrument Inc.), chlorophyll content was measured using in situ fluorescence by an Aquafluor (Turner Designs, USA). Data on water discharge (daily means at Cologne) were provided from routine measurements of the "Bundesschifffahrtsamt" (Duisburg, Germany).

Flow velocities were measured at the sampling place between the glass slides and also in front of the research platform with a hydrometric propeller (WTW). The measurement showed that the flow velocity in the box in front of the slide inserts was reduced to about 30% of the original flow velocity, and to about 40% between the inserts. The availability of light in the flow channel was determined with a LI-250A (Li-Cor) light meter to determine the photosynthetically active radiation (PAR). The value determined in the box containing the slides resembled the values determined in measurements directly above ground (4 m depth) and was reduced to about 2 % of the surface light intensity. The rationale of this experimental design was to simulate the conditions near the ground of the river.

Statistics

Statistical analysis was conducted using SPPS for Windows (Version 11.0). Correlation was evaluated by calculation of Pearson's correlation coefficient. Significant correlation of the nonparametric data was detected by Spearman rank test analysis. To evaluate the effects of the abiotic parameters, correlations were tested between the whole biofilm community on the level of taxonomic groups and all investigated abiotic parameters (seston content, temperature, water discharge, pH-value, conductivity and chlorophyll content). To analyze the effects of the substratum (slides and natural substrates) ANOVAs were conducted.

Results

Organism composition The average numbers of organisms over the total investigation period detected per square centimeter biofilm were $91x10^6$ bacteria, 622 algae, 20,933 heterotrophic flagellates, 3,359 ciliates, 107 nematodes and 10 rotifers (Fig. 3 a). Ciliates constituted the major part of the biomass (Fig. 3 b); its mean portion being 50%, the mean portion of macrofauna being 43% ,while the mean portions of algae and flagellates were only 0.09% and 0.2%. The contribution of bacteria to the biomass was 2.8%, while meiofauna contributed 3.6% (Fig. 3 c).

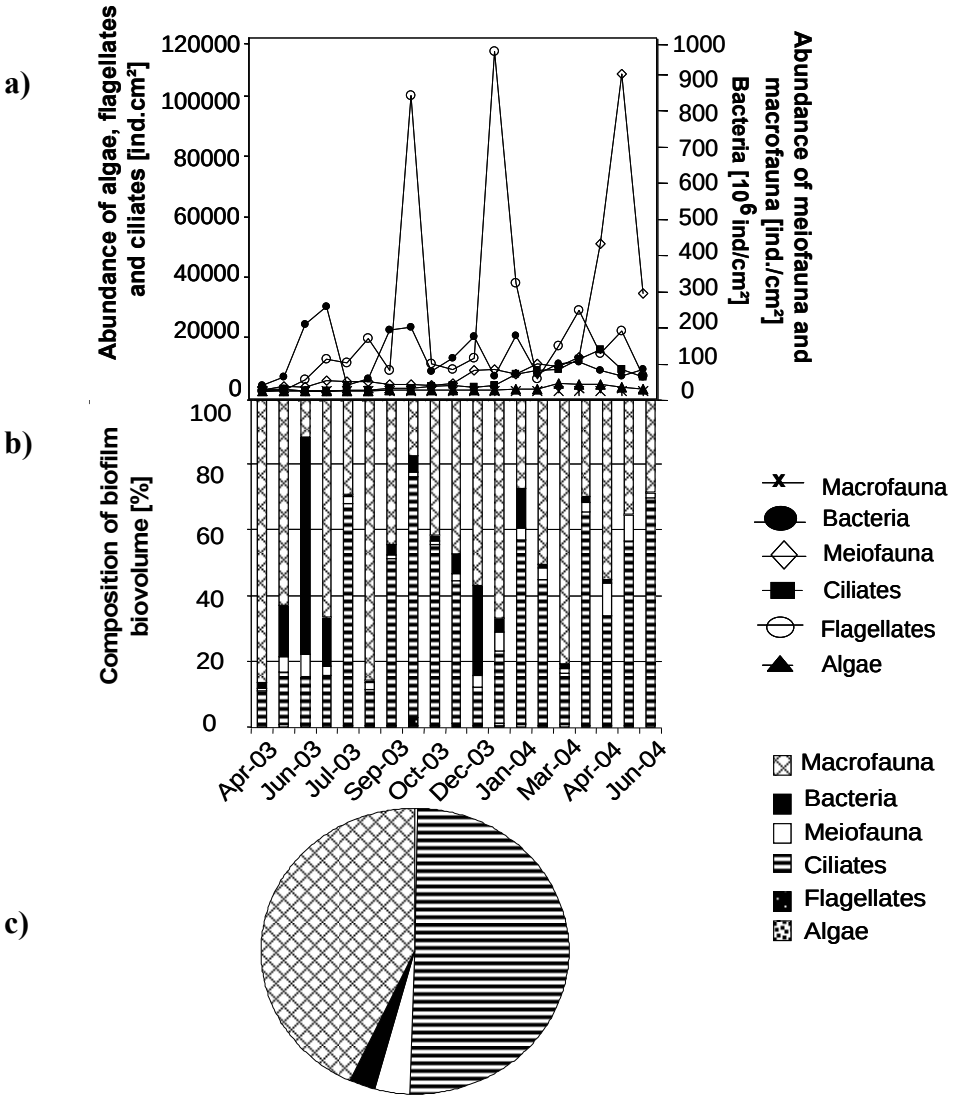

Figure 3 Average composition of the biofilm on slides (n=3), succession over the study period a) abundances arranged by sampling dates over the total investigation period; b) biomass arranged by sampling dates over the total investigation period c) Mean composition of the biomass over the total investigation period (n=19).

Heterotrophic nanoflagellates

The average organism count per cm^2 bottom area in biofilm during the study period was about 3,260 Bodonea, 820 Ancyromonadida, 16,400 Choanoflagellida, 270 Euglenida, 180 Chrysomonadida, 20 Bicosoecida, 60 Cercomonadida, 10 Thaumatomonadida, 5 Apusomonadida and 50 other flagellates which could not be determined to the genus level (Fig.4 a). With 83%, Choanoflagellida dominated the average biomass distribution, while the percentages of Euglenida, Bodonea and Ancyromonadida were 10%, 2.5% and 2.6%,

respectively. The percentages of Chrysomonadida (0.9%), Bicosoecida (0.5%), Cercomonadida (0.4%) Thaumatomonadida (0.07%), Apusomonadida (0.05%) and undetermined species (0.13%) were rather low throughout the whole year (Fig. 4 b).

In total, an average number of 16,540 flagellates were detected in the biofilm feeding on plankton, representing more than 78% of the flagellate count. An average number of about 4,500 flagellates in the biofilm are feeding on benthos. With respect to biomass, 84% of the flagellates lived on the pelagic zone, while only 16% lived on biofilm resources. In total, almost 99% of the flagellates detected in biofilm were bacterivorous.

While groups like Bodonea and Ancyromonadida demonstrated a relatively homogeneous seasonal distribution, other groups like Euglenida and Choanoflagellida showed seasonal peaks. The mean coefficient of variation (Fig. 5) demonstrates that variation was least in Bodonea throughout the study period between individual replicates (n=3) at the individual sampling dates (mean CV approximately 40%), resulting in a relatively homogeneous spatial distribution. In contrast, the other important groups (Ancyromonadida, Euglenida, Choanoflagellida, Chrysomonadida) formed patches.

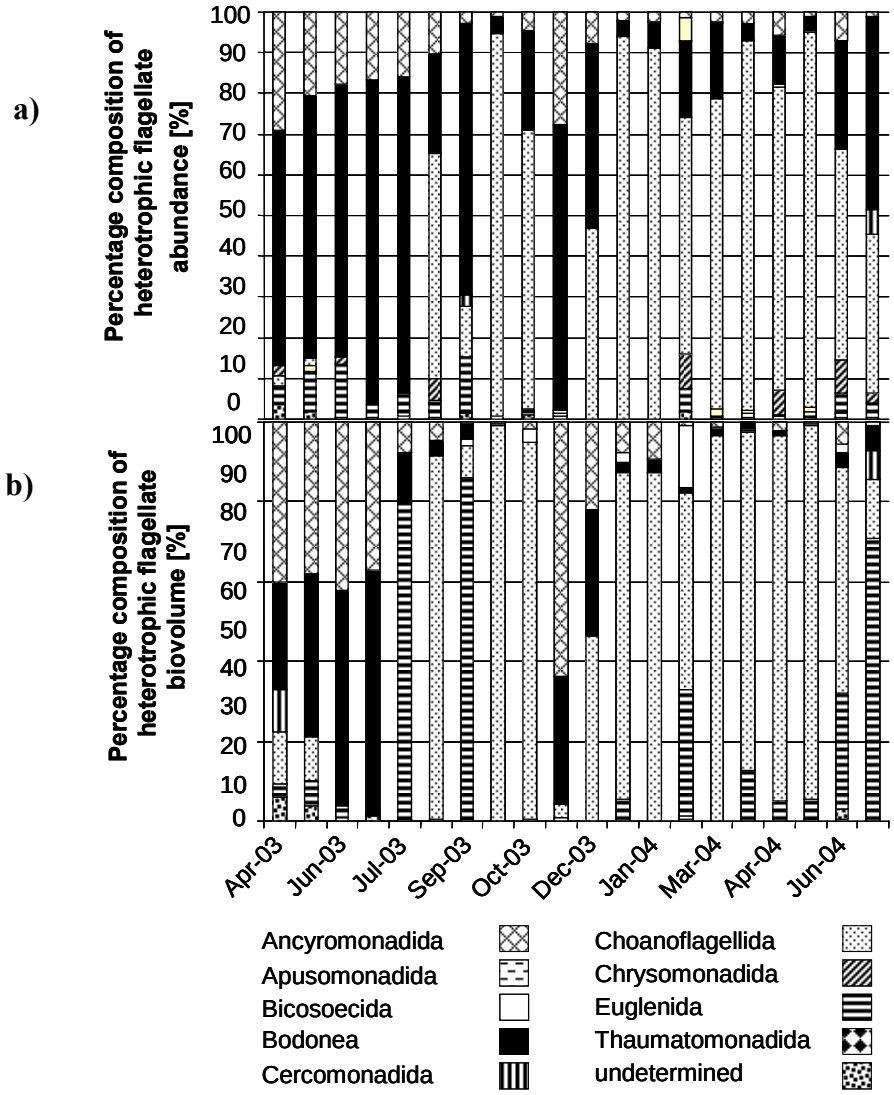

Figure 4 Average relative distribution of the flagellate groups on slides (n=3), succession over the study period, a) abundance arranged by sampling dates over the total investigation period; b) biomass arranged by sampling dates over the total investigation period.

Group-specific distribution patterns are shown in Figure 6. Apusomonadida, Bicosoecida, Cercomonadida and Thaumatomonadida appeared only sporadically during the study period. Chrysomonadida and Choanoflagellida were of seasonal importance; at certain phases, they formed enormous abundances, thus dominating the distribution of flagellates. Permanent representatives were the two groups Ancyromonadida and Bodonea. Seasonal distribution patterns could be also recognized below the group level. The composition of the two important groups Bodonea and Choanoflagellida (Fig. 7) demonstrated a succession on

the species level. The two dominating and permanent species of Bodonea were *Neobodo designis* and *Rhynchomonas nasuta*, both demonstrating their largest abundance in summer. Other species, like *Bodo caudatus*, *Bodo curvifilus*, *Bodo saltans* and *Bodomorpha minima* only appeared sporadically in the biofilm. On the species level, Choanoflagellida demonstrated a clear succession. From August to October 2003, *Monosiga spp*. dominated, while from December 2003 to January 2004, *Codonosiga cf. botrytis* was the most important representative of the Choanoflagellida, followed by *Salpingoeca cf. amphoridium* from March to May 2004.

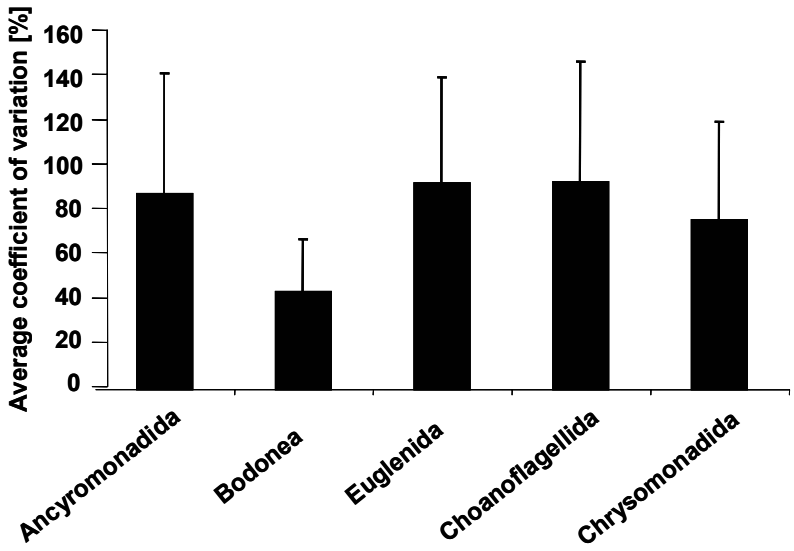

Figure 5 Average coefficient of variation of the abundance of different taxonomic groups of heterotrophic flagellates over the study period (n=20).

The analysis of seasonal fluctuations of the most important representatives of the flagellates demonstrated significant changes in abundance of individual species throughout the year (Fig. 8), illustrating that the biotope biofilm represents an extremely variable system. On the other hand, the typical representatives of flagellates in biofilms (*Neobodo designis, Rhynchomonas nasuta* and *Ancyromonas sigmoides*) were present throughout the whole year,

although their abundance varied. In contrast to this finding, the three dominating representatives of the Choanoflagellida were subject to strong seasonal changes. A list of species of heterotrophic flagellates found in biofilms of the river Rhine is given in Table 2.

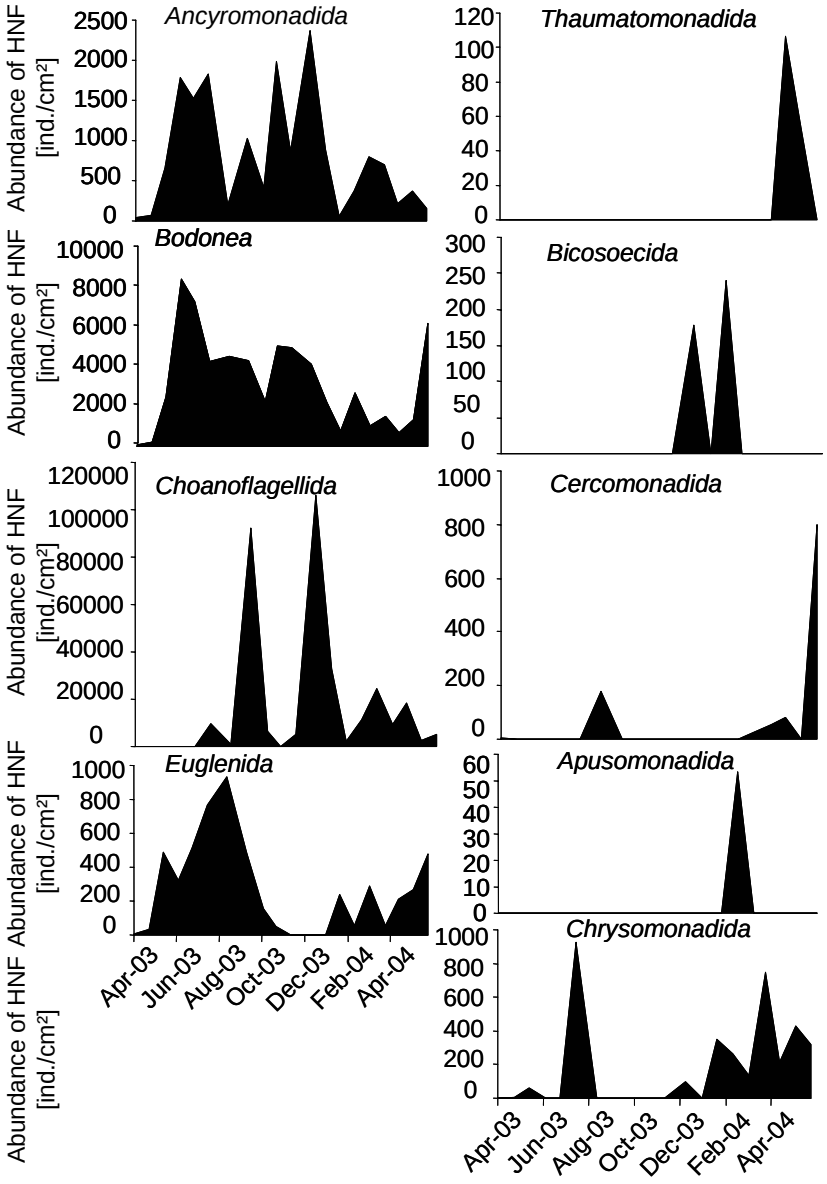

Figure 6 Fluctuations in the abundance of groups of heterotrophic nanoflagellates in biofilms on slides throughout the year.

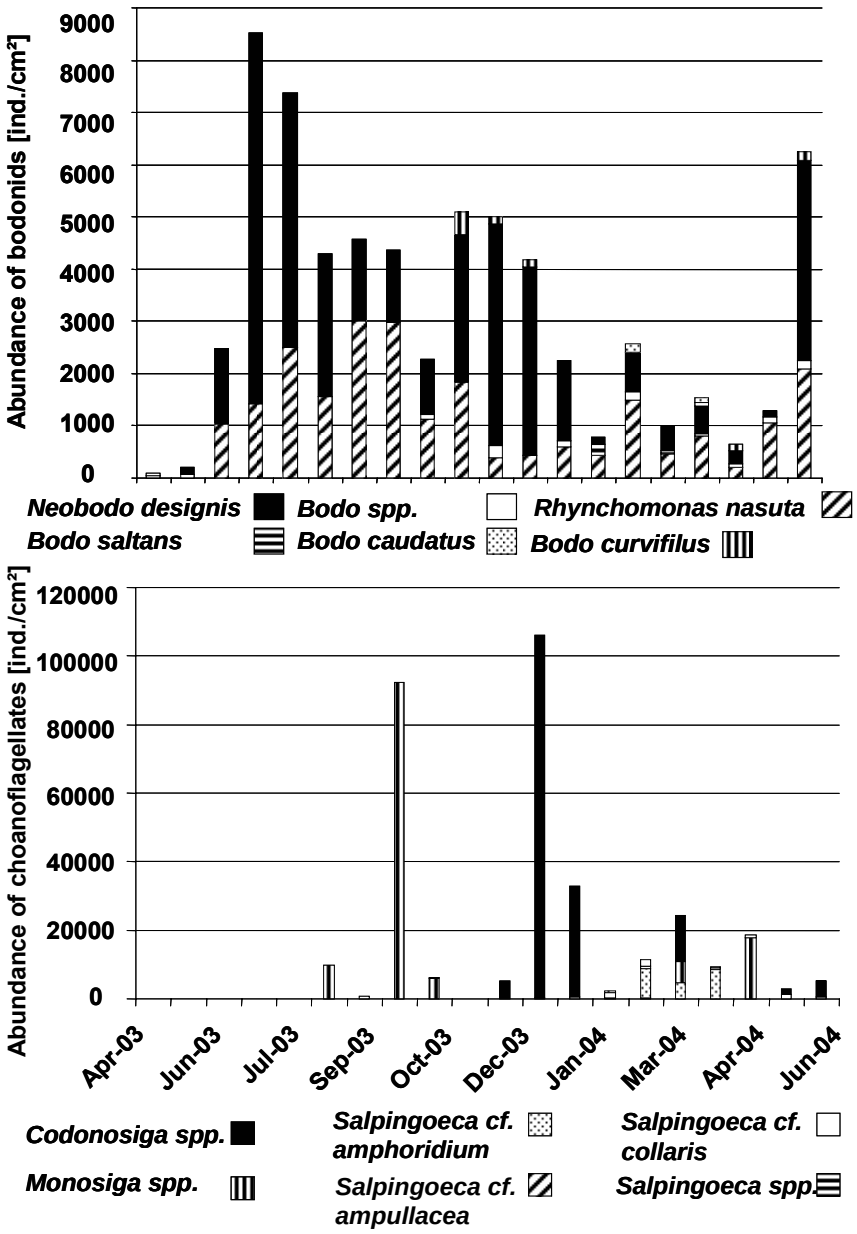

Figure 7 Changes in the composition of Bodonea and Choanoflagellida abundances in biofilm on slides throughout the year arranged by sampling dates (n=3).

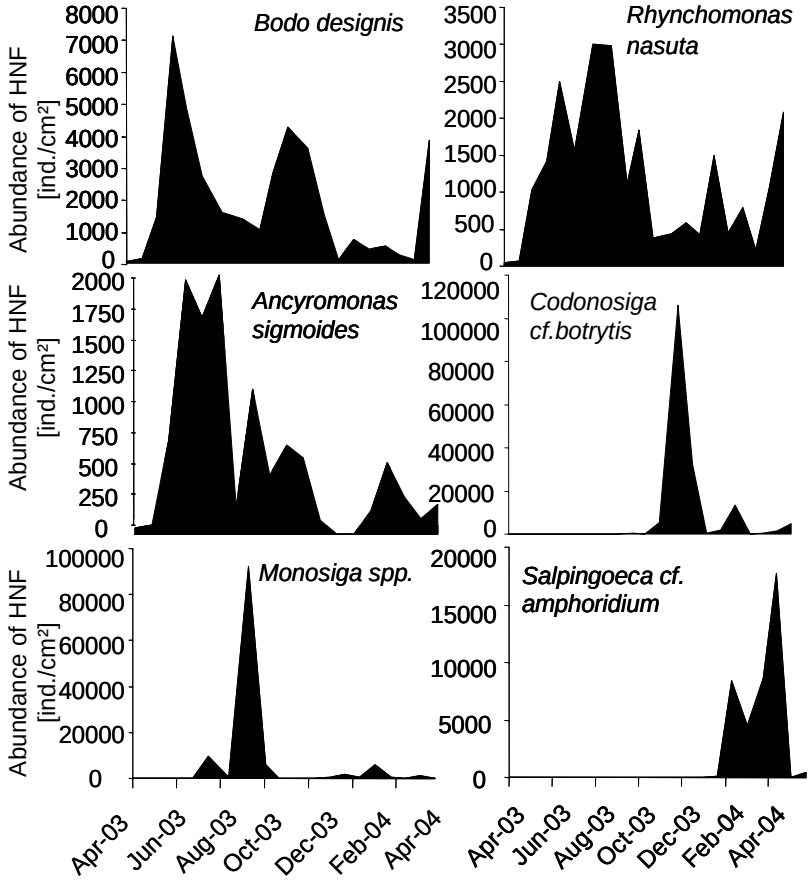

Figure 8 Seasonal fluctuations in the most abundant flagellate species in biofilm on slides.

Comparison of the biofilm on slides and on natural substrates from the bottom of the Rhine

The overall cell counts shown in Figure 9 demonstrate that large cell numbers (with high variances) were detected in autumn 2003 at both sites, while cell counts decreased from winter 2003 to spring 2004 to a relatively low level and then significantly increased again in summer 2004. The increase in cell count (abundance) in summer 2004 was more distinct on the bottom of the Rhine than on slides. However, in total they demonstrate the same trend, and the composition of heterotrophic flagellates was almost identical. The density of Bodonea varied from about 480 to 2,000 individuals per cm^2 on the bottom of the Rhine and from about 700 to 5,000 ind./cm^2 on slides. Choanoflagellida density ranged from about 1,350 to 108,400 ind./cm^2 on the bottom and from about 5,200 to 92,300 ind./cm^2 on slides.

The comparison of flagellates in biofilm formed on slides with naturally grown biofilms on stones from the bottom of the Rhine demonstrated a very similar composition (Fig. 9). In autumn 2003, the Choanoflagellida dominated on stones and also on slides, representing more than 90% of the overall flagellate composition. In winter 2003, the portion of Bodonea was approximately 40% at both sampling sites, while Choanoflagellida represented 30-40% of the individuals. On slides, Ancyromonadida represented 10% of the cell count; in contrast to this finding, 10% of the cell count on stones was represented by Thaumatomadida. In spring 2004, Choanoflagellida dominated the flagellate community in both biotopes, followed by Bodonea. In summer 2004, more than 90% of the flagellates were Choanoflagellida at both sites, together with a relatively small Bodonea portion.

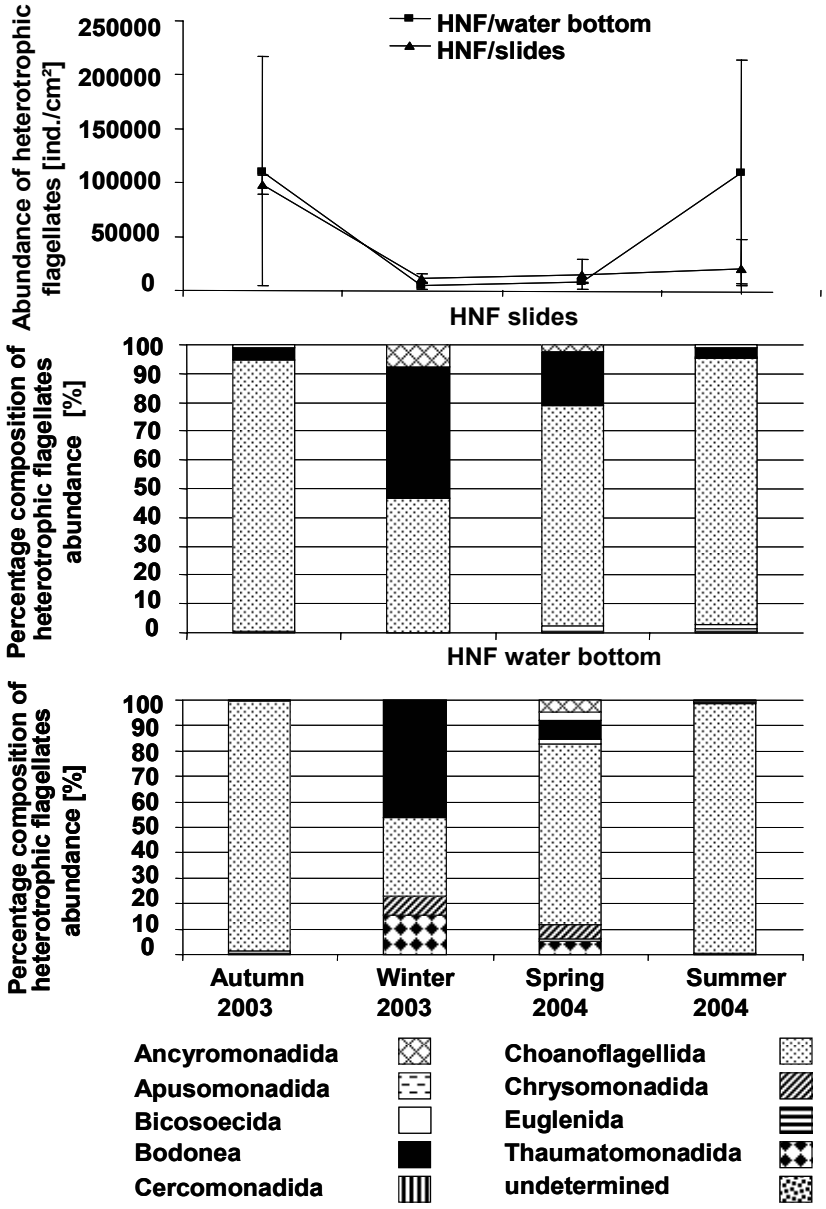

Figure 9 Comparison of the composition of the flagellate communities in biofilm on slides in the flow channel and on stones of the Rhine bottom, error bars indicate standard deviations, (n=3).

Comparison of biofilm and pelagic communities

Several species detected in biofilm also exist in the pelagic zone. The number of individuals in biofilm and in the pelagic zone demonstrates differences in seasonal patterns (Fig. 10). While the highest cell count on biofilm was detected in autumn, the highest cell count in the pelagic zone was determined in spring. With respect to Bodonea, maxima

alternate in the pelagic zone and in biofilm. Mostly, an increase in cell count in biofilm was followed by an increase in cell count in the pelagic zone. The situation was similar in Ancyromonadida which represented the second important benthic flagellate group in biofilm. In contrast, the patterns in the pelagic flagellate group Chrysomanadida are exactly reversal. In the Chrysomonadida, first the pelagic cell count increased and was followed by an increase in the cell count in biofilm. The situation was similar in the second important, primarily pelagic flagellate group, the Choanoflagellida. Also the relationship in cell count was reversal when biofilm and pelagic community structure was compared. While Chrysomonadida represented the most important flagellate group in the pelagic zone, they played only a minor role in biofilm. Despite their importance in biofilm, the abundance of Bodonea and Ancyromonadida in the pelagic zone was low. Only the Choanoflagellida cell count represented a considerable share both in the pelagic zone and in biofilm.

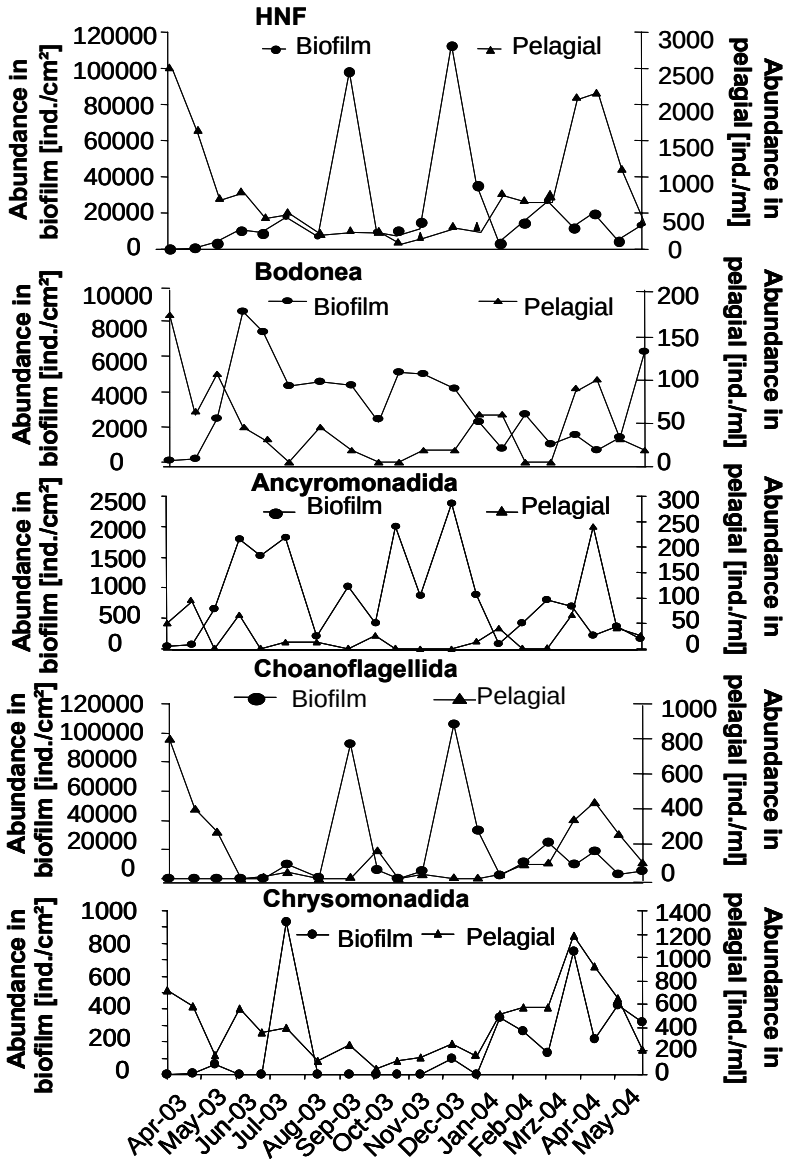

Figure 10 Comparative analysis of important flagellate groups in biofilm on slides and in the pelagic zone over the investigation period.

Bacteria

Seasonal bacterial fluctuations in bacterial count also demonstrated a clear succession pattern. The overall cell count varied from $1.6x10^7$ to $2.4x10^7$ bacteria/cm² (Fig. 11 a). At most sampling dates, cocci and rods were detected in almost equal orders of magnitude. The highest cell counts were obtained in June and July 2003; at that time, rods dominated representing almost 70% of the cell count. Other maxima in bacterial count were detected in

September, October and December 2003 and in January 2004. The variance between replicates (n=3) was remarkably low (Fig.11 a). It is also remarkable that 50% to 90% of the bacterial mass is derived from bacteria ≤ 2 µm (Fig.11 b, 11 c). Larger portions of filamentous species were only seasonally detected. This finding is in agreement with time-dependent changes in the mean biovolume (Fig. 11d). The maximum bacterial biovolume was detected in winter 2003 ($2x10^8$ µm³/cm²).

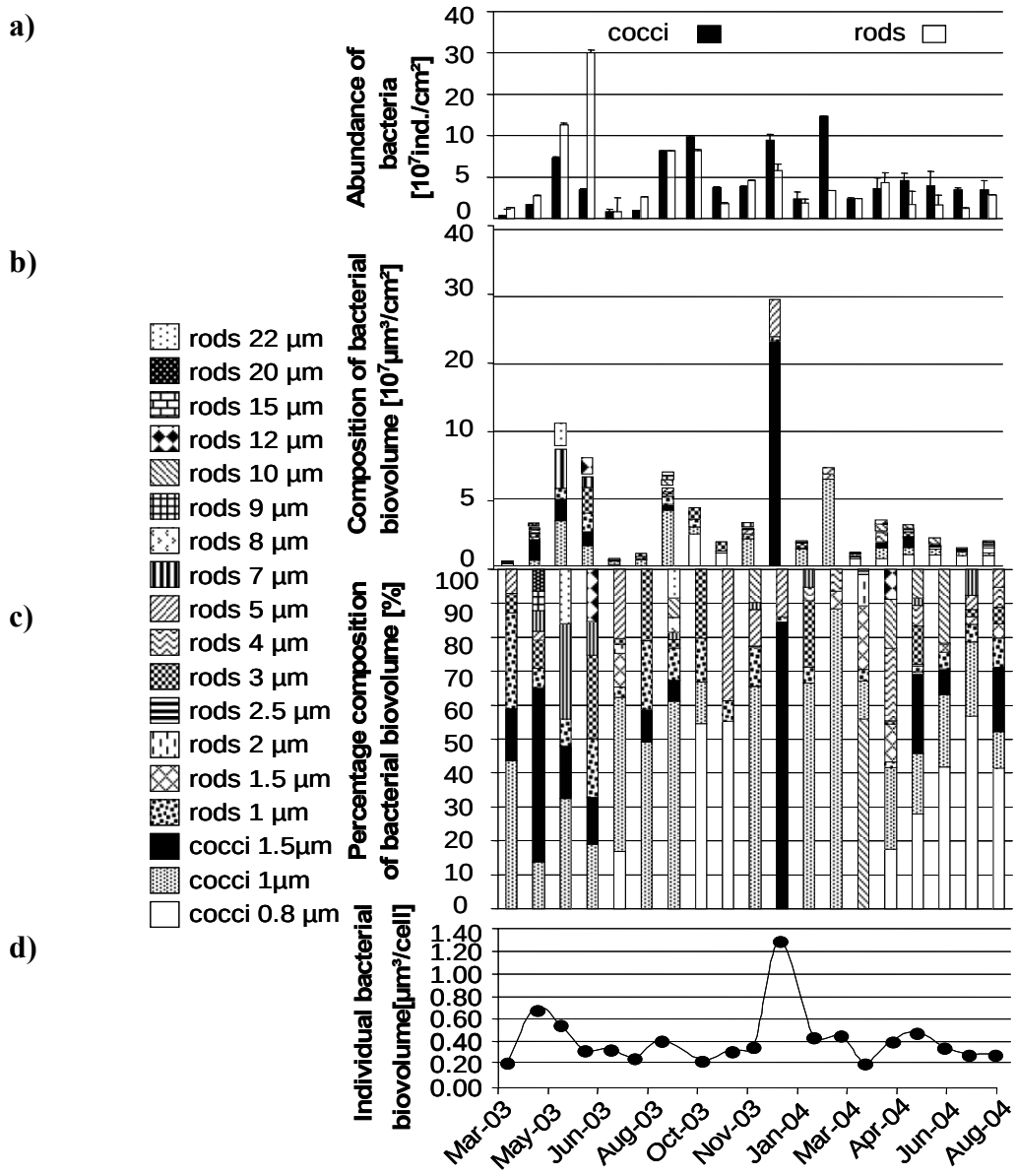

Figure 11 Seasonal changes in biofilm on slides over the investigation period (n=3); a) bacterial abundance, error bars indicate standard deviations, b) composition of bacterial biomass, c) biomass distribution (n=3); d) mean biovolume over the investigation period.

Ciliates

During the study period, ciliate density varied from ≤ 100 ind./cm² to 13,698 ind./cm² (Fig.12 a). Their mean biovolume varied from $2{,}6x10^7$ to $5.2x10^8$ µm³/cm² (Fig.12 b). Predominance alternated between peritrichous (*Vorticella*, *Zoothamnium*, *Carchesium*, *Epistylis*) and heterotrichous (mainly *Stentor*) ciliates (data from Ackermann, in preparation).

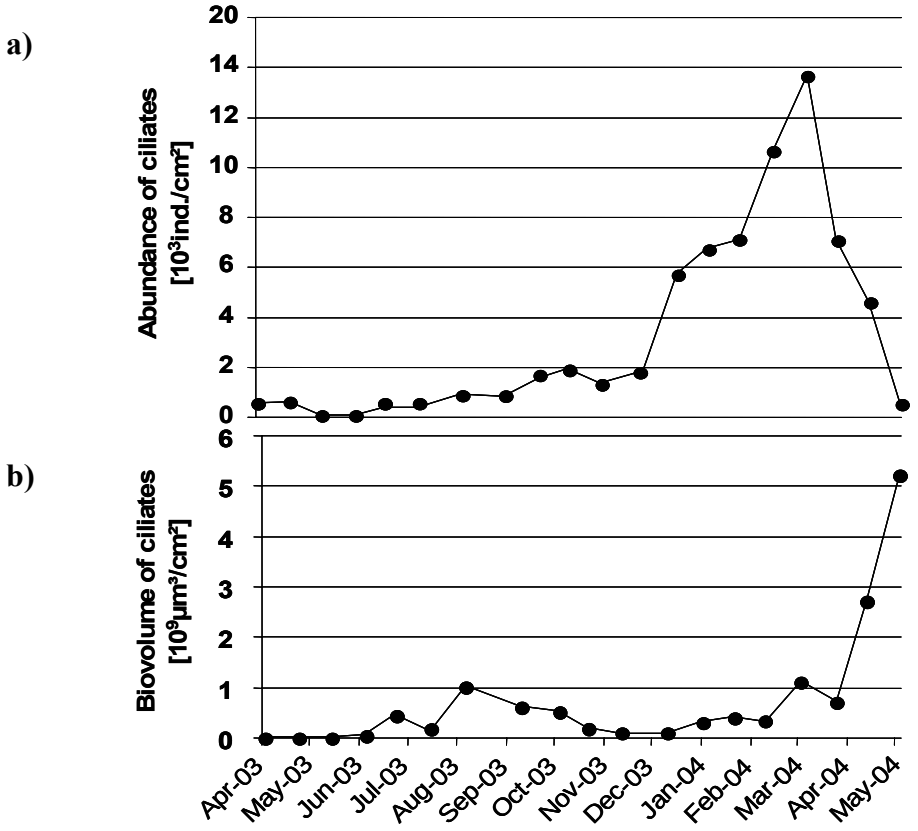

Figure 12 Mean abundance of ciliates in biofilm on slides over the study period (n=3); b) mean biovolume of ciliates in biofilm on slides over the study period (n=3).

Meiofauna

The biomass distribution of the meiofauna illustrates that only Rotatoria and Nematoda occurred frequently enough throughout the study period to have an effect on flagellates (Fig. 13). Minima and maxima of the detected meiofauna taxa are listed in Table 3. Nematodes were nearly exclusively represented by Chromadorida (*Punctodora ratzeburgensis* and *Chromadorina bioculata*); they represented the most important meiofauna taxon throughout the study period. The number of detected nematodes increased from ≤ 1 to ≥ 800 nematodes/cm² biofilm area in spring 2004. Rotatoria represented another important group (common taxa: *Rotaria*, *Trichocerca*, *Squatinella Colurella* and *Brachionus*); at certain times, their count was higher than 45 ind/cm². Almost throughout the whole year, specimens of bdelloid Rotatoria were detected in higher numbers than monogonont Rotatoria. Also Acari and nauplii and copepdites of cyclopoid and harpactoid copepods could be detected in the biofilm, although in low numbers. Although subject to seasonal changes, also Oligochaeta (common species: *Aelosoma hemprichi* and *Chaetogaster spp*.) and Cnidaria (*Cordylophora caspia*) represented a larger share in the species composition of the meiofauna. In particular the colony formation of *Cordylophora caspia* was the reason for a significant increase in biofilm surface area in summer 2004.

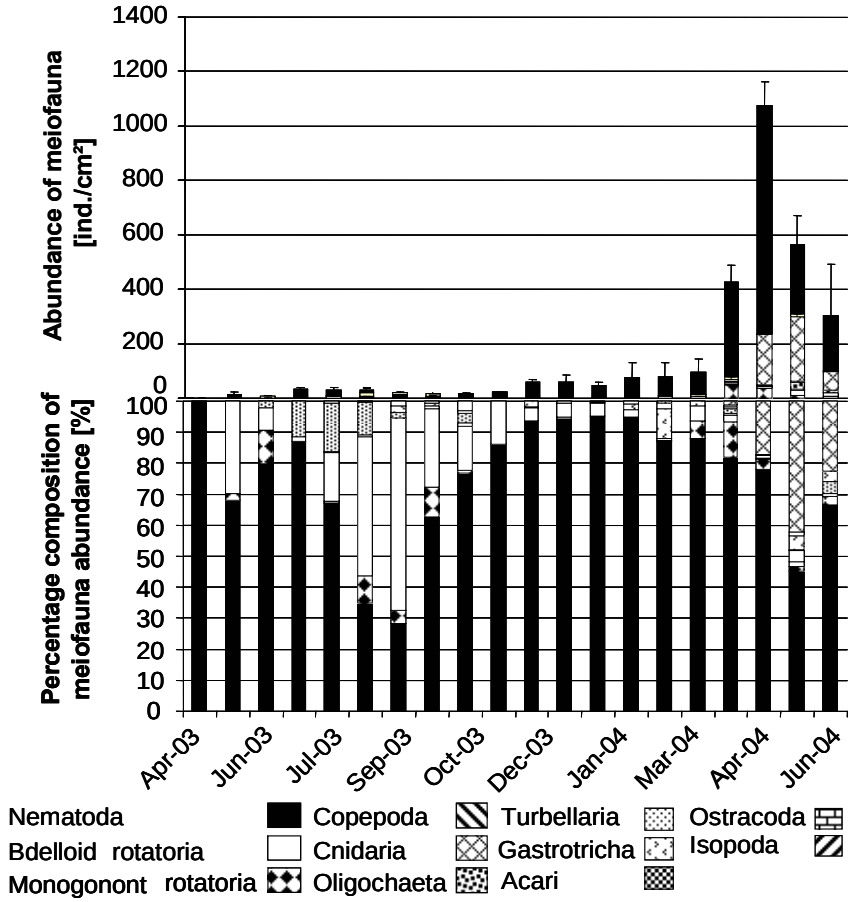

Figure 13 Fluctuations in meiofauna abundance in biofilm on slides throughout the study period arranged by sampling dates, error bars indicate standard deviations, (n=3).

Macrofauna

The mean biovolume of the macrofauna (n=3) during the study period ranged from $18x10^6$ to $5.5x10^8$ µm³/cm² (Fig. 14 a). The most important representatives were *Hydropsyche* (Trichoptera), *Chironomidae* (Diptera), *Jaera istri* (Isopoda), *Corophium curvispinum* and *Dikerogammarus vilosus* (Amphipoda), *Dreissena polymorpha* (Bivalvia) and *Ancylus fluviatilis* (Gastropoda) (Fig.14 b) (data from Ackermann, in preparation).

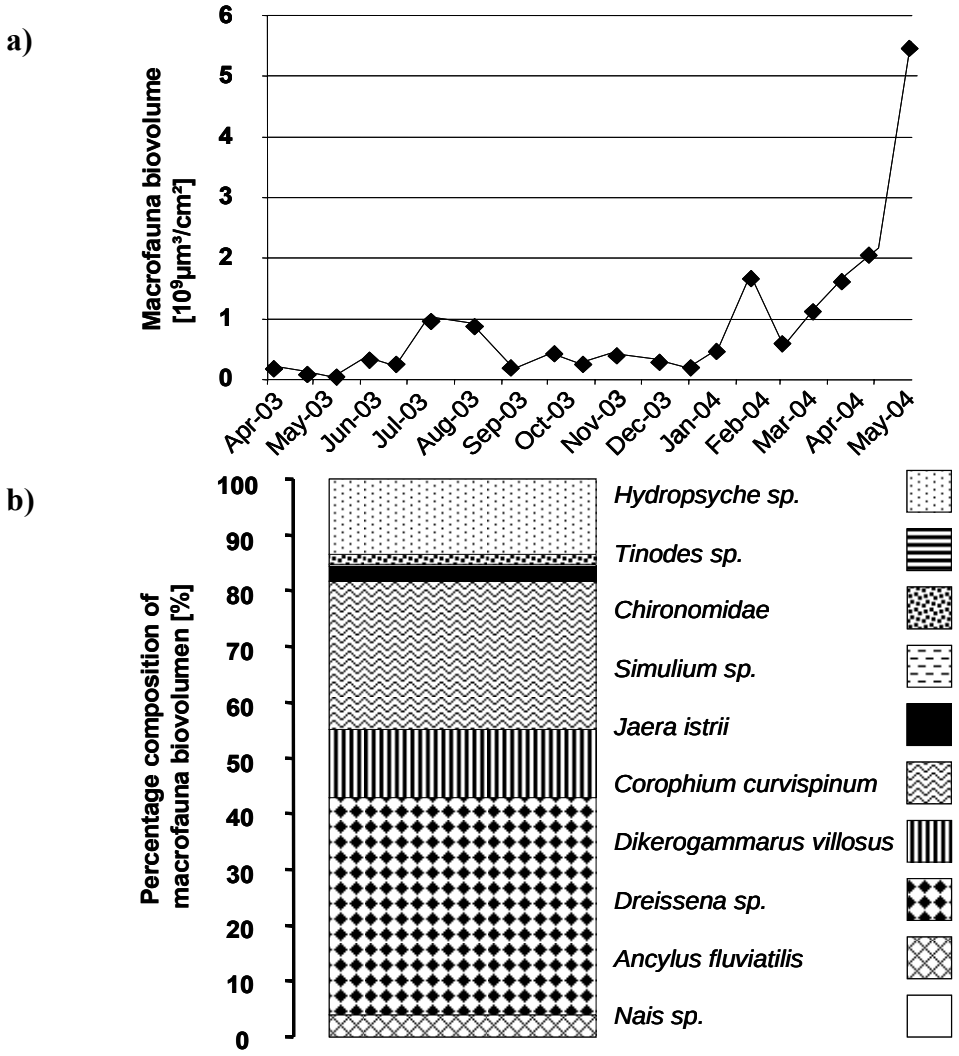

Figure 14 a) Average distribution of the macrofauna in biofilm on slides over the study period, biomass, (n=3), b) average composition of the macrofauna in biofilm on slides over the study period, biomass, (n=19).

Discussion

Biofilm

Biofilms are perceived as being hot spots for biotic interactions, genetic exchange and the biogeochemical cycling of elements (Parry, 2004). The exopolymetric matrix is an important component of biofilm, since it allows the distribution of many organisms in a small space (Costerton, 1995; Budde, 2005, unpublished data), while even 5 µm above the biofilm turbulences interfere with the attachment of flagellates (Willkomm, unpublished data). It represents a reservoir of water and nutrients and thereby provides a stable environment over a long time (Lawrence et al., 2004). As several protozoans have extracellular structures such as stalks, loricae and shells, which may remain in the biofilms also after cell death, protozoans often persistently influence the three-dimensional structure of biofilms (Arndt et.al., 2003).

Organism composition

The current study was the first which investigated a natural biofilm community for a period of 14 months, including all major components of the total biofilm community. The composition of the biofilm was subject to large seasonal variations. Overall biomass varied from $3.2x10^7$ to $5.4x10^9$ µm³/cm² biofilm. With respect to production and also calculated turnover rates, the biomass was dominated by ciliates. It is assumed that seaonal effects are overridden by the effect of succession of the biofilm-community. This becomes evident when comparing the biofilm biomass on the slides between starting phase and the end of the exposition period (for details see Chapter 3).

Heterotrophic nanoflagellates

Suspension feeding flagellates (Choanoflagellida and Chrysomonadida) dominated the biomass with an average of share of 84%, Bodonea and Ancyromonadida, representing the most important attached benthic flagellates groups, were present all year but formed only 5% of the overall biomass. This is in agreement with older studies on artificial substrate in the Rhine river (Weitere and Arndt, 2002) demonstrating that 8 to 54% of the HNF was composed of suspension feeding chrysomonads and choanoflagellates. It is generally postulated that the pelagic zone in the Rhine river is dominated by Chrysomonadida and Choanoflagellida (Weitere and Arndt, 2002), while sediments are dominated by Kinetoplastida and Euglenida (Dietrich and Arndt, 2000; Altmann and Arndt, in prep.; Garstecki et al., 2000). It is interesting that so far Ancyromonadida were ignored by textbooks, despite their important role in biofilm with an average count of 817 cells/cm². Genetic studies demonstrated that the morphotype *Ancyromonas sigmoides* contains very different genotypes (Scheckenbach et al., in preparation).

The average count was 21,000 flagellates/cm²; however, peaks of more than 100,000 individuals/cm² were detected, a number significantly higher than the flagellate density reported in earlier studies mostly investigating younger biofilms: 200-450 flagellates/cm² (Widera, 1997) in little streams near Dortmund, 350-800 flagellates/cm² (Railkin et al., 1990) in the White Sea and 50.000 cells/cm² (Zolotarev, 1995) of colony-forming flagellates in various other streams. The number of individuals in biofilms on slides that were exposed to the Rhine for only a short period of time (1 week) (Arndt et al., 2003) was 20-400 cells/cm². Schmidt-Denter (1999) detected mostly Kinetoplastida (Neob*odo designis, Rhynchomonas nasuta*); in total, only few planktonic taxa were detected. Studies on succession (Arndt et al., 2003) demonstrated that even after one day, a plateau level of HNF count occurred on the biofilm, and that no further increase in cell number took place. An increase in predator density resulted even in a decrease in flagellate abundance. In contrast to this finding, it could be

demonstrated that the biomass of the benthic HNF steadily increased over a period of three months, until a plateau was achieved. Also large choanoflagellate colonies were occasionally present which clearly dominated the biomass, resulting in an increase in biomass even after 12 months.

Some species (bodonids) are especially adapted to graze on attached bacteria (Fenchel, 1986). So far, this was not demonstrated for the benthic zone (Dietrich and Arndt 2000; Starink, 1995). Mostly, Bodonea were positively correlated with bacteria ≤ 2 µm (p-value 0.013, Spearman) (Fig.15). Except for a few times, the mean bacterial biovolume was 0.2 to 0.4 μm^3, being in exact agreement with the size spectrum of bacteria which Boenigk and Arndt (2000) determined as the preferred food size spectrum of the bodonid *Rhynchomonas nasuta*.

Variance was lowest in Bodonea; they were regularly distributed over the surface. Choanoflagellates and Chrysomonadida formed patches by colony formation, they attached from the plankton and formed local colonies. Euglenida and Ancyromonadida formed patches that might be due to nutrient preferences. Another potential explanation for the patchy distribution of flagellates is the local grazing of predators.

Using estimates of production and consumption for microbes of the river Rhine (cf. Weitere et al., 2005) the major role of the different components of the biofilm was estimated. The available data strongly suggest a model basically working as follows: During the study phase, the HNF were subject to a high grazing pressure by ciliates, meiofauna and in particular macrofauna. About 250% of the potential production of flagellates was even needed due to the potential consumption rate of nanophagous ciliates. The nanophagous meiofauna required more than 200% of the potential production of flagellates for the formation of its own biomass, the nanophagous macrofauna needed more than 100%. Even if the growth rate of the flagellates would be increased by a factor of 3 compared to the assumptions by Weitere et al. (2005), the flagellates would be subject to a high grazing pressure. Earlier studies on the

succession of HNF on biofilms (Schmidt-Denter, 1999) detected an increase in flagellate density for the factor 21 d^{-1}. However, as the highest measured growth rates of flagellates are only about $6d^{-1}$ (Fenchel, 1986), the increase in densitiy appears to depend not only on reproduction alone. Obviously, ciliates, meiofauna and macrofauna cannot live on the indigenious production of the biofilm alone but depend on continuous colonization from plankton for sufficient nutrient supply. Previous studies have shown in labaratory and field experiments that ciliates, rotifers and copepods (Arndt, 1993a; Sanders and Wickham, 1993; Cleven 1996; Jürgens et.al., 1997) can act as voracious predators of HNF (Arndt et. al., 2000). A part of the HNF is probably protected from grazing in the interstitial pore system of the microbioal mats on the biofilm forming refuges for the nanofauna. Only few large heterotrophic flagellate species were detected on the biofilm, this may indicated a negative selection by predators.

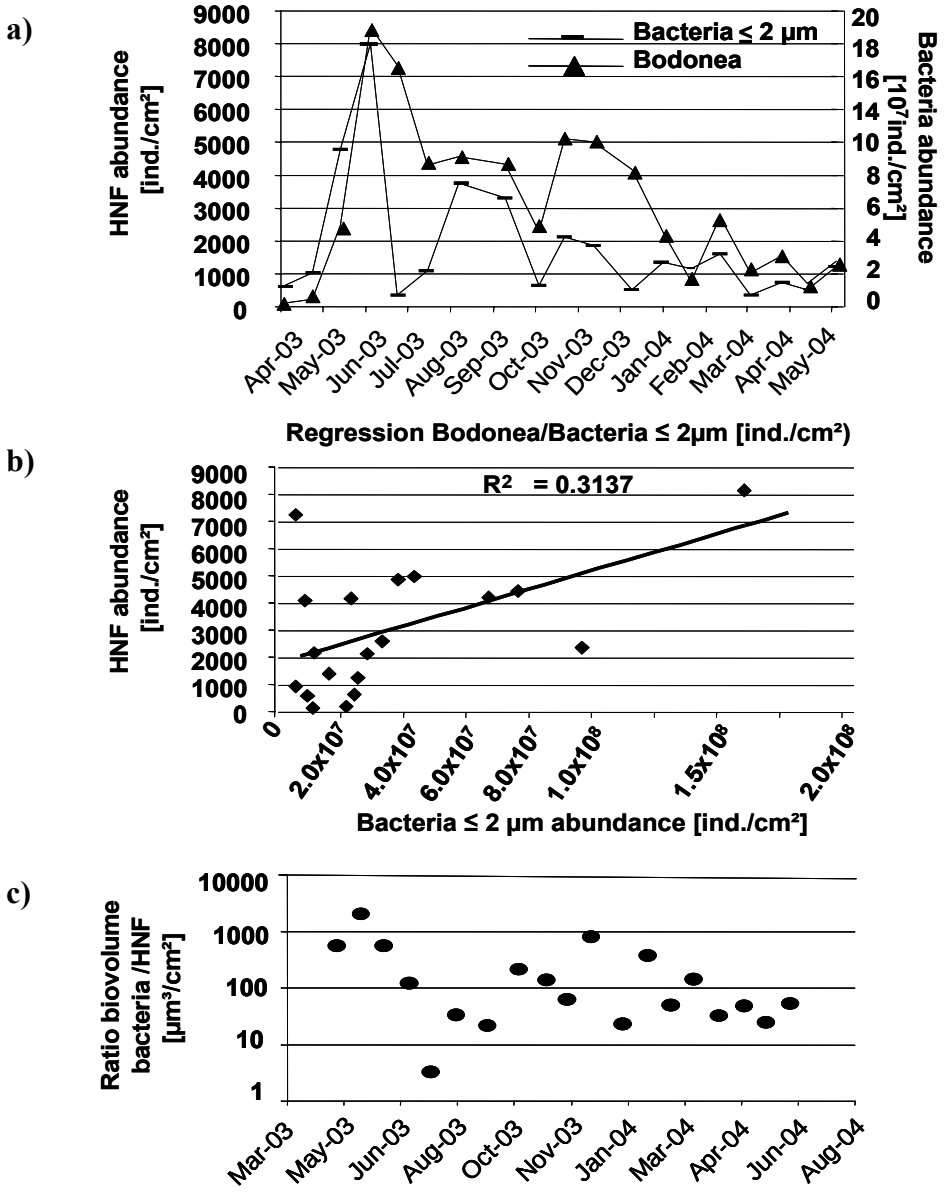

Figure 15 a) Correlation between abundances of Bodonea and bacteria ≤ 2µm in biofilm on slides; p= 0.013 (Spearman rank), b) Regression of the correlation between Bodonea and bacteria ≤ 2µm (abundance) in biofilm on slides; c) Ratio of the biovolumes of bacteria ≤ 2µm and bacterivorous HNF in biofilm on slides over the investigation period arranged by sampling dates.

Comparison of biofilm on slides and on the bottom of the Rhine

The study demonstrates that slides represent a suitable model system for the examination of biofilms; this is in agreement with previous studies (Schmidt-Denter, 1999, Schönborn, 1981). It can be postulated that the composition on the slides represents a good approach to natural conditions.

Comparison of biofilm and pelagic zone

The succession of various species in the benthic and pelagial zones (Fig. 10) demonstrates the close benthic-pelagic coupling in the Rhine. The benthal is repeatedly colonized by the attachment of pelagial organisms, while filaments or organisms become detached from the biofilm and populate the pelagic zone again. This process is particularly influenced by the relationship between water column and water bottom, i.e. water discharge. The effect is increased by changes in flow velocity. This is in agreement with earlier statements that HNF populate the pelagic zone as a result of resuspension of the sediment surface layer, by rafting of biofilms or active migration (Arndt et.al, 2000). Drastic changes in the water level regulate the ratio of water volume to the colonised river bottom area which influences the losses of plankton organisms in the river (Weitere and Arndt, 2002). With respect to Bodonea, a negative correlation with the water level was established (Fig.16) (Pearson, $p=0.003$). It can be seen that Bodonea are resuspended from the biofilm by increased flow velocity. However, no significant correlation between pelagic zone and biofilm can be established, emphasizing the status of the biofilm as a proper microhabitat.

Bacteria

Zubkov and Sleigh (1999) estimated that 70-85% of the nutrients present in biofilm bacteria ingested by protozoans were regenerated and released into the immediate vicinity of the bacterial biofilm. In addition, protozoans can stimulate bacterial production and therefore enhance carbon and energy flow (Sherr and Sherr, 1984). The present study demonstrates that bacteria on biofilm are subject to a high grazing pressure. However, the grazing pressure does not originate from heterotrophic nanoflagellates requiring only 11% of the bacterial production biomass ($\mu m^3/cm^2$) to build up their biomass. This is in agreement with the results of earlier studies (Starink et.al., 1995), the estimated bacterivory ranged from 0.4 to 5.2% of the bacterial production mass, the total HNF community still consumed only an insignificant

amount of the bacterial mass. The low impact of the HNF on the bacterial community of the biofilm may be explained by the fact that approximately 80% of the HNF are suspension-feeding flagellates feeding on planktonic bacteria. Only benthic flagellates like Bodonea and Ancyromonadida are especially adapted to graze on attached bacteria and feed autochtonously from biofilm. The grazing pressure on bacteria rather originates from ciliates requiring almost more than 100% of the potential bacterial production to build up their own biomass.

However, not all bacteria are available to their predators, a known defence strategy against protozoan predation is for example the formation of bacterial colonies and filaments (e.g. Jürgens and Güde, 1994; Hahn et al., 2001), possibly also forcing the formation of biofilms by excretion of polymeric substances. Only few data on grazing protection in biofilm are available; however, sediments represent a comparable environment. In sediments, a significant amount of the bacteria is associated with particles and therefore protected by non-availability to its predators. Estimates range from 50-99% (Weisse and Rheinheimer, 1978; Sich, 1990).

The calculation of the potential consumption rate of the bacterivorous macrofauna demonstrates that it cannot live on one biofilm resource alone, because it would need approximately more than the total potential bacterial production mass to build up its biomass. A potential explanation for its food source is that macrofauna in biofilm is only temporarily present at one site and regularly drifts away.

Earlier studies demonstrated that the attachment rate is higher than the division/reproduction rate of the bacteria in biofilm (Arndt et al., 2003). Even when both causes for the presence of bacteria are taken together, their biomass is so low that they are subject to a high grazing pressure which would be still high if their reproduction rate would be increased by a factor of 2-3. This may also be an explanation for the fact that principally a selection to small forms (≤2 μm) has taken place that settle well into the matrix of the biofilm,

as Bacteria within micro refuges or glued to particles may be protected from predation by protozoa (Starink et.al., 1995) and disruption. However, also large filamentous forms were detected gaining some protection from grazing due to their large size.

Ciliates

Ciliates represented the most important predators in biofilm. The major pathway for the matter flow through biofilms would be dominated by protozoans, even if the assumed growth rate would change. Protozoa can remove between 80-100% of bacterial production per day (Sherr et al., 1983). In the present study, the ciliates should have consumed more than 100% of the estimated bacterial production per day. Also the ciliates were subject to the high grazing pressure of the meio- and macrofauna, a several fold part of the potential production of ciliates might have been consumed (for more details see Ackermann, in preparation).

Meiofauna

The average meiofaunal densities of earlier studies were around 53 ind./cm² in White Clay Creek (Borchardt and Bott,1995), 214 ind./cm² in Goose Creek, Virginia (Palmer, 1990), 27 ind./cm² in Ohio streams (Hummon et. al., 1978), up to 76 ind./cm² in the sediment of the Rhine (Reiss, 2002) and up to 120 ind./cm² (Beier and Traunspurger, 2001) in small German streams. During the present study period, 148 ind./cm² were detected; this number fits well to the results of earlier studies.

Benthic protozoan abundances may also be controlled by meiobenthic predation (Starink et al.; 1995; Hamels et al.; 2001; De Mesel et al.; 2001; Moens and Vincx, 1997). Nematode taxa ingest bacteria (Duncan et.al., 1974), algae (Jensen, 1982, 1987; Admiraal et al., 1983) fungi (Spaull, 1973) and detritus and dissolved nutrients (Montagna, 1984a; Jensen, 1987) and sessile ciliates (Kusuoka and Watanabe, 1989). Traunspurger (2000) postulated that nematode communities can consist of generalists and specialists which can feed on detritus,

bacteria or algae, or live as predators. The present study detected only representatives of the Chromadorida which had no impact on bacteria and flagellates in experiments; they had only a significant impact on algae (for details see Chapter 3). This is in agreement with a study of Croll and Zullini (1972) who detected that *Chromadorina bioculata* was correlated with a filamentous green alga (*Cladophora*). Labaratory and field experiments showed that ciliates, rotifers and copepods (Arndt 1993a; Sanders and Wickham; 1993; Cleven, 1996; Jürgens et.al., 1997) can act as voracious predators of HNF (Arndt et.al, 2000). The potential consumption rate of the meiofauna is more than 6 times the flagellates potential production. When the consideration is limited to Rotatoria, they would need about two times of the flagellates production. On the other hand, the meiofauna is subject to a high grazing pressure by predatory meiofauna (*Chaetogaster spp*.) and the macrofauna. About the whole potential production of the meiofauna might be consumed by predatory representatives of the meiofauna (e.g. *Chaetogaster spp.*). Considering the high estimated predation rate of macrofauna there should have always been a tremendous predation pressure on meiofauna by macrofauna. This might have resulted in the selection of smaller forms that might colonize well the niches of the biofilm matrix. For example, *Chromadorina bioculata* and *Punctodora ratzburgensis* colonized the tubes of the amphipod *Corophium curvispinum* (Eßer, unpublished data).

Macrofauna

Biofilms respresent an important food source for many stream invertebrates (Frost and Elser, 2002; Hillebrand and Kahlert, 2001; Lawrence et al., 2002) and pelagic zooplankton (Jeppesen et.al., 2002). The existing biomass of the macrofauna was a dominating part of the biofilm community. The macrofauna could exert a high grazing pressure on all trophic levels. Also with respect to potential production estimates, the macrofauna was a dominating part of the community. In addition to the grazing effect, the macrofauna also certainly exerted a structure-forming effect. Important were the colonies of *Cordylophora caspia*; their stalks

were colonized by peritrichous ciliates and choanoflagellates. *Corophium* tubes multiplied the biofilm surface area and were colonized by Nematoda and other organisms. Snails have a very strong impact on biofilm removing the entire superstructure and essentially reducing it to a relatively thin layer of bacterial cells (Lawrence et al., 2002; Ackermann, unpubl.). The macrofauna alters the architecture of the biofilm completely by grazing or disrupting structures by its movements (for more details see Ackermann, in preparation)

Abiotic parameters

No correlation of HNF with abiotic factors could be established: This is in agreement with a number of studies, which postulated that HNF can be highly tolerant towards abiotic factors such as temperature or oxygen supply (Finlay, 1990; Arndt et.al., 2000). Only the water level had a significant impact on the Bodonea (Fig.16). This is in agreement with the results of earlier studies on the Rhine (Weitere and Arndt 2002b for HNF; Scherwaß and Arndt, in press, for ciliates). Flow velocity has an impact on the attachment of the biofilm and results in a loss by disruption (Willkomm, unpublished data). It seems that temperature had only a minor impact on the succession of the flagellate society.

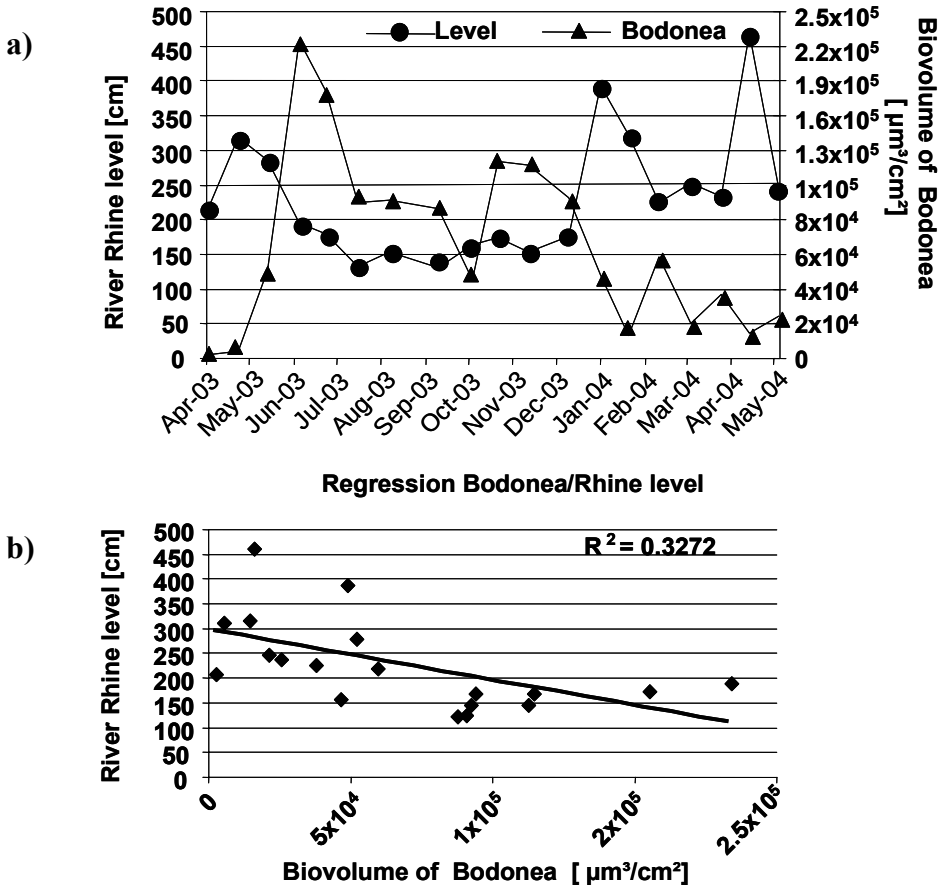

Figure 16 a) Correlation between Bodonea (biovolume) and Rhine level (cm), p= 0.03 (Pearson); b) Regression of the correlation between the biovolume of Bodonea and Rhine level.

Food web

One aim of the study was to estimate the role of the different biofilm components in biofilm. Earlier studies on the Rhine focused on planktonic food web structure (Weitere et al., 2005). Schmidt-Denter (1999) detected a correlation between bacteria and HNF and postulated that HNF in biofilm of the Rhine would be controlled **bottom up**. 99% of the HNF detected in biofilm were bacterivorous. This is in agreement with earlier studies (Schmidt-Denter, 1999). A bacteria:HNF ratio of about 1000:1 is common in plankton (Gasol, 1993). Lower density ratios (220:1) were found in streambed sediments (Bott and Kaplan, 1989). In addition, Bott and Kaplan (1990) calculated that a significant amount of the annual streambed bacteria production was consumed by HNF (52-119%). The mean bacteria:HNF ratio in the

present biofilm study was 4324:1, only an insignificant portion of the bacterial biomass should have been consumed by HNF. This is in agreement with earlier studies on the benthic zone stating that heterotrophic protists are usually dominated by the standing stock of the combined proto- and metazooplankton, but only play a minor role in the benthic zone (Garstecki et al, 2000).

The ratio of the potential production of bacteria ≤2µm to the potential consumption rate of the bactivorous flagellates was 25:1. 11% of the potential bacterial production ($\mu m^3/cm^2$) of the biofilm may have been consumed by benthic HNF, more than 100% by benthic ciliates. The remaining biomass is not sufficient to support the growth of meio- and macrofauna. Therefore, an import from plankton is expected to have taken place. The growth on the biofilm is not autochtonous but depends on permanent recolonization from the pelagic zone (Schmidt-Denter, 1999). Biofilms are open systems which interact with the surrounding media. This includes attachment and detachment of organisms as well as the turnover of biological matter, which can be influenced directly or indirectly by the protozoans (Arndt et al., 2003).

In White Clay creek, the average microflagellate densities were approximately 2 orders of magnitude higher than ciliate densities and microflagellates accounted for 67 % of protozoan bacterivory (Bott, 1995). In the pelagic zone, flagellates have an advantage compared to ciliates due to their higher growth rates, resulting in an approximate biomass ratio of 9:1 in the river Rhine (Weitere et al., 2005). In biofilm, ciliates are not exposed to the high grazing pressure of filter feeders and can therefore build up more biomass. In the pelagic zone, a ratio of 1860:1 was established between HNF and ciliates (Weitere et al., 2005), while the ratio was 4.7:1 on biofilm (Schmidt-Denter, 1999); a ratio of 6.2:1 was detected in the respective study. The large abundance of ciliates generates a high grazing pressure on HNF, which could mean **top down** control on the flagellates. The high grazing pressure might have resulted in a shift in the species spectrum in biofilm and explain the dominance of smaller

HNF with high cell division rates. The value of the potential consumption of nanophagous ciliates is about 200% of the potential production of HNF. Even if the growth rates of the HNF would be higher than the assumed conservative figures, the HNF would be exposed to an enormous grazing pressure. Other predators of HNF are macro- and meiofauna. The potential consumption rate of the meiofauna is nearly equal to the overall potential production of picobenthos, nanofauna and algae. Rotatoria could be considered another relevant predator of HNF. In the river Rhine Rotatoria as potential ciliate predators are the main group of metazooplankters (Weitere et al., 2005). Based on the estimates of production and consumption, macrofauna (in particular *Ancylus, Dikerogammarus, Corophium)* had a high grazing effect on bacteria, flagellates and algae, despite the low potential turnover rates. An additional effect is caused by a high impact on the biofilm structure (for details see Ackermann, in preparation).

Despite the available bacteria/HNF ratio of 4321:1, due to the high grazing pressure by picophagous microfauna, picophagous meiofauna and picophagous macrofauna on bacteria and due to competition for the resource bacterial biomass, a partial bottom up control can be assumed. This sounds particularly reasonable because of the non-availability of a relevant portion of bacteria due to their grazing protection. However, due to the considerable grazing pressure in the biofilm of the Rhine by nanophagous microfauna, nanophagous meiofauna and nanophagous macrofauna on HNF, rather a predominant impact of top-down control should be assumed.

Table 1 Mean assumed growth rates (d^{-1}) of the different groups of biofilm organisms. The growth rates are based on measurements of HNF in the Rhine (Weitere and Arndt, 2002a,b) as well as on assumptions for the other groups according to de Ruyter van Steveninck et al. (1992) for Rhine bacteria, Schöl et al. (2002) for Rhine algae, Müller and Geller (1993) and Scherwass (2001) for ciliates, Stemberger and Gilbert (1985) and Stelzer (1998) for metazoans.

Biofilm groups	Growth rate
Algae	0.70
Bacteria	1.05
HNF 2-5 µm	2.00
HNF 5-10 µm	1.20
Ciliates 10-50 µm	0.60
Ciliates 50-250 µm	0.30
Ciliates ≥ 250 µm	0.10
Meiofauna	0.30
Macrofauna ≤ 1000µm	0.10
Macrofauna ≥ 1000 µm	0.05
Macrofauna 5-10 mm	0.03
Macrofauna ≥ 10mm	0.01

Table 2 List of species of heterotrophic flagellates of biofilm on slides, exposed in the River Rhine.

Euglenida	*Petalomonas minuta, Petalomonas ornata, Peranema sp., Entosiphon cf. obliquum, Anisonema spp.*
Bodonea	*Rhynchomonas nasuta, Neobodo designis, Bodo saltans, Bodo curvifilus, Bodo spp., Bodo caudatus.*
Chrysomonadida	*Spumella spp., Anthophysa vegetans.*
Bicosoecida	*Bicosoeca spp.*
Choanoflagellida	*Monosiga varians, Monosiga spp., Salpingoeca spp.m Salpingoeca cf. collaris, Salpingoeca cf. ampullaceae, Salpingoeca cf. amphoridium, Codonosiga botrytis.*
Cercomonadida	*Cercomonas crassicauda, Cercomonas cf. longicauda, Cercomonas sp., Bodomorpha minima.*
Thaumatomonadida	*Protaspis spp., Thaumatomonas spp, Thaumatomastix setifera.*
Ancyromonadida	*Ancyromonas sigmoides, Ancyromonas magnus.*
Apusomonadida	*Apusomonas cf. proboscidea.*

Table 3 Minimum and maximum abundances of the meiofauna/cm² in biofilm on slides, exposed in the River Rhine.

	Minimum	Maximum
Nematoda	**0.81**	**839.06**
Rotatoria	**0.11**	**48.95**
Acari	**0.15**	**25.00**
Copepoda	**0.03**	**20.00**
Oligochaeta	**0.00**	**12.33**
Isopoda	**0.00**	**6.00**
Turbellaria	**0.00**	**0.37**
Ostracoda	**0.00**	**0.29**
Gastrotricha	**0.00**	**0.05**
Cnidaria	**0.00**	**237.33**

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Kapitel 2

Long-term dynamics of microbial biofilm communities of the river Rhine with special reference to the meiofauna

Abstract

A long term study on taxonomic composition, seasonal dynamics, succession and biotic interactions of the principal biotic components in biofilms of the river Rhine (i.e. bacteria, algae, heterotrophic flagellates, ciliates, meiofauna, macrofauna) has been performed. This paper assessed the relative importance of meiofaunal organisms in the biofilm food web structure grown on artificial substrates (glass slides). Changes in abundance and biomass of different groups over the year were investigated involving a live-counting-technique**.** The average numbers of meiofauna over the total investigation period detected per square centimeter biofilm were 107 nematodes, 9.7 rotifers, 2.5 Acari, 1.6 copepods, 1.9 oligochaetes, 0.6 isopods, 0.02 turbellarians, 0.08 ostracods, and 25 cnidarians. Major groups of meiofauna were represented by only few species with specific features. Chromadorid nematodes are able to attach themselves to the substratum and to escape grazing through their mobility within the film. Abundances are comparable to earlier studies with an average abundance of 149 meiofauna organisms/cm², represented by more than 75% of the individual count of meiofauna by chromadorid nematodes. Seasonal dynamics were recorded, with a maximum of more than 800 nematodes/cm² biofilm area in spring 2004; the number of detected rotifers was temporarily higher than 45 rotifers/cm².

A major impact of nematodes on algae could be established by feeding experiments with feeding rates of 151 algae nematode^{-1} day^{-1} ; this effect war also visible in long-term studies. Rotifers were present with only short term occurrence feeding on flagellates and probably also predatory ciliates. In feeding experiments, a feeding rate of 1780 HNF rotifer^{-1} day^{-1} was established, being in good agreement with earlier studies.

The detection of many important grazers among the Meiofauna, suggest that they might structure microbial components via selective grazing; it has a major impact on the microfauna and the microalgae of biofilms through biological activities such as grazing,

excretion and movement. In addition to direct effects, also indirect effects should be expected, by predation of predatory ciliates which indirectly might support HNF; in addition, HNF are fed directly. It is assumed that meiofauna plays a very prominent role in the functioning of microbial biofilms of lotic river bottoms.

Introduction

Meiofauna has been so far often neclected by freshwater ecologists (Schmid-Araya and Schmid, 2000), though it represents an abundant component of freshwater biofilms and would be expected to have a major effect on the microfauna and the microalgae of biofilms through biological activities such as grazing, excretion and movement (Abrams and Mitchell, 1980; Alkemade et al., 1992; Aller and Aller, 1992; Traunspurger et. al, 1997; De Mesel et al., 2004). Meiofauna are known to live on hard substrates in association with periphytic and epiphytic algae, however, abundance, diversity and colonizing abilities of hard substrate meiofauna have been poorly documented (Attila et.al., 2003). Meiofauna comprises more than 95% of all metazoan organisms in most rivers; the groups with the highest abundances are nematodes and rotifers (Duft et al, 2002). However, the role of this community in benthic food webs of lotic ecosystems has been scarcely investigated, although they embody an abundant and diverse component of the benthos (Palmer and Strayer, 1996; Robertson et al., 2000a). The understanding of ecological processes in the benthos stems from research primarily focusing on macrofauna and microbes (Allan, 1995; Schmid-Araya and Schmid, 2000; Reiss, 2002; Bergtold and Traunspurger, 2004). Lotic meiofauna feed on bacteria, algae and detritus (Perlmutter and Meyer, 1991; Borchard and Bott, 1995). Due to their grazing activity, meiofauna are intimately associated with detrital material and can influence decomposition rates (Goedkoop et al., 1997; Gullberg et al., 1997) and bacterial activity in freshwater ecosystems (Traunspurger et al., 1997). It has been shown that the excretion

products of marine nematodes serve as a substrate for bacterial production (Rieman and Schrage, 1978). Their size range would appear to place meiofaunal organisms at the level of intermediate species in a food web, potentially linking the microbial and the macrofaunal foodweb. In particular, many meiofauna organisms play an important role as consumers in the detrital food web and, in turn, serve as a major food source for predatory invertebrates and native fish (Giere, 1993; Schmid-Araya and Schmid, 2000).

Although the taxonomic composition of the meiofauna can substantially vary between freshwater ecosystems, nematodes are usually the dominant group, both with respect to abundance and biomass. The present study investigates the role of the lotic freshwater meiofauna in biofilms in the river Rhine. Glass slides as a model substrate were directly exposed to the Rhine and all components of the biofilm community were studied over a period of 14 months (see Chapter 1). In addition, feeding experiments were performed with the dominant taxa of biofilm meiofauna (i.e. nematodes and rotifers) to investigate the effect of dominant meiofauna groups on the composition of the biofilm.

Material and methods

Study site

The investigation of the meiofauna community of the biofilm was performed in the river Rhine at Cologne at the Ecological Rhine Station, Rhine km 685 (see Fig. 1, Chapter 1). The sampling period was from April 2003 to June 2004. For details see Chapter 1.

Sampling of the biofilm community

In this investigation, the samples of the biofilm community (n=3) were prepared following the procedure described in Chapter 1. In short, glass slides as a model system were exposed at a depth of 10 cm for a period of fourteen months in a flow channel at the

Ecological Rhine Station. Bacteria were scraped off from the slides and fixed in a 4% ice-cold glutaraldehyde solution (final concentration 2%), stained with DAPI (4`6-diamino-2-phenylindol, Porter and Feig, 1980) filtered to membrane filters (Nucleopore, 0.2 µm pore size) and counted with an epifluorescence microscope (Zeiss Axioskop, 1000x magnification). Heterotrophic flagellates were scraped off from one side of the slides and investigated for species composition and abundance on the opposite side of the glass slide by direct live count (Arndt et. al., 2000) with a microscope (Zeiss Axiostar, 400x magnification, phase contrast, ocular micrometer, video recording) immediately after sampling. Meiofauna was scraped off, transferred into a counting chamber (Bogorov tray, Hydrobios, Kiel-Holtenau) and analyzed by live count under a binocular microscope (Olympus S Z X9, 12.6x-114x magnification). The sampling and counting of ciliates, algae and macrofauna followed in principle the methods for heterotrophic flagellates (for methodological details see Ackermann, in preparation). Macrofauna was collected from the whole glass slide holder.

In addition to the described method, reference samples were taken by scuba diving from the bottom. For this purpose, stones close to the research platform of the Ecological Rhine Station were collected from the bottom on one day each in August 2003, December 2003, March 2004 and July 2004. The biofilm was removed from the stones with a biofilm sampler (see Fig. 2, Chapter 1) and the meiofauna was determined by live count as described above (for details see Chapter 1). In addition, samples from stones were collected in the bank region at Cologne, Düsseldorf and Neuss and treated as described above.

Method comparison

For comparison with the routine sampling method described above, slides were sampled with the biofilm sampler in November. Then the meiofauna organisms were transferred into a counting chamber (see above) and analyzed by live count.

Abiotic parameters

In addition to the biotic data, data on seston content, chlorophyll content, temperature, pH-value, conductivity, flow velocities, photosynthetically active radiation and water discharge were collected (for details see Chapter 1).

Statistics

Statistical analysis was conducted using SPPS for Windows (Version 11.0). Correlation was evaluated by calculation of Pearson's correlation coefficient. Significant correlation of the nonparametric data was detected by Spearman rank test analysis. To evaluate the effects of the abiotic parameters, correlations were tested between the whole biofilm community on the level of taxonomic groups and all investigated abiotic parameters (seston content, temperature, water discharge, pH-value, conductivity and chlorophyll content). To analyze the effects of the substratum (slides and natural substrates) ANOVAs were conducted. The Student's *t*-test was used to determine differences between the chambers of the feeding experiment and to detect differences between areas with and without *Corophium* tubes.

Feeding experiments

Feeding experiments (presence/absence method) were conducted with the dominating meiofauna components nematodes and rotifers. The feeding experiments were run in the lab of the Ecological Rhine Station in July 2004. For this purpose, micro-flow chambers were designed as microcosms (Fig. 1b, 1c). The chambers (18.75 cm^2) were fitted to cover glasses (12.5 cm^2) and were sealed with a cover slide each at top and bottom. One type of flow chambers (reservoir chambers) (Fig. 1b) was completely hollow, the other type had a meandering channel inside (test chambers) (Fig.1c). The surface of the test chambers available for cultivation was 6.5 cm^2, the volume was 3.25 cm^3. 250 liters of 40 µm pre-

filtered Rhine water were pumped into a vessel (Fig. 1d). The water was continually pumped through a flow channel with an Oase USP 20 pump (67 L/min) and guided back into the reservoir vessel (Fig. 1a, reservoir vessel 1d). From the flow channel, water was pumped through the test chambers and then back into the flow channel over a period of three days (72 h). To prevent heating of the water, the temperature of the flow channel was continuously kept at 15°C with an immersion water cooling device (Colora, Germany) (Fig. 1e). Throughout the 72 h period, biofilms were allowed to grow in the test chambers (n=12). After the 72 period, the test chambers were removed from the flow system and connected to the reservoir chambers. Half of the reservoir chambers (n=12 in total) were seeded with 100 predators each (experiment 1: nematodes, chromadoridae (*Punctodora ratzeburgensis, Chromadorina bioculata*); experiment 2: bdelloid rotifers (*Rotaria rotatoria*)). The water circulated from the reservoir chambers to the test chambers and back over a period of 7 days (Fig. 1g, 1h) with a flow rate of 0.1 L/h. From day 1 to day 7, the biofilm community in the test chambers was analyzed. Algae, flagellates and ciliates were counted by continuous flow live count with a microspcope (Zeiss Axiostar, 400x magnification, phase contrast, ocular micrometer, video recording) (Fig. 1f). After day 10, the cover slides with biofilm growth were fixed in a 4% ice-cold glutaraldehyde solution (final concentration 2%) and stained with DAPI (4`6-diamino-2-phenylindol, Porter and Feig, 1980); then the bacteria were counted with an epifluorescence microscope (Zeiss Axioskop, 1000x magnification). Experiments were run in six replicates both for experimental and control chambers.

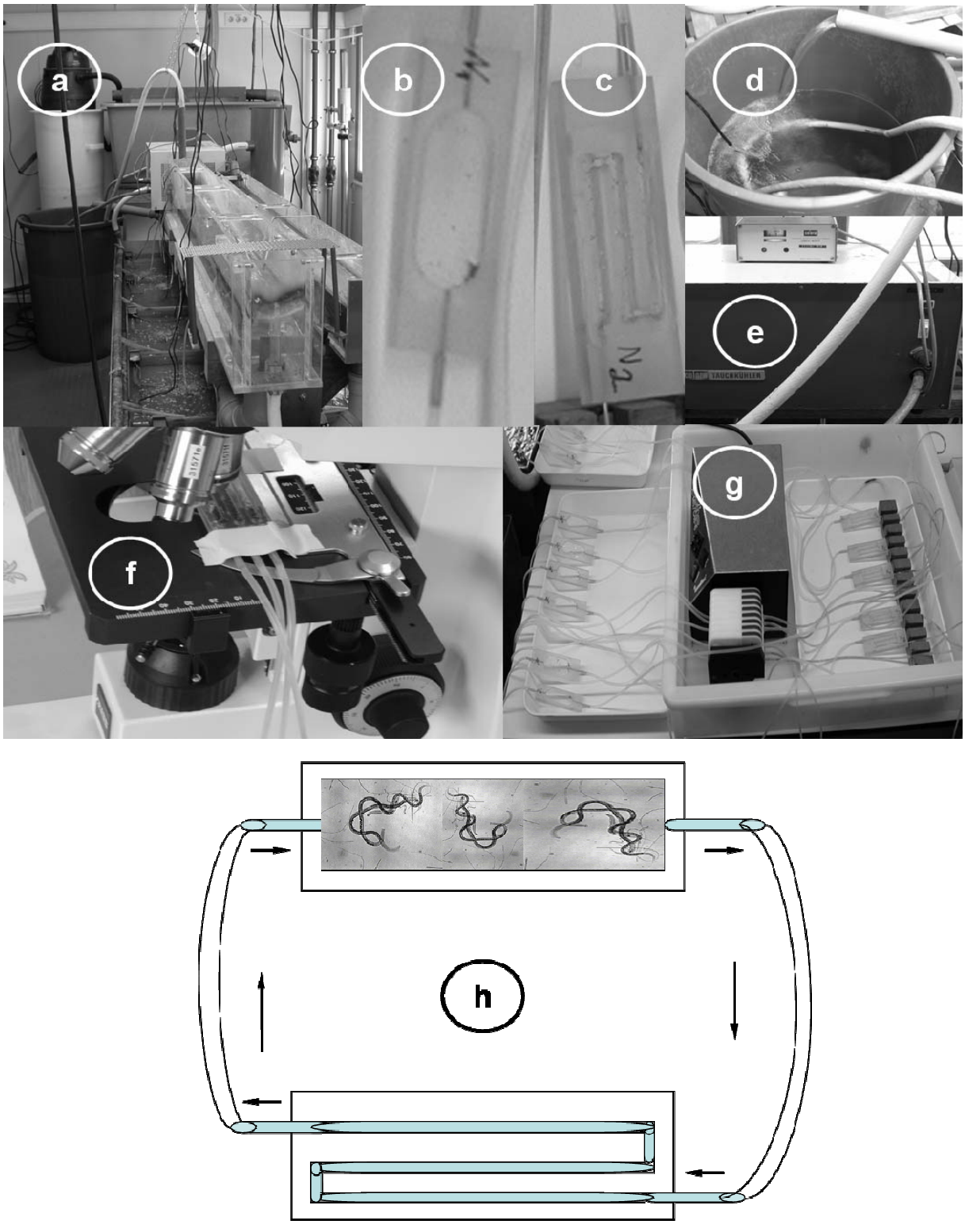

Figure 1 Experimental design, a) flow channel in the lab; b) reservoir-flow chamber; c) experimental flow chamber; d) water reservoir; e) water-cooling device; f) live count under the microscope; g) flow chamber with pump; h) schematic representation of the flow system .

Results

Organism composition

On average, $9.1x10^7$ bacteria/cm², 622 algae/cm², 20,933 heterotrophic flagellates/cm², 3,359 ciliates/cm² and 149 meiofauna organisms/cm² were detected in the biofilm on the slides (Fig. 2a). With respect to meiofauna, on average 107 nematodes, 9.7 rotifers, 2.5 Acari, 1.6 copepods, 1.9 oligochaetes, 0.6 isopods, 0.02 turbellarians, 0.08 ostracods, and 25 cnidarians were detected per cm² (Fig. 3). The maximum counts are shown in Table 1. With respect to biomass, the organism composition in biofilm on the slides was dominated by ciliates (50% in average), followed by macrofauna representing 43% of the biomass (Fig. 2b, 2c) (for details see Chapter 1). On average, the meiofauna comprised 3.6 % of the biomass; its maximum share in biomass was 9.8 % in April 2004 (Fig. 2c). The maximum biovolume of the meiofauna was 3.8 x 18^8 µm³/cm² in May 2004.

Table 1 Comparison of the abundance of taxonomic groups of meiofauna in biofilm on slides.

Taxonomic group	Mean abundance [ind./cm²]	Maximum abundance [ind./cm²]
Nematoda	106.91	839.06
Rotatoria	9.67	48.95
Acari	2.52	25.00
Copepoda	1.64	20.00
Oligochaeta	1.86	12.33
Isopoda	0.62	6.00
Turbellaria	0.02	0.37
Ostracoda	0.08	0.29
Gastrotricha	0.00	0.05
Cnidaria	24.73	237.33

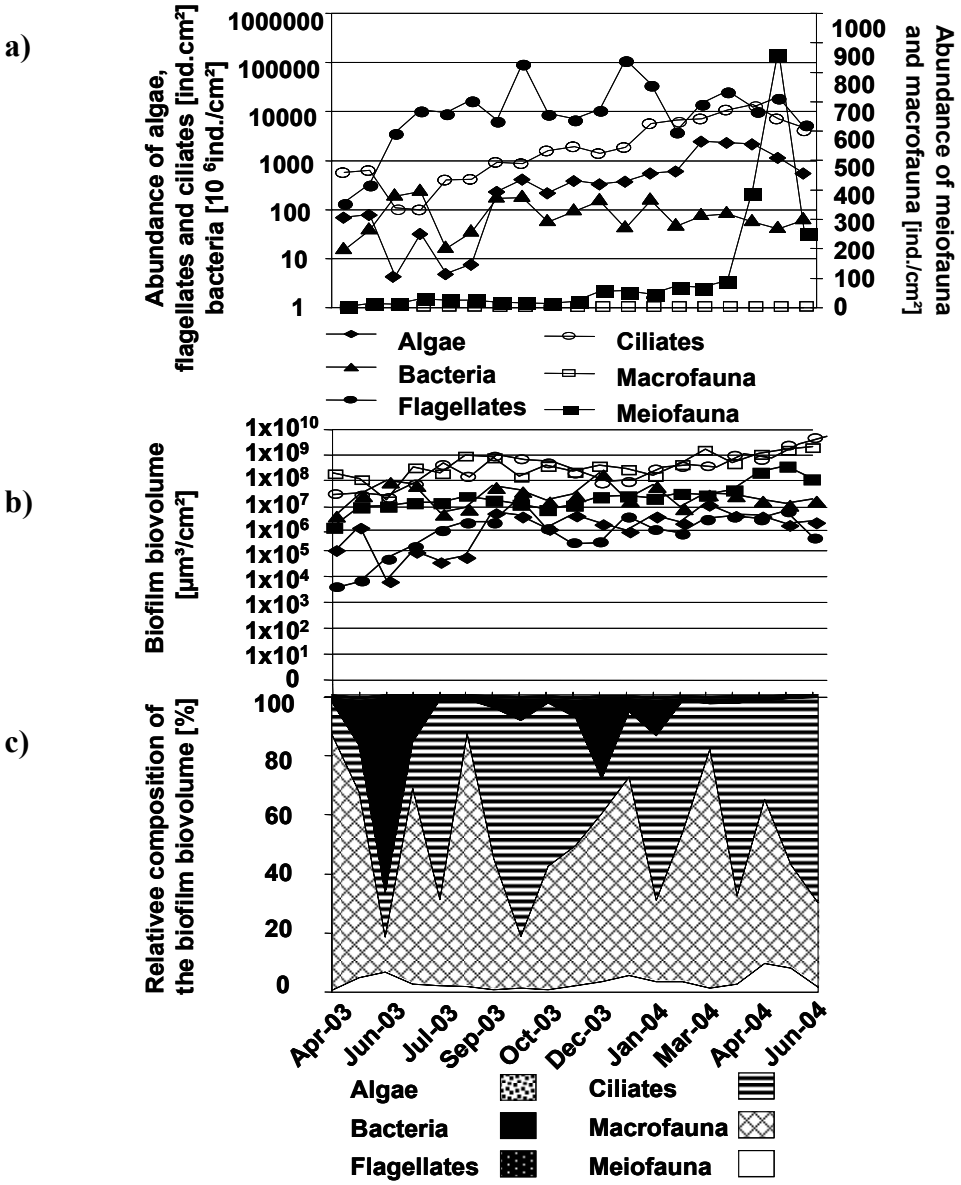

Figure 2 Average composition of the biofilm on slides, succession over the study period arranged by sampling dates (n=3); a) abundance of the different groups of biofilm organisms; b and c) biomass of the different groups of biofilm organisms.

As demonstrated by the meiofauna biomass distribution, only Rotatoria and Nematoda were sufficiently abundant during the study period to potentially affect the biofilm composition (Fig. 3). Average and maximum numbers of the detected meiofauna individuals grouped by taxa are listed in Table 1. Rotatoria were an important group (common taxa: *Rotaria*, *Trichocerca*, *Squatinella Colurella* and *Brachionus*); their number sometimes was

higher than 45 ind/cm². Almost throughout the whole year, specimens of bdelloid Rotatoria were detected in higher numbers than monogonont Rotatoria. Nematodes were almost exclusively represented by Chromadorida (*Punctodora ratzeburgensis* and *Chromadorina bioculata*), being the most important meiofauna taxon throughout the study period. The detected nematode count exceeded 800 nematodes/cm² biofilm area in spring 2004. Also few Acari and nauplii and copepodites of cyclopoid and harpactoid copepods were present in the biofilm. Oligochaeta (common species: *Aelosoma hemprichi* and *Chaetogaster spp*.) and Cnidaria (*Cordylophora caspia*) represented a larger share in the species composition of the meiofauna; however, their numbers were subject to strong seasonal changes. One of the principal reasons for a significant increase in biofilm surface area in summer 2004 was colony formation of *Cordylophora caspia*.

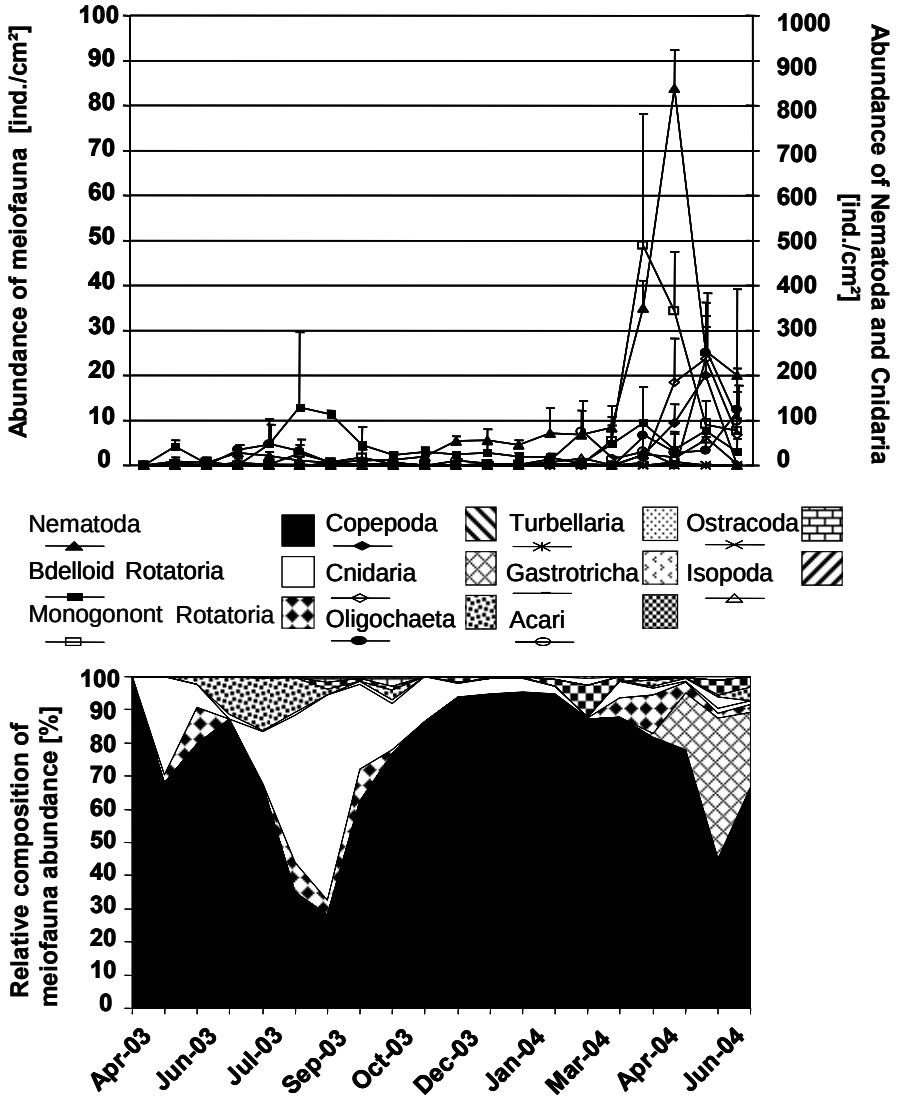

Figure 3 Fluctuations in meiofauna abundance in biofilm on slides throughout the study period, arranged by sampling dates, mean values and standard deviations, (n= 3).

The meiofauna composition on slides was strikingly similar to that found on natural substrates. With respect to the number of individuals, both biofilms on slides and on river bank stones were dominated by nematodes (>75%); their composition was dominated by Chromadoridae (>74%) (Fig. 4). Figure 5 shows individual numbers (n=3) of various samples collected at different sites close to the Ecological Rhine Station in June 2004. At all sampling dates, meiofauna composition was dominated by nematodes (54%-69%). Also rotifers, copepods and Oligochaeta were relevant groups of these biofilm communities; this is illustrated by the mean abundance (Fig. 6a). On average, 81 nematodes, 10 rotifers, 9

Oligochaeta, and 15 copepods were counted. A comparison of the organism count from lotic site (undercut slope) and lentic site (slip-off slope) demonstrates that despite almost identical relative taxa composition (Fig. 5) the abundances at the undercut slope were significantly higher (Fig. 6b). While the mean organism number at the undercut slope was 89, it was only 10 at the slip-off slope.

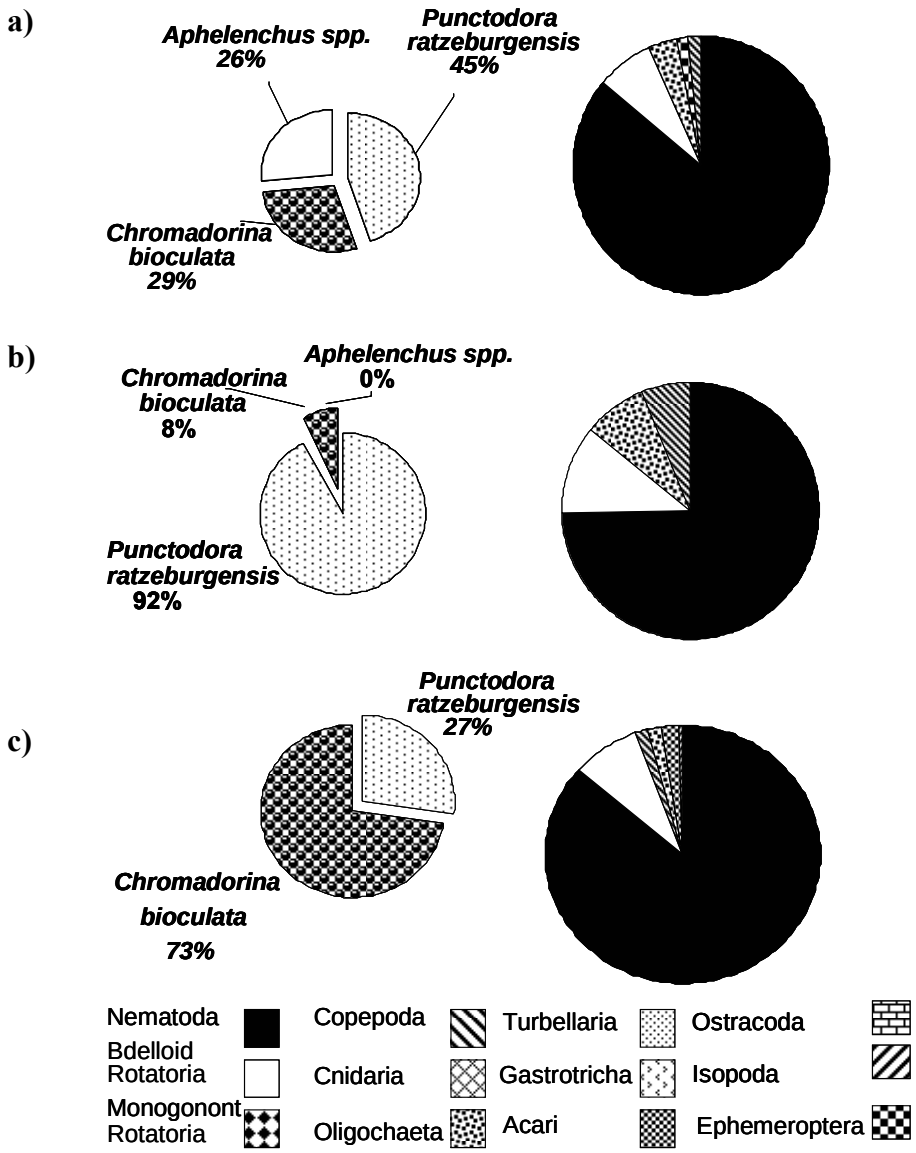

Figure 4 Average distribution of meiofauna composition in biofilm a) river Rhine at Düsseldorf, on stones, December 2003, (n=6); b) river Rhine at Cologne, on stones, July 2004, (n=18); c) Cologne, Ecological Rhine station, on slides, April 2003-June 2004, (n=60).

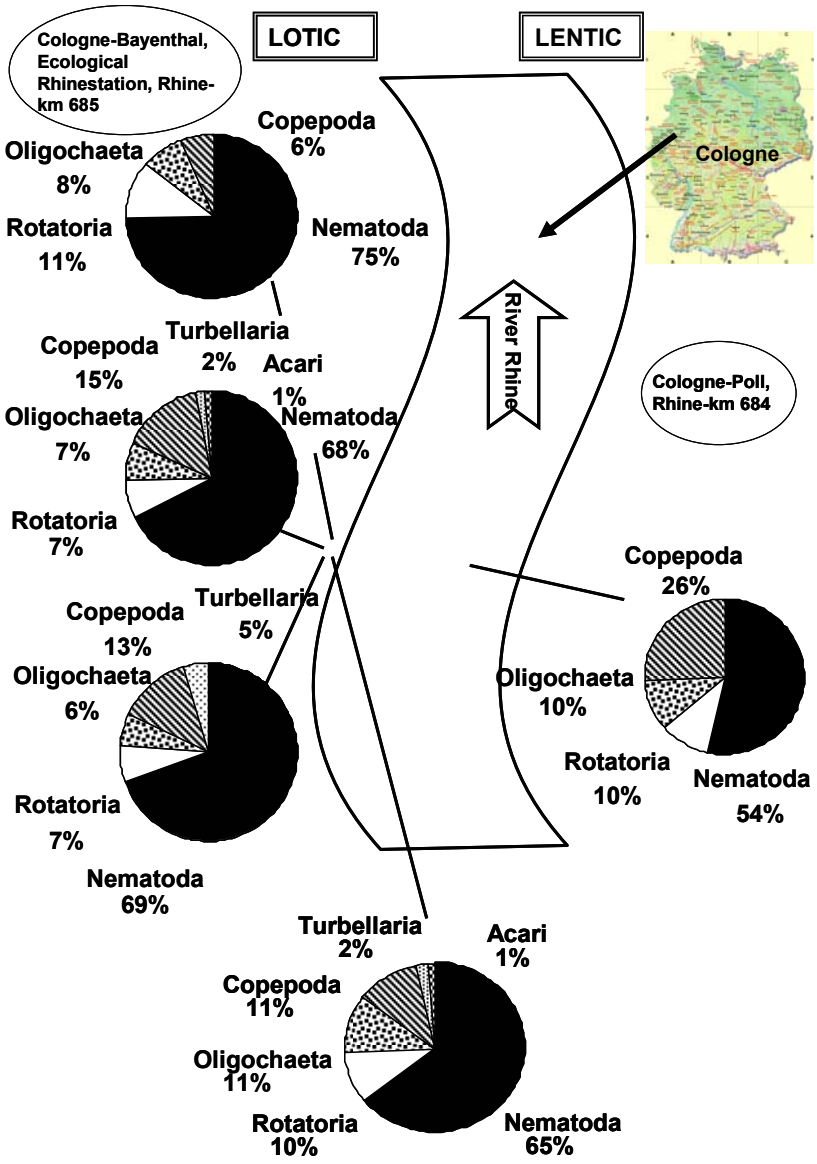

Figure 5 Average composition of meiofauna in biofilms on stones of the river Rhine at Cologne, July 2004, (n=3).

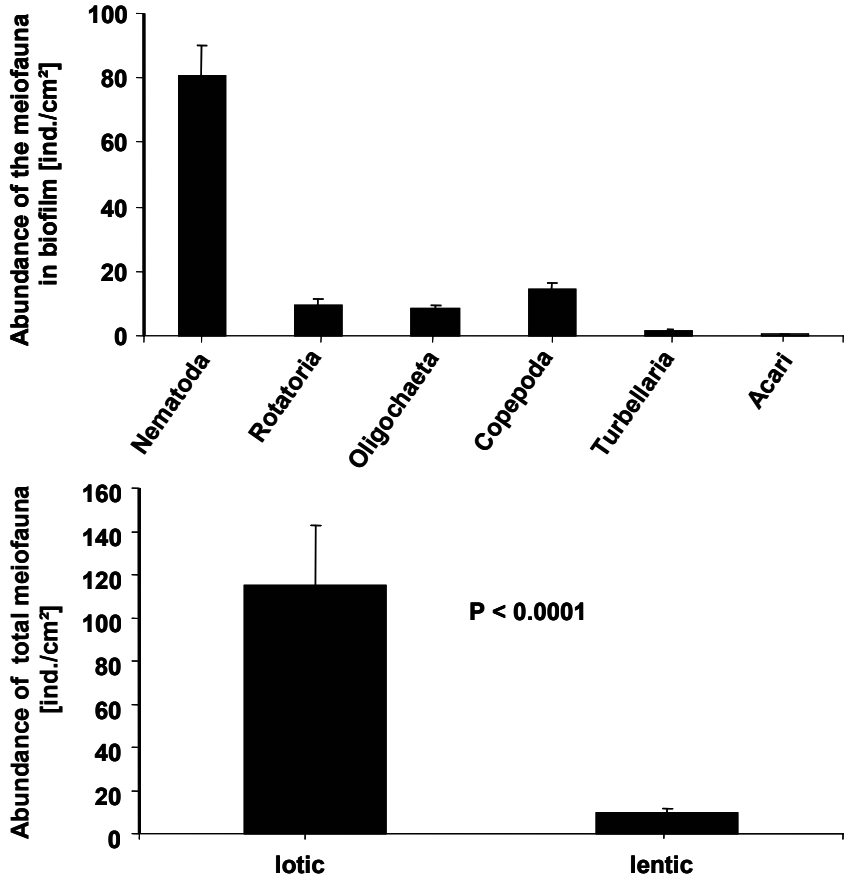

Figure 6 Meiofauna in biofilm on stones a) average composition of the meiofauna communities in biofilms of the river Rhine, mean values and + standard deviations, (n=15); b) comparison of the abundance of meiofauna communities in biofilms on stones on the undercut slope (lotic) and slip-off slope (lentic), mean value and standard deviations, (n=3), p< 0.0001 (Student's *t*-test).

In addition, the relationship between structure of the biofilm surface and meiofauna abundance was significant. It was demonstrated by a comparison between biofilm areas with and without *Corophium* tubes that more than the double number of nematodes and rotifers was detected in areas where *Corophium* tubes were present ($p<0.001$, Fig. 7). An average number of 45 nematodes und 1.8 rotifers were detected on areas with *Corophium* tubes, while their numbers were only 17 and 0.4, respectively, on areas without *Corophium* tubes.

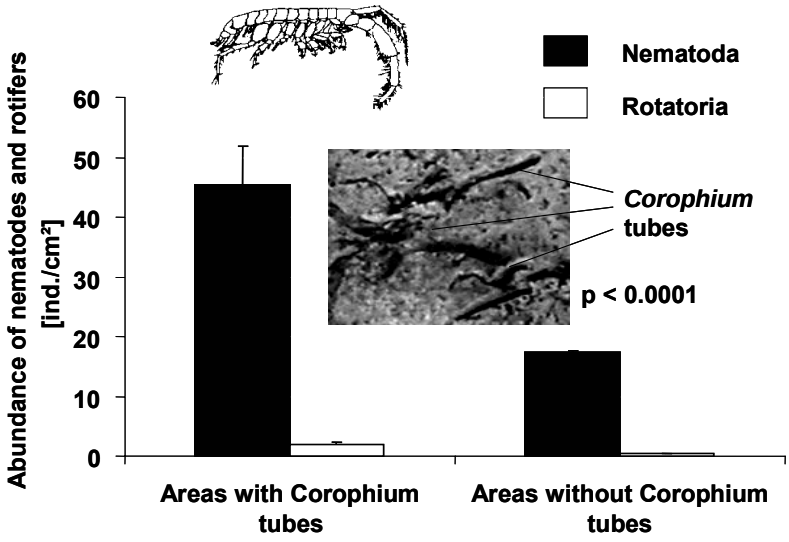

Figure 7 Comparison of the abundance of meiofauna in biofilms of areas with and without *corophium* tubes, mean values and + standard deviations, (n=3), p< 0.0001 (Student`s *t*-test) for nematodes and rotifers.

Comparison of the biofilm on slides and on the bottom of the Rhine

Methods comparisons revealed that almost identical results were obtained by comparing the meiofauna counts from samples taken by the routine method and from samples taken by the biofilm sampler (Fig. 8).

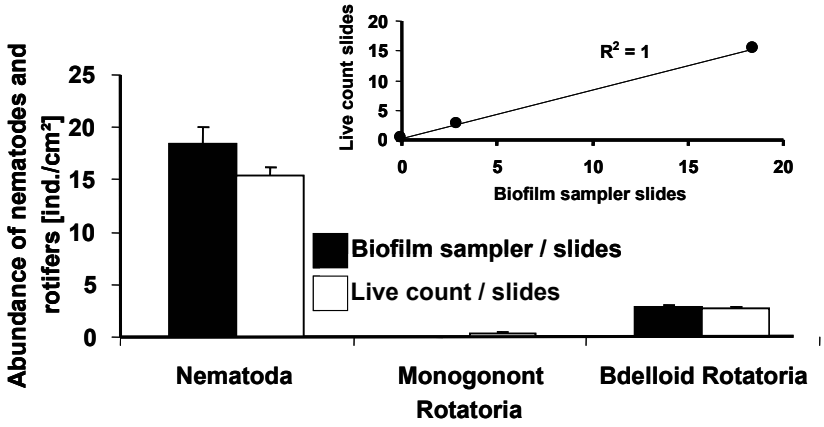

Figure 8 Methods comparison of abundance of the meiofauna of biofilms probed with biofilm sampler and with live count on slides, November 2003, mean values and standard deviations, (n=3).

The comparison of biofilms on slides and on the bottom of the Rhine throughout the year demonstrated that the number of individuals was significantly higher on slides (Figure 9). With respect to the principal representatives of meiofauna (nematodes; monogonont rotifers and bdelloid rotifers), the picture is as follows: in August 2003 about 10 nematodes, 3 monogonont rotifers and 13 bdelloid rotifers per cm^2 were counted on the slides, while the density on stones of the bottom was about 2 nematodes, 0 monogonont rotifers and 0.1 bdelloid rotifers per cm^2. In December 2003, the count on slides was about 54 nematodes, no monogonont rotifers and 3 bdelloid rotifers per cm^2 compared to 13 nematodes, no monogonont rotifers and 2 bdelloid rotifers on stones. In March 2004, about 68 nematodes, 0.3 monogont rotifers and 0.4 bdelloid rotifers per cm^2 were detected on slides, 0.1 nematodes and 0 rotifers were detected on stones. In June 2004, the number was about 200 nematodes, 8 monogonont rotifers and 3 bdelloid rotifers per cm^2 on slides and 10 nematodes and no rotifers per cm^2 on stones. Taken together, the data demonstrated an increase in nematode individual density on slides, while no general tendency was detected with respect to stones.

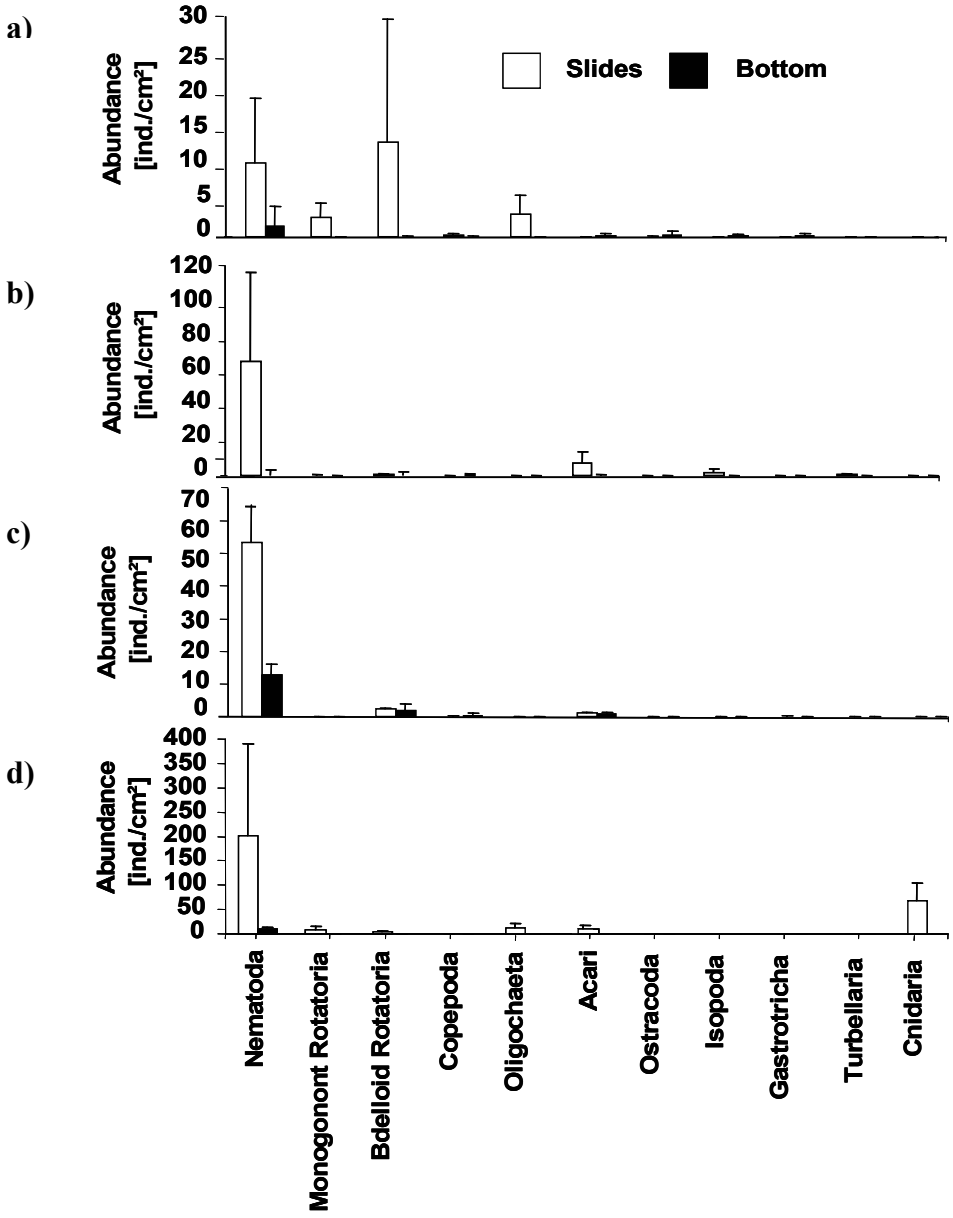

Figure 9 Comparison of the composition of the meiofauna communities in biofilms on slides in the flow channel with those on stones of the river bottom a) August 2003; b) December 2003; c) March 2004; d) June 2004, mean values and standard deviations, (n=3).

Feeding experiments

The feeding experiments with nematodes demonstrated that the individual density of algae and flagellates decreased during the study period (Fig. 10). However, a significant difference between experimental and control chambers only was detected in algae. This becomes even clearer when considering the organism groups separately. The difference in individual count/cm^2 per group on day 4 of the study period is shown in Figure 11. No significant difference was detected between the bacterial densities in experimental and control chambers (Fig. 11a). Also, no significant difference in the number of individuals between experimental and control chambers could be detected with respect to flagellates and ciliates (Fig. 11c and 11d). In contrast, a clear significant effect was detected for algae; diatoms and also chlorophytes demonstrated a significant reduction in individual counts in the experimental chambers (Fig. 11b). The share of diatoms and chlorophytes in the experimental chambers was reduced to about 50%: The number of individuals was reduced from about 3820 diatoms/cm² and 4960 chlorophytes/cm², respectively, in the control chambers to 1880 diatoms /cm² and 1590 chlorophytes/cm², respectively. A comparison of the abundance of algae in control chambers vs. experimental chambers revealed a minimum estimate of the feeding rate of about 151 algae per day and nematode (Tab. 2).

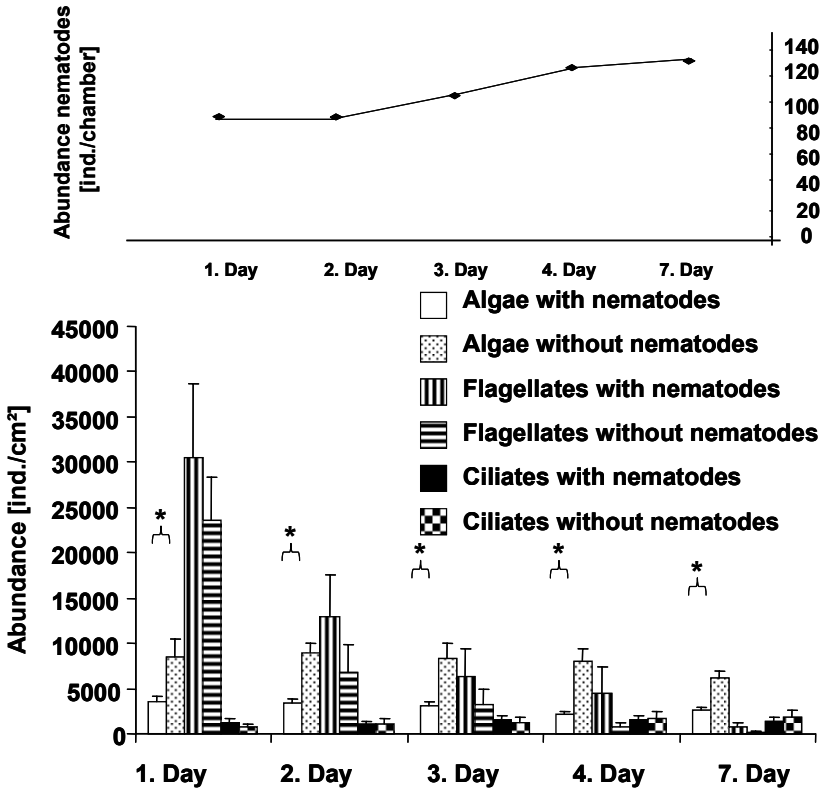

Figure 10 Feeding experiment with chromadorid nematodes (*Chromadorina bioculata*, *Punctodora ratzeburgensis*). Lower panel: Mean abundance of algae, flagellates and ciliates in the course of the experimental period. Error bars indicate standard errors, (n=6). The Student`s *t*-test was used to established differences between absence and presence of nematodes for each prey organism group; significant differences are indicated by an asterisk (*). Upper panel: Corresponding abundance of nematodes per chamber.

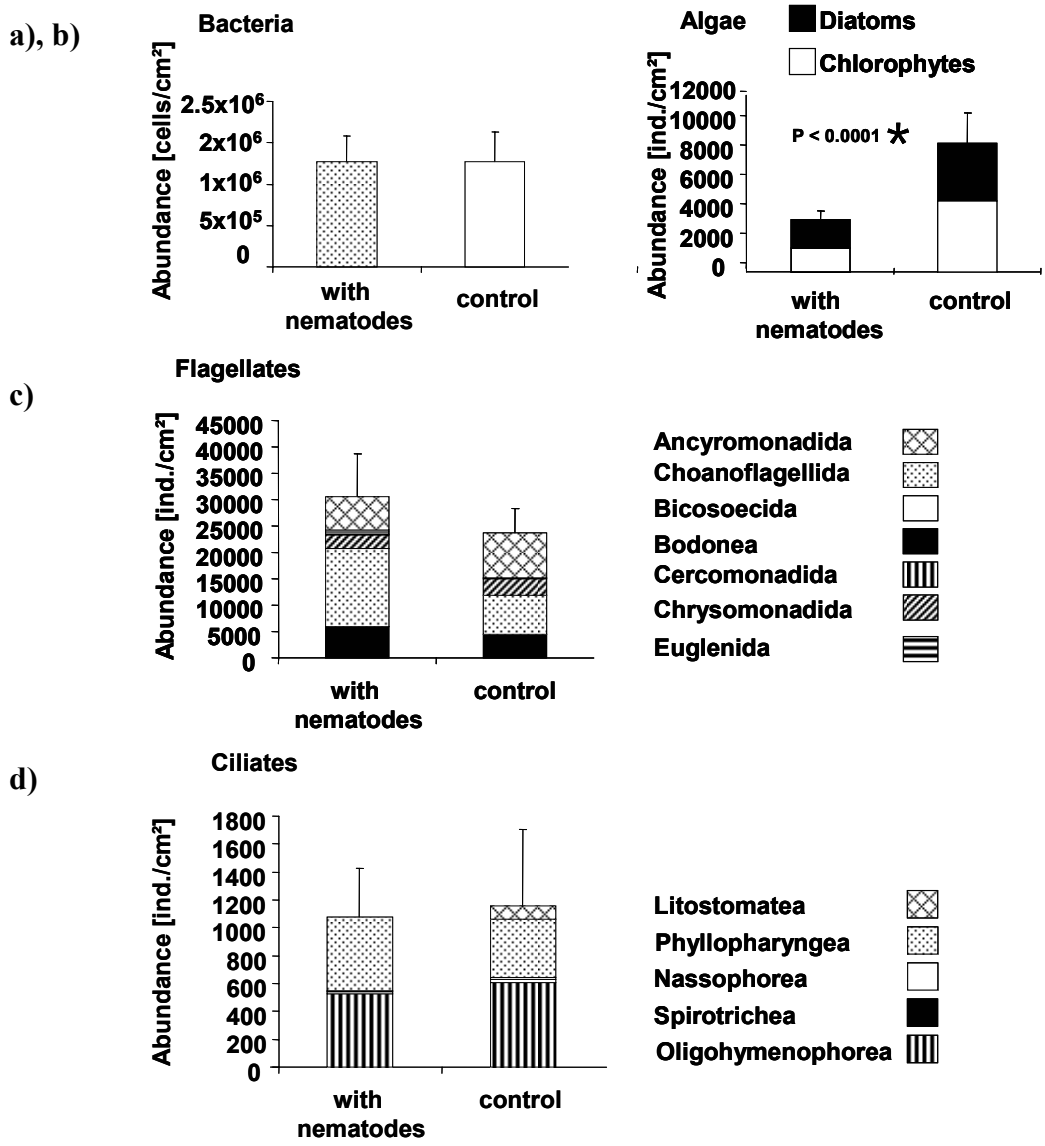

Figure 11 Experiment with chromadorid nematodes (*Chromadorina bioculata*, *Punctodora ratzeburgensis*). Mean abundance on day 1 of the experimental period, a) bacteria; b) algae; c) flagellates; d) ciliates; error bars indicate standard errors, (n=6). The Student`s *t*-test was used to established differences between absence and presence of nematodes for each prey organism group; significant differences are indicated by an asterisk (*).

The feeding experiments with bdelloid rotifers showed that throughout the whole study period, only for flagellates significant a difference existed between experimental and control chambers (Fig. 12). Bacterial density (Fig. 13a), algae counts (Fig. 13b) and ciliate counts (Fig. 13d) demonstrated no significant differences between experimental and control chambers. However, the flagellates were clearly decimated in the presence of bdelloid rotifers (Fig. 13c). The individual counts of the flagellates were reduced as follows: Kinetoplastida from about 1230/cm² to 444/cm², Choanoflagellida from about 2920/cm² to 380/cm²,

Chrysomonadida from 1880/cm² to 40/cm², Euglenida from 26/cm² to 20/cm², and Ancyromonadida from 40/cm² to 0/cm². A comparison of the abundance of heterotrophic flagellates in control chambers vs. experimental chambers revealed a minimum estimate of the consumption rate of about 1780 flagellates per day and rotifer (Tab. 2).

Table 2 Estimated feeding rates from the feeding experiments with nematodes and rotifers on the first day of the experimental period; cells day^{-1} predator^{-1}, (n=6).

Nematodes	Feeding rate [algae nematode^{-1} day^{-1}]
Chlorophyta	93
Diatoms	58
Total algae	151
Rotifers	**Feeding rate [flagellates rotifer^{-1} day^{-1}]**
Kinetoplastida	258
Choanoflagellida	893
Chrysomonadida	613
Euglenida	2
Ancyromonadida	13
Total flagellates	1780

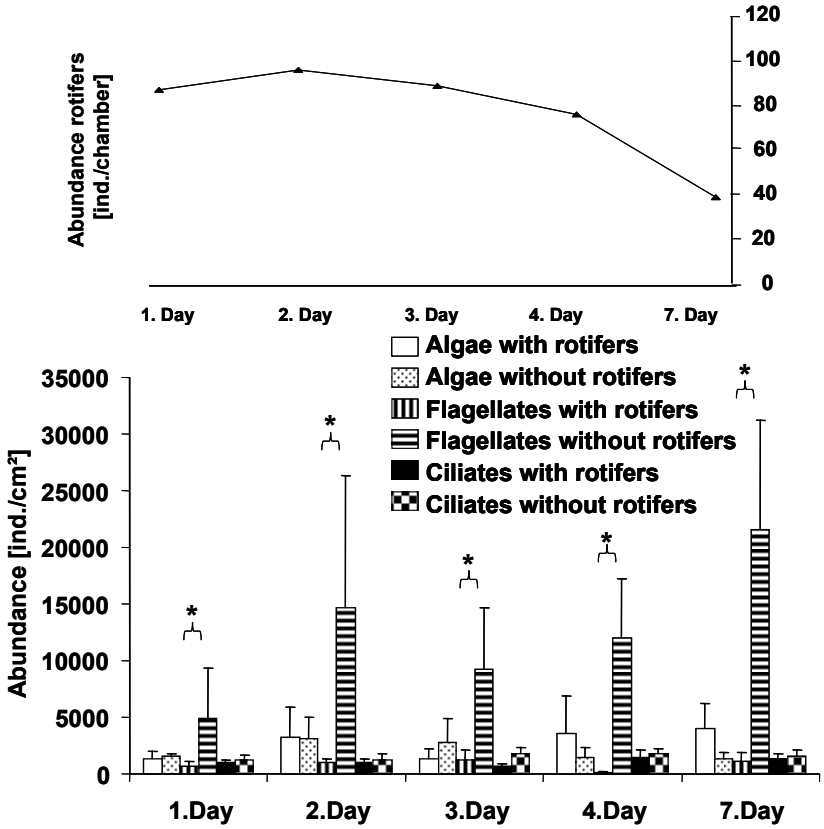

Figure 12 Feeding experiment with bdelloid rotifers (*Rotaria rotatoria*). Lower panel: Mean abundance of algae, flagellates and ciliates in the course of the experimental period. Error bars indicate standard errors, (n=6). The Student`s *t*-test was used to established differences between absence and presence of rotifers for each prey organism group; significant differences are indicated by an asterisk (*). Upper panel: Corresponding abundance of rotifers per chamber.

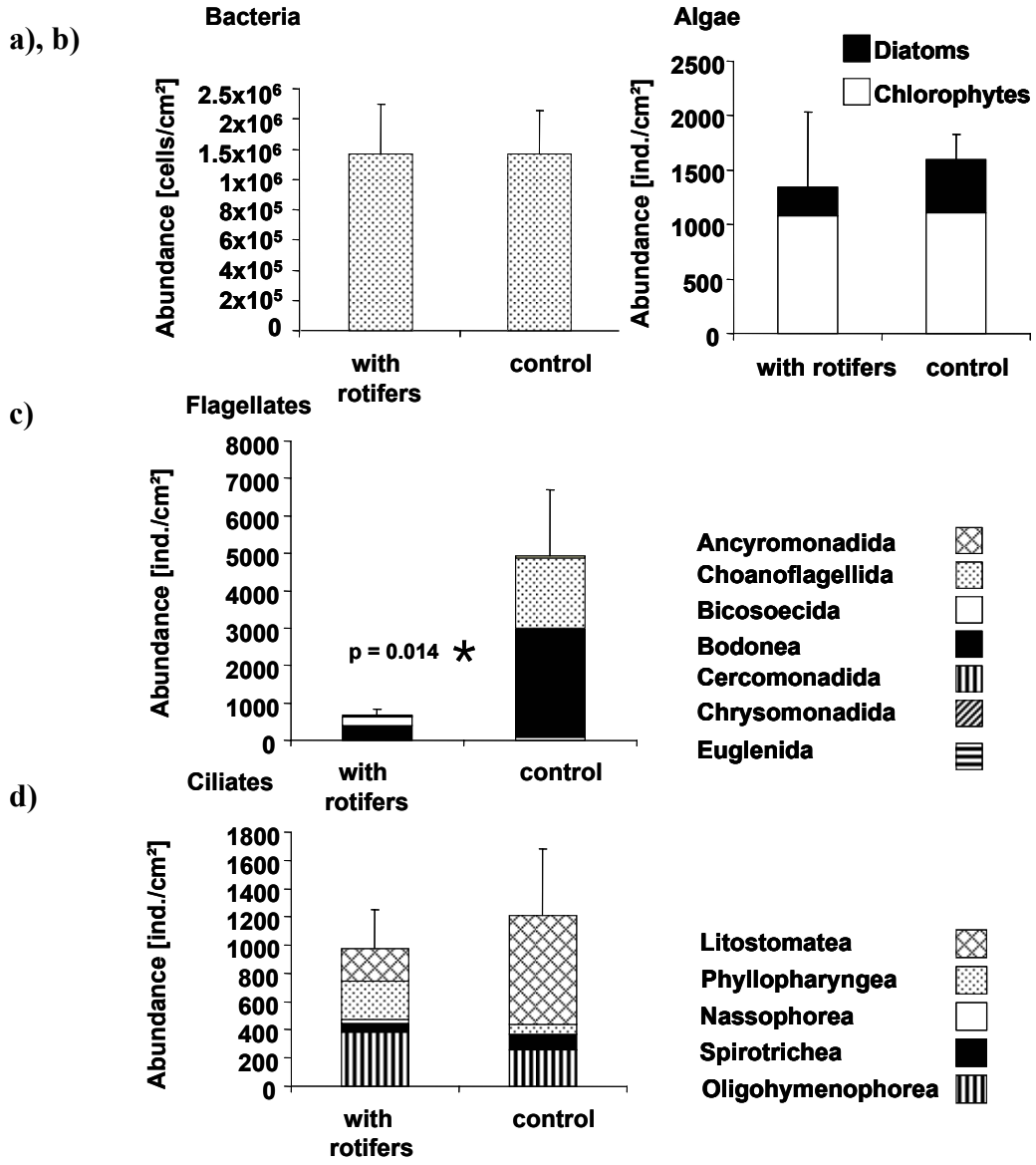

Figure 13 Experiment with bdelloid rotifers (*Rotaria rotatoria*). Mean abundance on day 1 of the experimental period, a) bacteria; b) algae; c) flagellates; d) ciliates; error bars indicate standard errors, (n=6). The Student`s *t*-test was used to established differences between absence and presence of rotifers for each prey organism group; significant differences are indicated by an asterisk (*).

To check whether or not experimentally demonstrated relationships between nematodes and rotifers and their potential food sources can be detected under field conditions, data from long term slides exposures were analyzed.

Experimentally demonstrated influences of nematodes on algae are also substantiated by the data from the long-term slide exposition (Fig. 14a, b). A significant correlation exists between the abundance of nematodes (exclusively chromadorids) and the abundance of algae

in biofilms on slides (Fig. 14a, b). The data of the long-term slide exposition do not allow the verification of the experimentally demonstrated relationship between heterotrophic flagellates and rotifers (Fig. 15a, b) by a significantly positive correlation (Fig. 15a, b).

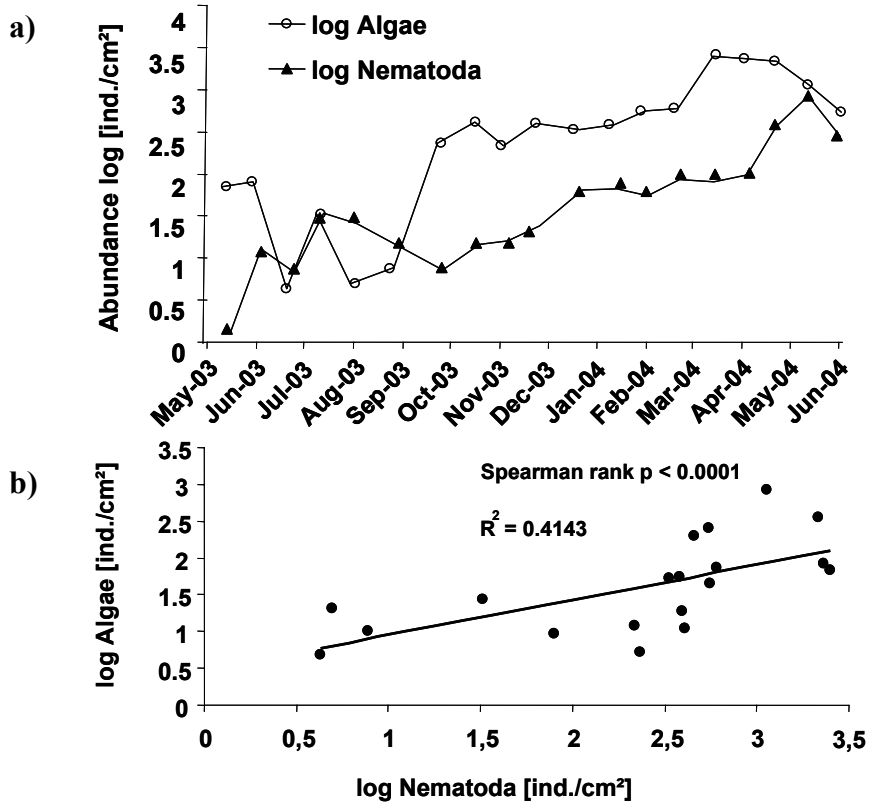

Figure 14 Relationship between algae and nematodes in biofilms on slides in the river Rhine a) correlation between log abundance of algae and log abundance of nematodes, p < 0.0001 (Spearman rank); b) regression of the correlation between log algae and nematodes.

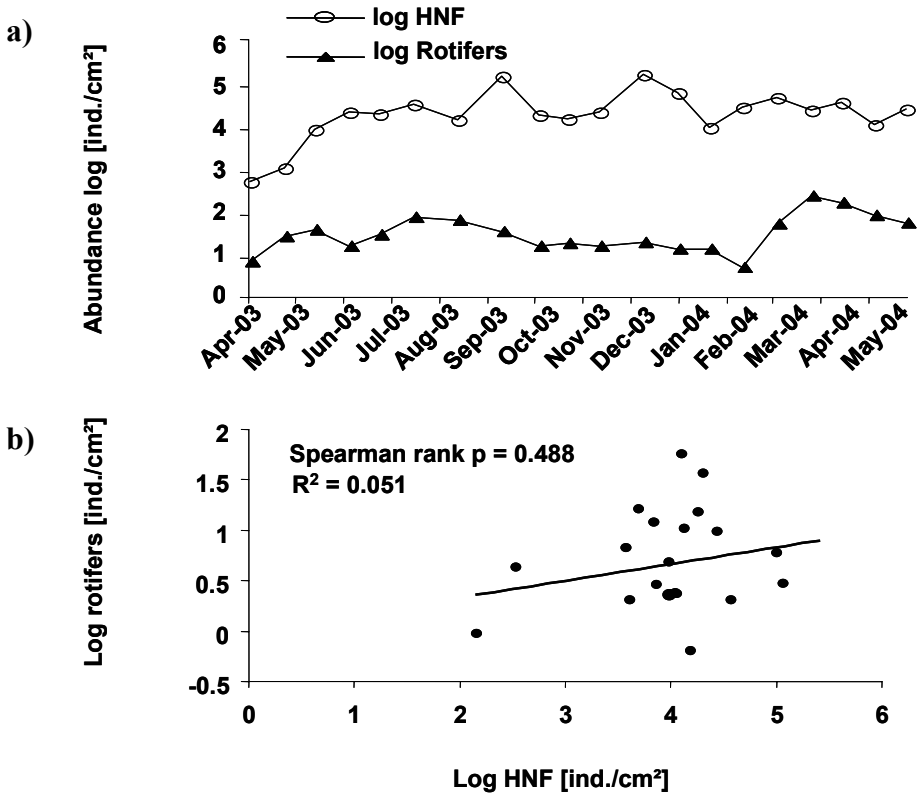

Figure 15 Relationship between flagellates and rotifers in biofilms on slides in the river Rhine a) correlation between log abundance of HNF and log abundance of rotifers, $p < 0.488$ (Spearman rank); b) regression of the correlation between log HNF and rotifers.

Discussion

Organism composition

To our knowledge, this is the first study on meiofauna as a part of the whole biofilm community of a river including pico-, nano-, micro-, meio- and macrobenthos. With respect to biomass, biofilms of the river Rhine were dominated by ciliates, followed by macrofauna. However, with respect to the number of individuals, meiofauna dominated metazoans. Over the total study period, meiofauna represented 98.8-99.9% of the individuals/cm². This is in agreement with earlier studies, which found that in most rivers meiofaunal organisms represent more than 95% of all metazoan organisms (Duft et al., 2002). Meiofauna was dominated by nematodes representing more than 75% of the individual count of meiofauna (86.4%) followed by rotifers 8.2% of individuals during the total study period. This is in accordance with earlier sediment studies (Vidakovic et al., 2001; Bergtold and Traunspurger, 2004). Nematodes regularly dominate the meiofaunal communities of lotic habitats (Anderson, 1992; Traunspurger, 2000, 2002; Reiss, 2002). Rotatoria represented the second most important component of meiobenthos in the river Rhine and were dominated by bdelloid Rotatoria. Schreiber et al. (1997) investigated the colonization of granular activated carbon filters in the river Rhine from May 1994 until August 1995, the dominating organism groups were rotifers and nematodes, also within the rotifers, representatives of Bdelloidea predominated.

The most frequent representatives of nematodes in our study of the river Rhine were Chromadoridae (*Punctodora bioculata* and *Chromadorina bioculata)*. Eisendle (2003) studied the free-living nematodes in biofilms of the river March (Weidlingbach, Austria). He found *Chromadorina bioculata* as the predominant species, also with a ratio of 55%. In a study of Jensen (1984) on the ecology of benthic and epiphytic nematodes in the Baltic Sea, *Chromadorina bioculata* and *Punctodora ratzeburgensis* were also identified as the

predominant species. In studies by Traunspurger (1992) of the littoral of the oligotrophic Königssee on hard substrates, *Chromadorina bioculata* was predominant at 2 m, 8 m and 20 m and represented a share of the relative abundance between 11.5 and 64.2 %. Traunspurger (2000) discusses generally that in more sheltered algal sites an increasing portion of epistrate feeders and selective deposit feeders can be detected. The experimental design of the long-term sampling in our studies corresponded to the low illumination at the bottom of the river Rhine; this might be a potential cause for the pronounced predominance of the Chromadoridae belonging to the epistrate feeders. In contrast to the results of the current study, which demonstrated that more than 90% of the nematodes were Chromadoridae, the nematode community in lotic freshwater is normally more diversified (Traunspurger, 2000; Reiss, 2002). Andrassy (1978) listed 117 genera, including 605 species of freshwater nematodes in a checklist of European inland waters**.** The major reason for the low diversity of nematodes at the lotic sampling site in Cologne seems to be the high flow velocity of the river Rhine at this site (>1 m/sec) together with the dominating hard substrate which supports the dominance of nematodes possessing a caudal gland, enable aquatic nematodes to attach themselves temporarily to the substratum. Traunspurger (2000) discusses that caudal glands are present in most Adenophorea (to which chromadorid nematodes belong to), their mucus secretions, extruded through a spinneret at the tip of the tail enable them to attach. Croll and Zullini (1972) write in their study that individuals of *Chromadorina bioculata* spend considerable periods attached to the substratum by caudal glands when not actively changing location.

The mean overall organism count of meiofauna (148 ind./cm²) found in the present study is within the range of earlier studies. Borchard and Bott (1995) counted 53 ind./cm² in White Clay Creek (USA), Palmer (1990) 214 ind./cm² in Goose Creek, Hummon et al (1978) 27 ind./cm² in Ohio streams, Beier and Traunspurger (2001) 120 ind./cm² in small German streams and Reiss (2002) 76 ind./cm² in the sediment of the Lower Rhine river.

Comparison of biofilms on slides and on the bottom of the Rhine

The comparison of the sampling methods used for the collection of samples from slides and from the river bottom revealed no significant differences, thus comparisons of the data obtained by both methods should be possible.

The comparison of meiofauna abundances on biofilms on the bottom of the river Rhine and on slides demonstrated that on slides significantly higher abundances could be detected throughout the whole year. A potential cause for the higher abundances on slides could be that the removal of stones from the bottom of the Rhine and the transport by scuba diving to the water surface, where the sampling with the biofilm sampler was performed, resulted probably in a pronouncedly stronger disturbance of the biofilm than the removal of slides from the carrier system. While the mean abundance on the stones from the bottom of the Rhine (6.1 nematodes/cm² on average) is within the data presented in earlier studies (e.g. Beier and Traunspurger (2003) found 4.7 nematodes/cm² in two little streams in Germany), the individual counts of nematodes on slides were pronouncedly higher (107 nematodes/cm² on average) than previously detected in field studies. The model biofilm on the slides has obviously strongly supported the chromadorid nematodes, a finding which is also supported by the species poorness of the nematode community.

Feeding experiments

Microbial biofilms in aquatic habitats are typically populated with high nematode densities (e.g. Höckelmann et al., 2004). A close spatial coupling between microphytobenthos patches and nematodes has been observed repeatedly (Blanchard, 1990; Pinckney and Sandulli, 1990; Moens et al., 1999a). The feeding experiment with *Punctodora ratzeburgensis* and *Chromadorina bioculata* in the present study demonstrated a significant impact on the abundances of diatoms and green algae in the microcosms. This is in agreement with earlier experiments, which demonstrated that species of the family Chromadoridae as epistrate

feeders correlated with the presence of algae (Croll and Zullini, 1972; Moens et al., 1999a). According to the structure of their buccal apparatus, chromadorids are considered predominantly algal feeders (Traunspurger, 2000). The estimated feeding rate per day in the current study was about 150 algae nematode^{-1} day^{-1}. The only published data on nematode algivory of a mixed nematode community are those by Admiraal et al. (1983), who found an average grazing rate of about 86 x 10^{-3} diatoms^{-1} day^{-1}. Their data are difficult to compare with the present study, since the nematodes were quite larger and included also large suction feeders. Algivory rates of nematodes estimated by Borchardt and Bott (1995) were negligible and most specimens appeared to be non-feeding, however, the size of the prevailing diatom assemblage in their study is not given. In the present study, a large percentage of diatoms and green algae was in the size range of 1-10 µm. The mean diameter of diatoms was 8.5 µm, that of green algae 1.5 µm, making them accessible to nematodes. The determined feeding rate of about 150 algae nematode^{-1}day^{-1} equals a food consumption of about 0.16 µg carbon nematode $^{-1}$ day $^{-1}$ which would support a growth rate of nematodes (assuming a growth efficiency of 25%) of about 1.2 day^{-1}. This rough estimate indicates that the obtained grazing rate is plausible.

The data of the long-term study of the biofilms on slides also demonstrated a significant positive relationship between algae and nematodes of the family Chromadoridae which supports the idea predominantly algivorous nutrition mentioned above. Hillebrand et al. (2000, 2001) also detected a positive correlation between algae and meiofaunal organisms. No significant difference in bacterial density could be observed between the nematode-containing chambers and control chambers in our studies. There was also no significant difference detected when rods and cocci or different size categories were considered in isolation. It was expected that nematodes would have a stimulating effect on bacterial abundance (Traunspurger et. al, 1997; de Mesel et al., 2004). Meiofauna is supposed to stimulate bacteria by transferring nutrients and oxygen into sediment through excretion and bioturbation

(Abrams and Mitchell, 1980; Alkemade et al., 1992; Aller and Aller, 1992). In addition, grazing by nematodes should have a stronger stimulatory influence on bacteria than bioturbation (Traunspurger et al., 1997). A potential cause of a non-detectable influence of nematodes on bacteria in our feeding experiments may have been that biofilms were relatively young and not very thick. A prolonged duration of this experiment might have resulted in the promotion of bacterial growth. Also, no significant effect on HNF and ciliates could be demonstrated by this experimental approach, although earlier studies suggested that the selectivity of nematodes is not so strongly pronounced but that they are opportunistic feeders, which may change feeding strategies in response to available food (Moens and Vincx, 1997).

In the feeding experiments with rotifers, it could be shown that rotifers of the family Philodinidae (*Rotaria rotatoria*) had significantly reduced the abundance of flagellates in the micro-flow chambers. The estimated feeding rate in the current study was about 1780 HNF rotifer^{-1} day^{-1}. The order of magnitude of the estimated feeding rate is in agreement with previously published data. In an earlier study of biofilm communities of the River Rhine (Schmidt-Denter, 1999), the feeding rate of rotifers was established in laboratory experiments involving fluorescently stained living flagellates. A feeding rate of 213 HNF rotifer^{-1} day $^{-1}$ was determined. It is very likely that the use of fluorescently stained flagellates causes an underestimate of the feeding rate. Arndt (1993) compiled feeding rates of a variety of rotifers ranging from 2.6 HNF rotifer^{-1} day^{-1} to 6126 HNF rotifer^{-1} day^{-1} with the majority of studies showing grazing rates in the range of values determined in the present study. The data on long-term sampling of the biofilms on slides, however, did not allow to establish a significant effect between rotifers and HNF. A possible explanation may be that in contrast to nematodes, rotifers were not present in a sufficient density on the biofilm on slides in the river Rhine throughout the complete study period. Their individual counts were only of temporary importance. Generally, HNF are typical components of the food size spectrum of rotifers (<1-20 µm, Pourriot, 1977). Arndt (1993) reviewed data on rotifer feeding activity demonstrating

that rotifers grazed protozoans in significant amounts. This indicates the ability of rotifers to structure protozoan communities. Holst et al. (1998) demonstrated that planktonic rotifers of the river Elbe grazed predominantly on heterotrophic components of the microbial food-web like heterotrophic flagellates. Starink et al. (1995) demonstrated in microcosm studies that the abundance of benthic HNF was strongly reduced in the presence of meiobenthos. However, these data refer only to findings based on mixed communities of cladocerans, copepods, ostracods, rotifers, nematodes and nauplii.

It has to be stressed that two groups of meiofaunal organisms which dominate the biofilms in the Rhine are specialized on very different food sources, nematodes (mainly family Chromadoridae) on the consumption of algae and rotifers (mainly family Philodinidae) on the consumption of protozoans .

Additional studies with labeled food organisms (tracers) would reveal more information on the preferred food of meiofaunal organisms. Another aspect requiring further research is the question, whether meiofauna organisms use microbial exopolymer secretions (EPS), which by themselves represent an easily assimilable organic food source for meiobenthic animals (Decho and Moriarty, 1990).

Food web

Food web descriptions in streams have lagged behind those for other freshwater habitats, and the issue of the role of small metazoans is generally unresolved (Schmid-Araya and Schmid, 2000) Integrating meiofauna into the concept of the benthic food web is complicated due to the fact that individual taxa cannot be considered a single trophic group, but can comprise representatives of various trophic levels (Reiss, 2002). One aim of the study was to estimate the role of the meiofauna components in lotic biofilm allowing a general idea how the potential flux of matter through the related to meiofauna benthic food web occurs.

Based on the mean biomass of the biofilm organisms, calculations of the potential production of the respective groups and their grazing impact were conducted. For this purpose, mean growth rates were assumed (for details see Weitere et al., 2005). The following growth rates were assumed: for algae 0.7 d^{-1} , for bacteria 1.05 d^{-1}, for HNF (2-5 µm) 2 d^{-1}, for larger HNF (5-10 µm) 1.2 d^{-1}, for ciliates in the 10-50 µm size range 0.6 d^{-1} , for medium-sized ciliates (50-250 µm) 0.3 d^{-1}, for large ciliates (≥250 µm) 0.1 d^{-1}, respectively. The growth rate of meiofauna organisms was assumed to be 0.3 d^{-1}, while for larger metazoans (macrofauna), the assumed rates were 0.1 d^{-1} (≤1000 µm), 0.05 d^{-1} (≥1000µm), 0.03 d^{-1} (5-10 mm) and 0.01 d^{-1} (≥10mm). The growth rates were based on measurements in the Rhine for HNF (Weitere and Arndt, 2002a,b) and on assumptions for the other groups according to de Ruyter van Steveninck et al. (1992) for bacteria, Schöl et al. (2002) for algae, Müller and Geller (1993) and Scherwass (2001) for ciliates, Stemberger and Gilbert (1985) and Stelzer (1998) for metazoans. The potential production was estimated involving the factors biomass and growth rate of the respective group for calculation. Potential consumption was calculated assuming growth efficiency values of 33% (Straile, 1997) (for details see Chapter 1).

From a quantitative point of view, the consumption rates of meiofauna were estimated to be more than 6 times the potential production of the flagellates. The potential consumption rate of the meiofauna on slides almost corresponds to the cumulated production of picobenthos, nanofauna and algae. It should be noted here that biofilms represent an open system interacting with the surrounding media. This suggests the conclusion that a substantial of food demand of meiofauna is due to food supply from the surrounding water.

On the other hand, meiofaunal organisms were subject to a tremendous predation pressure by the macrofauna. Rough estimates based on the above mentioned assumptions suggest that the complete potential production of the meiofauna would only cover about 25 percent of the food biomass requirements of the predatory macrofauna. This considerable grazing pressure may have induced structuring of the meiofauna, resulting in species poorness

of the meiofauna organism and only allowing the colonization of meiobenthos organisms capable of protecting themselves against grazing by embedding into the structures of the biofilm. The dominating meiofauna organisms were adapted to the ecotone biofilm. Due to their mobility, they can escape potential predators, while due to their caudal glands, chromadorid nematods can attach themselves temporarily to the substratum, thus reducing drift-related loss. On hard substrates, meiofauna and ciliates may escape grazing through their mobility within the biofilm (Hillebrand et al., 2002). Adult *Chromadorina bioculata* are capable of rapid movements, reaching a maximum of 6.5 undulations/sec at 20-22 °C; occasionally, adults were seen attached to the substrate or to algae by means of their four cephalic setae (Crool and Zullini, 1972). Chromadorid nematodes are rapidly growing species. *Chromadorina bioculata* has a generation time of 26-34 days (Pieczynska, 1964).
The impact of macrograzers on meiofauna has rarely been assessed in a small river (Bott, 1996).

Not only direct but also indirect effects

The present study established a relationship between the presence of *Corophium* tubes and the number of associated nematodes and rotifers. This finding confirms that structure has a decisive impact on colonization. In addition to food conditions, biofilms composed of filamentous microorganisms may offer structure and shelter for a variety of nematode species (Moens and Vincx, 1998). In a study of Hillebrand et al. (2002), it was reported that in contrast to the negative effects of the macrograzers on algal biomass, grazers had net positive effects on meiofauna. Grazers may enhance heterotrophic growth by increasing nutrient availability (Hillebrand et al., 2002). Earlier studies in lakes on microbenthic communities describe nutrient regeneration as an important effect of grazers in lenitic systems; however, due to the high flow rates, such effects should only play a minor role in lotic systems.

It appears that meiofauna structures microbial composition via selective grazing; due to its biological activities like grazing, excretion and movement, meiofauna has a major impact on microfauna and microalgae of biofilms. These direct effects are supposed to be complemented by indirect effects, by predation of predatory ciliates which indirectly might support HNF; moreover HNF are fed directly. The presented data strongly suggest a prominent role of meiofauna in the functioning of microbial biofilms of lotic river bottoms.

Literature

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Kapitel 3

Benthic Food Web Structure and Potential Carbon Flow in Biofilms of the River Rhine

Abstract

The relative biomass of biofilms in the river Rhine was analyzed with respect to the principal benthic groups (bacteria, algae, heterotrophic flagellates, ciliates, meiofauna, macrofauna) for a complete annual cycle; calculations on potential carbon flow within the biofilm food web were performed for four seasons. Ciliates dominated the biofilm biomass followed by macrozoobenthos. They also contributed the largest part of the benthic matter turnover within the benthic biofilm community, being in agreement with the predominance of the ciliates and their high growth rates. The share of protozoa of the potential production (µg C cm^{-2} d^{-1}) in biofilm is about 78%. The calculations strongly suggest that the protozoans of the biofilm consume the total bacterial and phytobenthos production throughout the year. Biofilm communities of bacteria, protozoa and microalgae bridge the gap between the microbial loop and the algal grazer pathway. In this way the use of planktonic organisms from pelagial is possible by the benthic subsystem. Only 2% of the total intercompartmental carbon flow has been autochthonously produced in the biofilm, the residual carbon requirement should be covered as an input from the pelagial. The import of planktonic organisms into the benthal microbial food web drives the mass flow in the investigated benthic riverine community. The study highlights the role of benthic predators as an important structuring agent for the planktonic food web structure.

Introduction

The assumption of the microbial food web by Azam et al. (1983) led to an increasing attention to food web interactions and the role of protozoans in both pelagic and benthic food webs (Alongi, 1991; Sherr and Sherr, 2000). The resulting intensive research demonstrated that in particular protozoans play a major role in food webs and therefore also in the metabolic turnover in aquatic systems (Pomeroy, 1974; Azam et al., 1983; Güde, 1989; Weisse et al., 1990). Being the principal consumers of bacterial production, productive protozoans play an important role among the plankton. They represent an essential component of the pelagic food web and thus are of pivotal importance in the degradation of organic matter in pelagic ecosystems. Several ciliates and flagellate species feed on algae and other protozoans and could therefore perform similar functions in the food web as the metazoans (Sanders, 1991; Sherr and Sherr, 1994; Arndt et al., 2000).

Weitere et al. (2005) examined the planktonic web structure in the lower Rhine River. In the pelagic zone, zooplankton was dominated by HNF, contributing more than 65% of the overall zooplankton biomass in all seasons. In accordance with the dominance of the HNF and its high growth rates, this group contributed the largest part of the planktonic matter turnover within the zooplankton. While basic information on organisms of the microbial food web is available for the pelagial (Sherr and Sherr, 1994), much less is known about the organisms colonizing the large surfaces of benthic systems. This particularly applies to nano-, micro- and meiofauna. However, the main metabolic processes of streams take place in microhabitats, especially in the boundary layers on stones and other flat habitats and in the interstitial of sediments (Schönborn, 1998; Stanford et al., 2005). The food web of river ecosystems is powered by microbial production (Ellis et al., 1998). The present paper focuses on biofilms on stones and exposed artificial substrates. As a major transfer of carbon in large river food webs, the flow between phytoplankton and planktonic and benthic consumers became a

relevant issue (Schöl et al, 2002). Coupling of benthic and pelagic food webs is currently not well understood (Hershey et al., 2005). Only few studies investigated the benthic zone, and they focused exclusively on bacteria, algae and ciliates and macrozoobenthos, in particular mussels (Riedel-Lorje, 1980; Foissner et al., 1992; Berger et al., 1997; Welker and Walz, 1998). Top-down control due to benthic-pelagic coupling can reduce planktonic biomass in rivers, where high water mixing rates enhance the exploitation of pelagic resources by benthic consumers. Several authors suggest a significant impact of benthic filter feeders (Alpine and Cloern, 1992; Köhler, 1995; Basu and Pick, 1997; Caraco et al., 1997; Welker and Walz, 1998; Schöl et al., 1999; Ietswaart et al., 1999). Recent studies demonstrate that turnover and decomposition of organic matter in running waters is only little controlled by makrozoobenthos (e.g. Monaghan et al., 2001; Weitere et al., 2005). This turnover is expected to be due to a large extent by biofilms growing in particular on the large surfaces of water bottoms and the adjacent interstitial biotope (Weitere et al., 2003).Protozoan participation in the formation and maintenance of freshwater biofilms has received little attention, even though it has been estimated that 99% of microbial activity is associated with surfaces (Parry, 2004). Biofilm communities involving bacteria, protozoa, microalgae, and fine detritus should play a decisive role in the transportation of matter in rivers (Pusch et al, 1998). They become accessible to larger consumers, bridging the gap between the microbial loop and the algal grazer pathway (Junk, 2005). Due to their impact on water quality, biofilm-dwelling protozoans are a matter of interest for water management (e.g. Sibille et al., 1998; Fried et al., 2000). Knowledge on the biofilm composition with respect to organism communities is poor. Currently, more or less only the presence of algae, bacteria and some ciliates in such biofilms is known. Only few studies (e.g. Railkin et al., 1990; Zolotarev, 1995; Widera, 1997) examined heterotrophic flagellates of the biofilm community, although it is likely that protozoa play an important role within freshwater biofilm communities (Hunt and Parry, 1997). However, the development of benthic submodels is impeded by the lack of adequate

quantitative information on benthic organisms and on local properties of benthic communities (Silvert, 1991; Weitere et al., 2003; Junk, 2005). The objective of the present study was to investigate the carbon flow during the biofilm communities of the river Rhine on slides directly exposed to the Rhine in a flow channel over a period of 14 months serving as a model substrate. The authors analyzed the benthic food web structure of one of the largest Central European rivers, the river Rhine. All components of the biofilm community were considered, i.e. algae, bacteria, heterotrophic flagellates, ciliates, meiofauna and macrofauna. The resulting data were used to identify the principal pathways of carbon flow in model biofilms in the river Rhine and to perform a coarse quantitative analysis. Taking the river Rhine as an example, it was intended to supplement the knowledge acquired on the mass flow in the pelagial (Weitere et al, 2005) by data on the benthos and to perform an initial integration of the data of both studies for a better understanding of mass flow in a large river.

Material and methods

Study site.

The investigation of the biofilm community was performed in the river Rhine at Cologne at the Ecological Rhine Station (University of Cologne), Rhine km 685. For details see Chapter 1. With a total length of about 1320 km and a catchment area of approximately 224.000 km², the river Rhine is one of the largest rivers in Europe. The mean discharge into the Lower Rhine is about 2000 m^3sec^{-1} at Cologne (Tittitzer and Krebs, 1996) at a mean flow velocity of about 1.5 m sec^{-1}. Near Cologne, the bed of the Rhine is strongly solidified, and it contains large stones forming a bottom typical for this part of the river. The stones are suitable for colonisation by periphyton. For details see chapter 1.

Sampling of the biofilm community

Biomass data on the biofilm organisms, i.e. bacteria, heterotrophic flagellates (HNF), ciliates and meio- and macrozoobenthos, were obtained in the river Rhine at the research platform of the Ecological Rhine Station in Cologne. The method allows to perform experiments within the original water flow. A model system involving biofilms colonizing glass sides was used to study the biofilm community in the river Rhine. Slides were placed in vertical position to obtain an optimum fouling colonization (Railkin et al., 1990). The slides were exposed in a permanent flow-through system at the Ecological Rhinstation at a depth of 10 cm for a period of fourteen months. Expositions were run from April 2003 to June 2004. Eight slides were randomly removed from the flow channel every three weeks and the biofilm community was investigated. (for details see chapter 1).

For the determination of **bacterial abundance** and size class distribution, the biofilm was scraped off from the slides and fixed in a 4% glutaraldehyde solution (final concentration 2%). 500 µl aliquots of the fixed samples were stained with DAPI (4'6-diamino-2-phenylindol, Porter and Feig, 1980), filtered to membrane filters (Nuclepore, 0.2 µm pore size) and counted with an epifluorescence microscope (Zeiss Axioskop, 1000x magnification). 200 bacteria per aliquot were counted and arranged by size classes. For the analysis of **heterotrophic flagellates**, the biofilm was scraped off from one side of the slides. The residual biofilm on the opposite side of the glass slide was investigated for species composition and abundance in the Ecological Rhine Station by direct live count (Arndt et. al., 2000) with a microscope (Zeiss Axiostar, 400x magnification, phase contrast, ocular micrometer, video recording) immediately after sampling. At least 30 flagellates were counted per slide (n=3). For the investigation of the **meiofauna,** biofilms were scraped off from each slide using a cover glass and transferred into a petri dish. A subsample of 50% of one slide side was resuspended in 5 ml filtered (0.2 µm) river water, transferred into a counting

chamber (Bogorov tray, Hydrobios, Kiel-Holtenau) and analyzed by live count under a binocular microscope (Olympus S Z X9, 12.6x-114x magnification) (n=3).

Data of **ciliates, algae** and **macrofauna** were taken from Ackermann (in preparation).In principle, sampling and counting of ciliates and algae followed the methods for heterotrophic flagellates. Macrofauna was collected from the whole glass slide holder.

Calculation of potential production and grazing.

Data on abundance and biovolume of the different taxonomic groups, including a detailed description of the methods, are presented in chapters 1 and 2.

Biovolumes of organisms were calculated by measuring the dimensions of living cells and an approximation to simple geometrical forms. Biomass given as carbon per cm² was calculated from the biovolume by using conversion factors of Simon and Azam (1989) for bacteria (55.97 fg C/bacterium), Børsheim and Bratbak (1987) for HNF (0.22 pg C/µm³) and Turley et al. (1986) for ciliates (0.11 pg C/µm³) and of Rocha and Duncan (1985) for phytobenthos (0.22 pg C/µm³]. For metazoans, a conversion factor of 0.0077 pg C μm^{-3} was used, based on the assumptions that the dry weight is about 17% of the fresh weight and that the carbon content is about 45% of the dry weight (see the review of Heerkloss, 1996). A chlorophyll a to carbin ratio of 1:25 was assumed for calculating the biomass of the phytoplankton (Riemann et al., 1982).

Calculations of the potential production of the respective groups and their grazing impact were based on the mean biomass of the biofilm organisms. For this purpose, mean growth rates were assumed for the respective seasons (Table 1) (for details see Weitere et al., 2005).This table is based on growth rate measurements and assumptions. For HNF, gross growth rates based on biomass were taken from size fractionation experiments (Weitere and Arndt, 2002a,b). The individual growth rates of ciliates were calculated using the regression established by Müller and Geller (1993). Mean growth rates of ciliates were calculated from

the individual growth rates. The maximum growth rates were divided by two for winter, summer and autumn, since we found low ciliate growth rates in the Rhine, which could be enhanced by approximately two times when algae were added (Scherwass et al., 2001). High growth rates close to the maximum growth rates were only measured in spring when algal abundances were high. Bacterial growth rates were assumed as measured in the river Rhine in 1990 by de Ruyter van Stevenick et al. (1992), i.e 1.05 d^{-1} in spring, 1.0 d^{-1} in summer and 0.8 d^{-1} in autumn. A bacterial growth rate of 0.6 d^{-1} was assumed for winter. Assumptions for meiofauna growth rates were made by taking temperature and food concentrations into account (cf. Stemberger and Gilbert, 1985; Stelzer, 1998). Low growth rates of 0.1 d^{-1} were assumed for winter and autumn. For spring and summer, we assumed growth rates of 0.3 d^{-1} and 0.5 d^{-1} (for details see Weitere et al., 2005). The growth rate of the larger metazoans was assumed to be half the meiofauna growth rate (cf. Gillooly, 2000). Algal growth rates were assumed to be 0.1 d^{-1} in winter, 0.5 d^{-1} in spring, 0.7 d^{-1} in summer and 0.5 d^{-1} in autumn d^{-1} (cf. Schöl et al., 2002).

Potential production was estimated based on the factors biomass and growth rates of the respective groups for calculation. Taking into account growth rates (r) and biomasses (B) of the respective groups, potential production (PP) values were estimated for each group (Figure 1) as follows:

$$PP = B \times r \; [\mu gC d^{-1}]$$

Potential consumption (PC) values by protozoans and metazoans were calculated involving gross growth efficiency (GGE) values of 33% for protozoans and 25% for metazoans according to Straile (1997) according to the formula:

$$PC = PP \times 100 / GGE \; [\mu gC d^{-1}]$$

Individual consumption values of food size classes were attributed to each individual HNF and ciliate species according to their feeding preferences (compare Scherwass, 2001; Weitere and Arndt, 2002b). Meiozoobenthos was classified based on the results of the feeding

experiment (chapter 2); chromadorid nematodes fed selectively on algae, while rotifers preferentially fed on HNF. Carnivorous metazoans were assumed to feed on large protozoans (>20µm) and small metazoans. The other metazoans were assumed to feed non-selectively on protozoans, bacteria and algae. It was assumed that the food spectrum consumed by non-carnivoraous metazoans reflected the amount of available food.

Based on individual PC values and feeding preferences, the amount of consumed particles in the respective food size class was estimated.

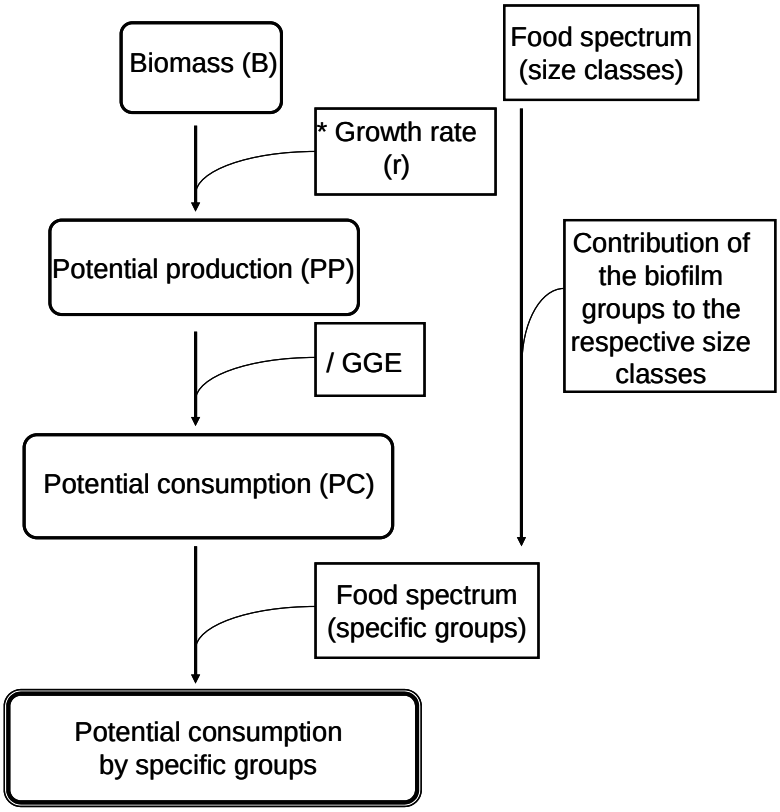

Figure 1 Flow chart explaining the calculation of the potential consumption of a zoobenthos group based on biomass and growth rate of the respective group as well as on assumptions on the food spectrum according to size class. See the Methods section for further details.

Abiotic parameters

Immediately after sample collection, temperature, pH-value and conductivity were measured with multi-probes (WTW, Germany, and Yellow Spring Instrument Inc.), chlorophyll content was measured using in situ fluorescence with an Aquafluor (Turner Designs, USA). Data on water discharge (daily means at Cologne) were provided from routine measurements of the "Bundesschifffahrtsamt" (Duisburg, Germany).

Flow velocities were measured at the sampling site between the glass slides and also in front of the research platform with a hydrometric propeller (WTW). The measurement showed that the flow velocity in the box in front of the slide inserts was reduced to about 30% of the original flow velocity, and to about 40% between the inserts. The availability of light in the flow channel was determined with a LI-250A (Li-Cor) light meter to determine the photosynthetically active radiation (PAR). The value determined in the box containing the slides resembled the values determined in measurements directly above ground (4 m depth) and was reduced to about 2 % of the surface light intensity. The rationale of this experimental design was to simulate the conditions near the ground of the river.

Results

Abiotic parameters

The water temperature of the river Rhine ranged from 5.5 °C in winter to 27 °C in summer during the investigation period (Fig. 2). The water level ranged from 1.32 m in October 2003 to 3.18 m in May 2004.

Biomass contribution

Ciliates constituted the largest portion of the biofilm biomass (2.6 µg C cm^{-2} to 576 µg C cm^{-2}), with highest values in summer 2004 (Fig. 2). Macrofauna biomass ranged from 1.4 to 165 µg C cm^{-2} with highest values in summer 2004 (Fig. 2). Meiofauna biomass ranged from 0.1 to 31 µg C cm^{-2}, reaching its maximum in summer 2004 (Fig. 2). Bacterial biomass ranged from 0.2 to 11 µg C cm^{-2}; values were highest in winter 2003 (Fig. 2). The major part of the bacterial biomass was contributed by bacteria smaller than 0.2 µm. HNF biomass ranged from 0.001 to 5 µg cm^{-2}, with highest values in spring 2003 (Fig. 2). The biomass distribution over the year showed a seasonal pattern with high values in spring and autumn (Fig. 3). Most of the HNF biomass was composed of benthic taxa, such as members of ancyromonads, choanoflagellates, bicosoecids and bodonids (Fig. 3). The highest values of ciliate biomass were detected in spring. Over the investigation period, most of ciliate biomass was composed of benthic taxa, for example members of the peritrich ciliates (Fig. 4). The highest amounts of meiofauna biomass were detected in spring (Fig. 5). Meiofauna composition was dominated by benthic taxa, like bdelloid rotifers and chromadorid nematodes. The highest values of macrofauna were detected in spring and summer 2003 (Fig. 6). A significant part of the biomass during the study period was contributed by amphipods. In spring and summer 2003, we found a high contribution by *Ancylus fluviatilis* (Fig. 6).

During the investigation period, the dominance of the food spectrum altered between planktivorous and benthivorous organisms in terms of HNF. Most of the ciliate biomass was due to planktivorous ciliates, while most of the meio-and macrofauna were benthivorous (Fig. 7).

Four seasons were characterized according to the typical biomass dynamics of the different benthic predator groups in biofilm: in spring (20 April –02 June 2004), the mean water temperature was 15°C, the mean water level was 315 cm and the average phytoplankton

biomass in the water column was 1210 µg C l^{-1}; high ciliate densities and average densities of HNF, meio- and macrozoobenthos were recorded. In Summer (02 July – 12 August 2003), a mean water temperature of 25 °C was measured, the water level was 169 cm, and the average phytoplankton biomass in the water column was 217 µg C l^{-1}; densities of HNF, ciliates, meio- and macrozoobenthos were rather low. In autumn (22 September – 21 December 2004), the mean water temperature was 14°C, the mean water level was 156 cm and the average phytoplankton biomass in the water column was 143 µg C l^{-1}; high HNF densities were found, while densities of ciliates, meio- and macrozoobenthos were average. In winter (03 January – 28 February 2004),), the mean water temperature was 6°C, the mean water level was 298 cm, and the average phytoplankton biomass in the water column was 105 µg C l^{-1}; HNF densities were average, while ciliate densities were low and meio- and macrozoobenthos densities were high.

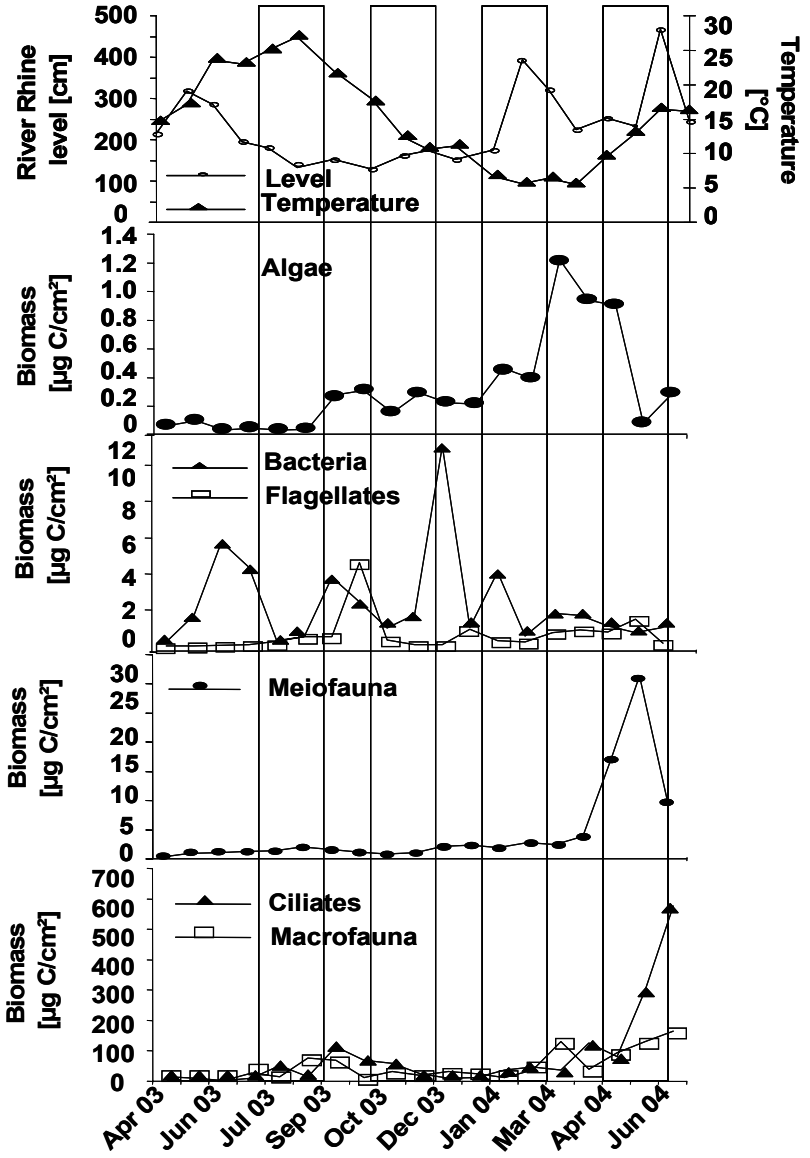

Figure 2 Seasonal changes in the abiotic parameters river Rhine level and temperature as well as the biomasses of the algae, bacteria, HNF, ciliates, meiofauna and macrofauna. The time frames in which the data were averaged for the carbon flow calculations were marked.

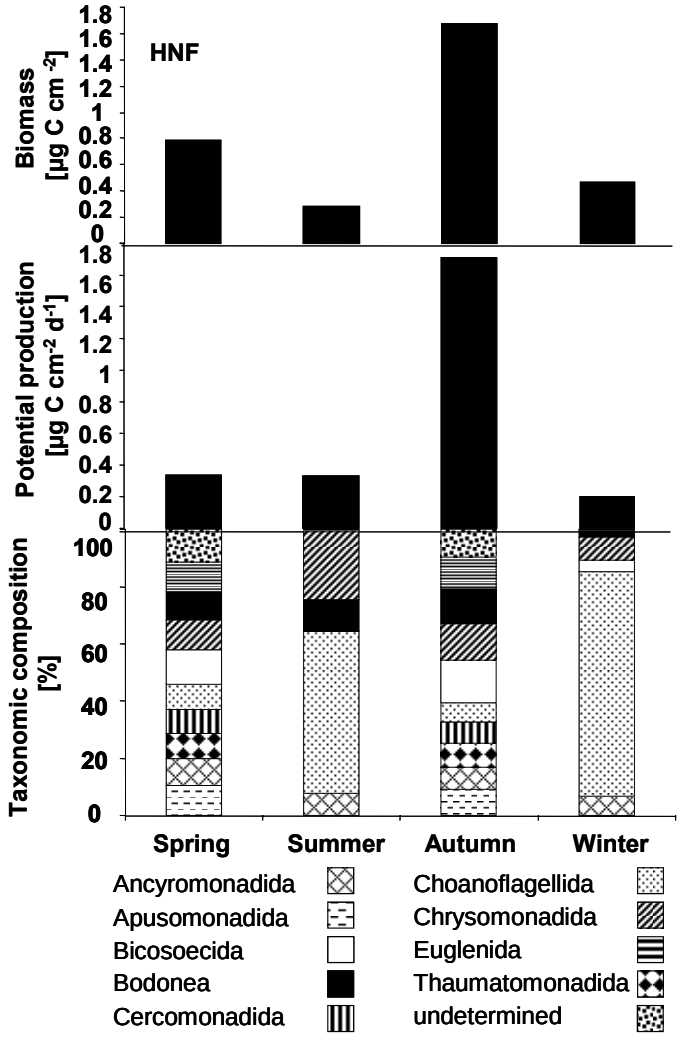

Figure 3 Seasonal means of biomass, potential production and taxonomic composition of HNF.

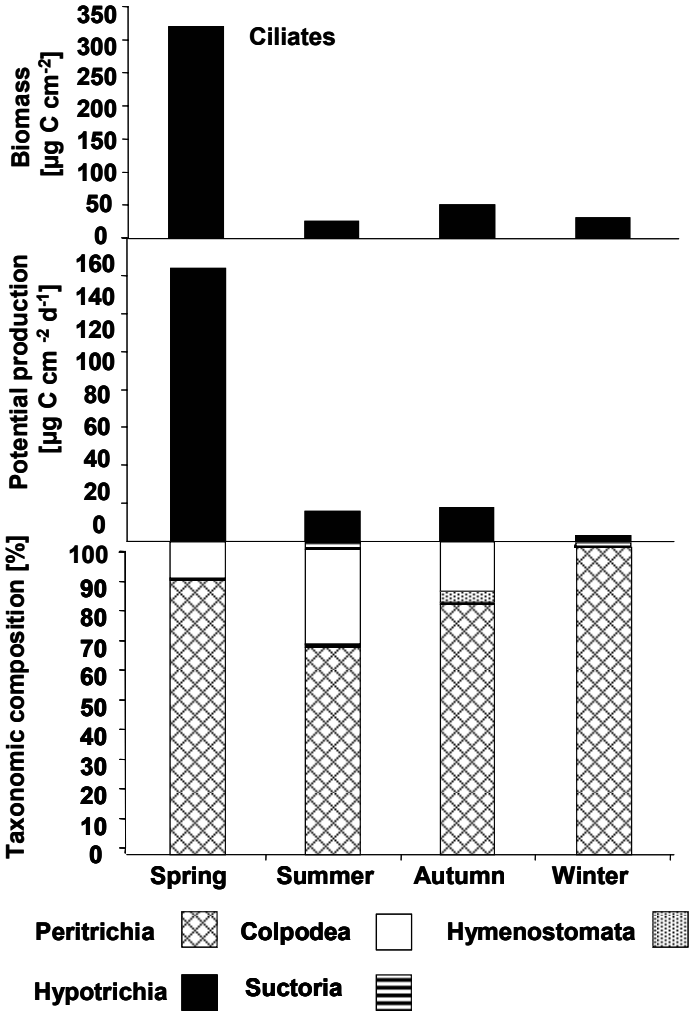

Figure 4 Seasonal means of biomass, potential production and taxonomic composition of ciliates.

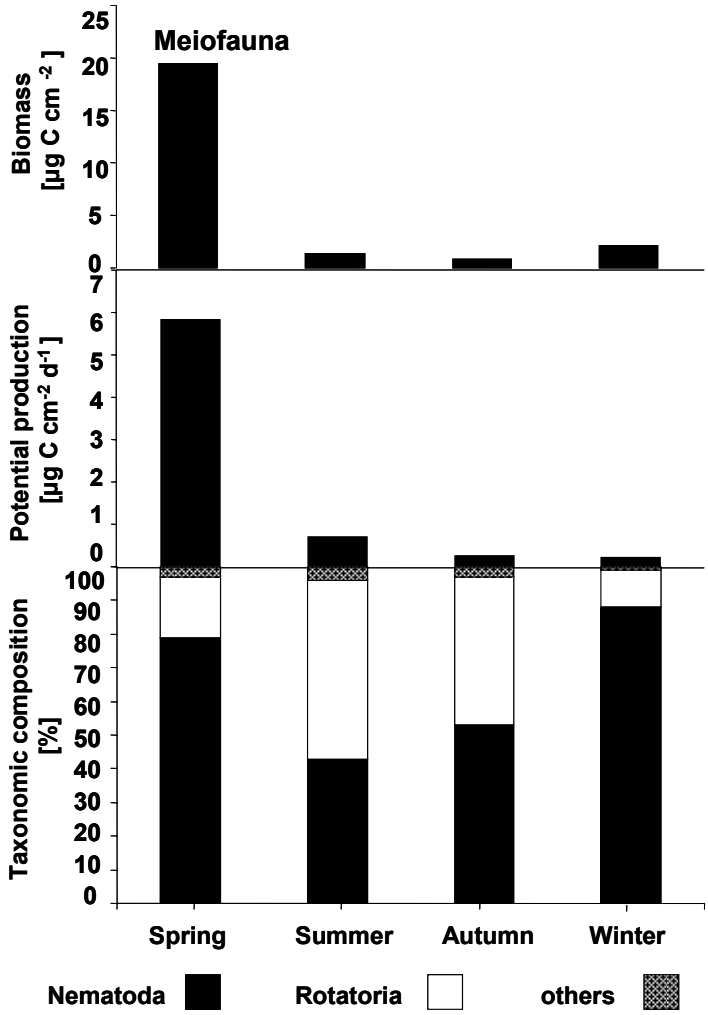

Figure 5 Seasonal means of biomass, potential production and taxonomic composition of meiofauna.

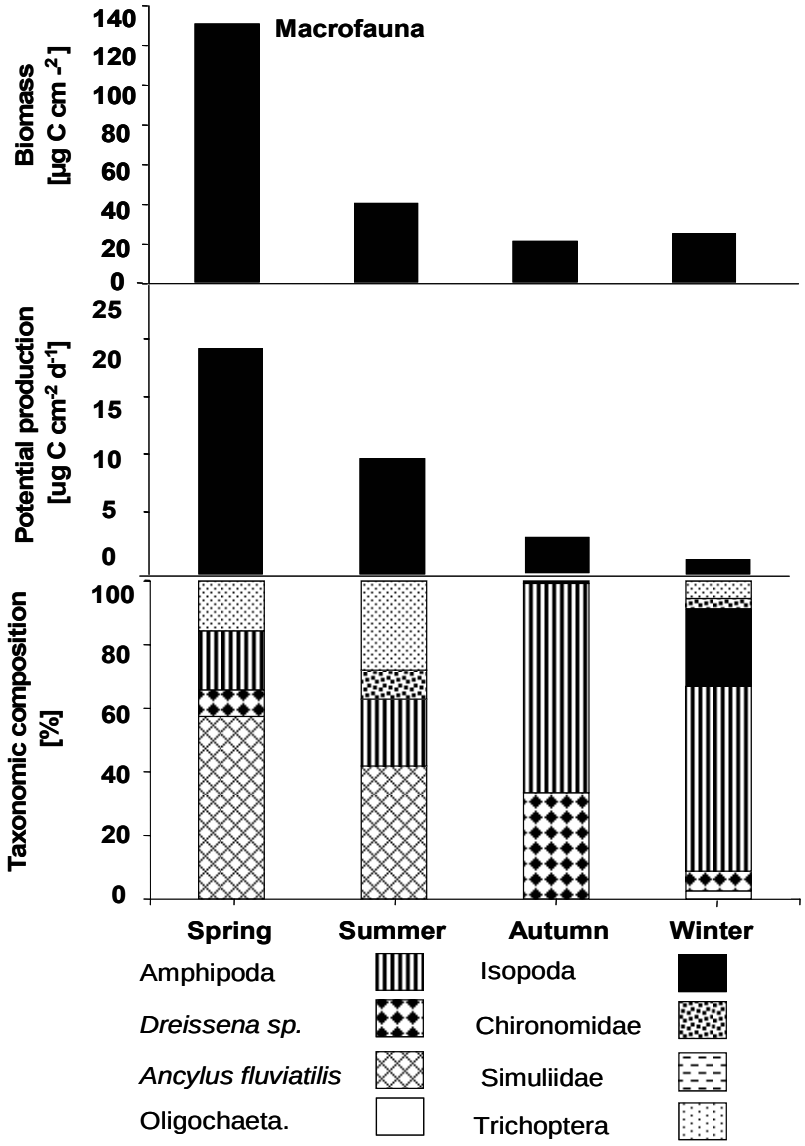

Figure 6 Seasonal means of biomass, potential production and taxonomic composition of macrofauna.

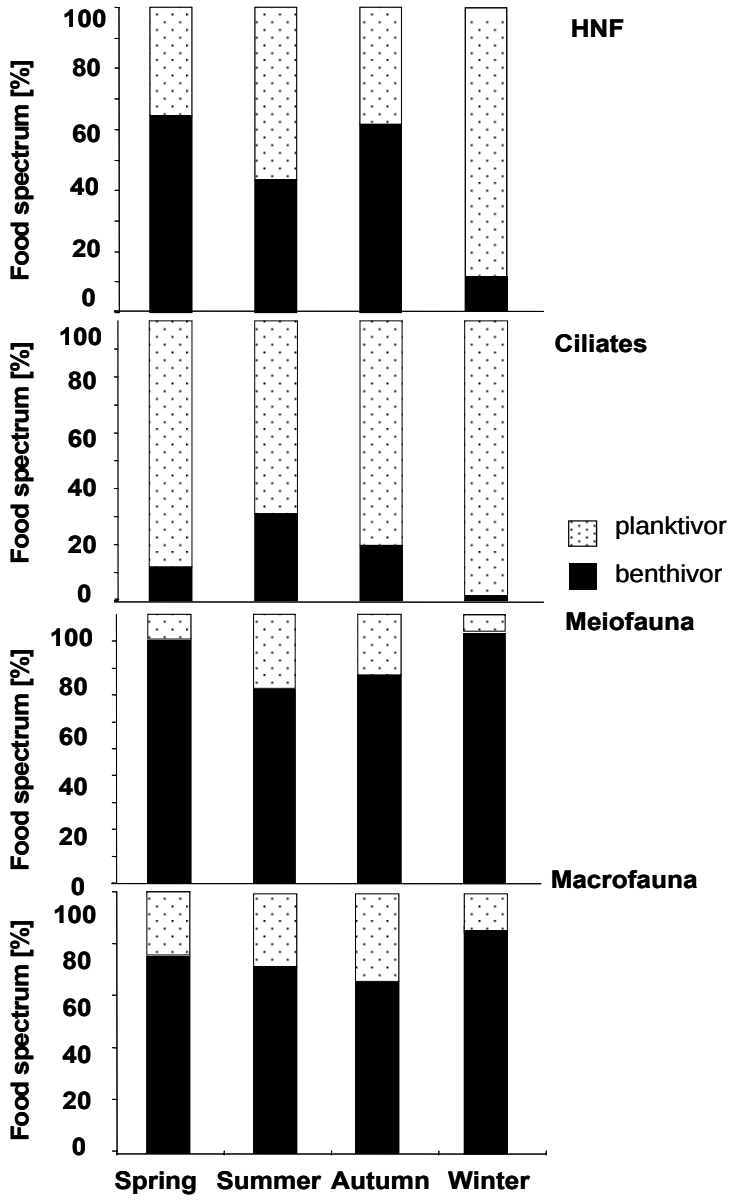

Figure 7 Seasonal food spectra of HNF, ciliates, meiofauna and macrofauna.

Potential carbon flow during spring (Figure 8)

According to our estimates, we found the highest potential production of algae during the investigation period (0.2 µg C cm^{-2} d^{-1}) in spring. Bacterial potential production was estimated to 3 µg C cm^{-2} d^{-1} and therefore assessed average. The highest potential production among the biofilm groups was found for ciliates (144 µg C cm^{-2} d^{-1}), followed by macrozoobenthos (19 µg C cm^{-2} d^{-1}) and meiozoobenthos (5.8 µg C cm^{-2} d^{-1}). Heterotrophic flagellates demonstrated the lowest protozoan values (1 µg C cm^{-2} d^{-1}). The calculations

indicated that the complete phytobenthos and bacterioplankton production covers less than 1% of the potential consumption by predators within the biofilm. The calculated production of heterotrophic flagellates covers less than 2% of the calculated potential consumption of specific predators within the biofilm (ciliates, meio- and macrozoobenthos). According to the estimates, the ciliates were the most dominant consumers of algae, bacteria and HNF. Metazoans represented the other dominant consumers within the biofilm.

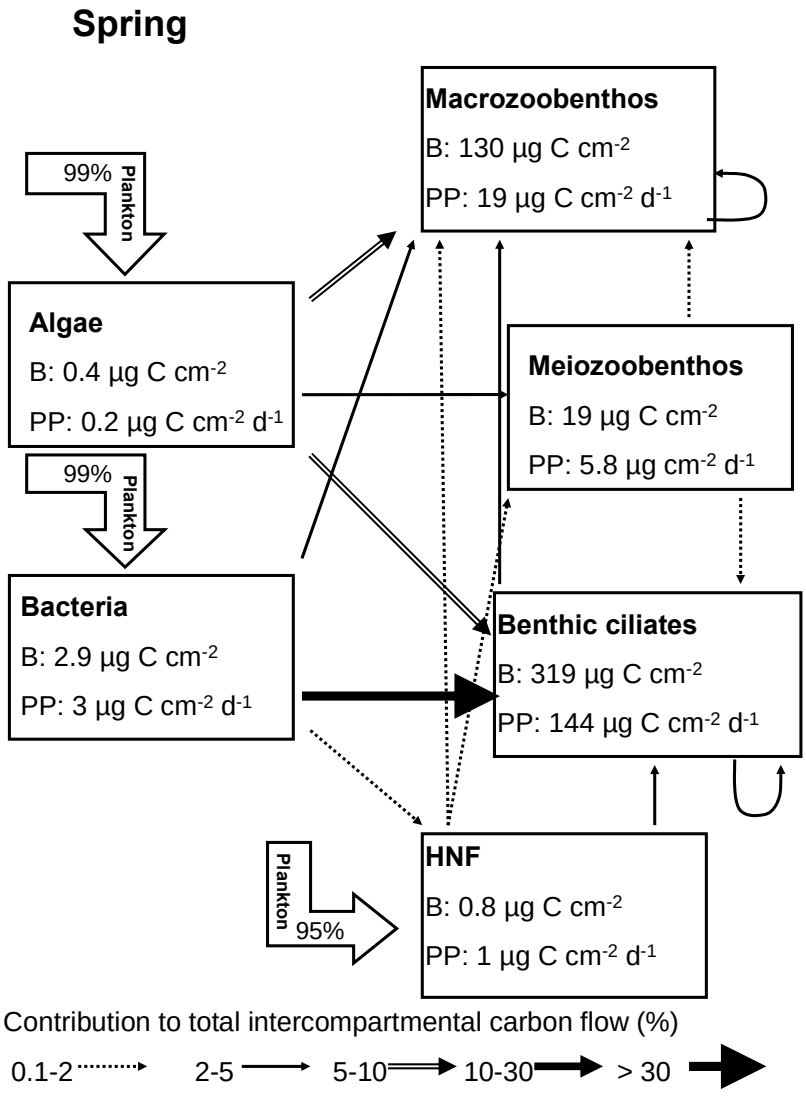

Figure 8 Carbon flow chart for spring (20 April to 2 June). The arrows indicate the relative contribution of the individual flow to the total intercompartmental carbon flow (534 µg C cm-²d-1 for spring) within the biofilm food web. Biomass (B) and potential production (PP) of the biofilm groups are indicated in the respective boxes.

Potential carbon flow during summer (Figure 9)

During summer, the calculated production by algae was much lower than in spring (0.01 µg C cm^{-2} d^{-1}). Bacterial potential production was slightly higher than in spring (5.3 µg C cm^{-2} d^{-1}). Similar to spring, however on a lower level, the highest potential production among the biofilm groups was found for ciliates (16 µg C cm^{-2} d^{-1}), followed by macrozoobenthos (10 µg C cm^{-2} d^{-1}) and meiozoobenthos (0.7 µg C cm^{-2} d^{-1}). Heterotrophic flagellates demonstrated the lowest values (0.4 µg C cm^{-2} d^{-1}) among protozoans. The calculations indicate that the overall phytobenthos and bacterioplankton production covered less than 6% of the potential consumption of predators within the biofilm. The calculated production of heterotrophic flagellates within the biofilm represented less than 6% of the calculated potential consumption of specific predators (ciliates, meio- and macrozoobenthos). According to the estimates, similar to spring, ciliates are the most dominant consumers of bacteria, algae and HNF also in summer.

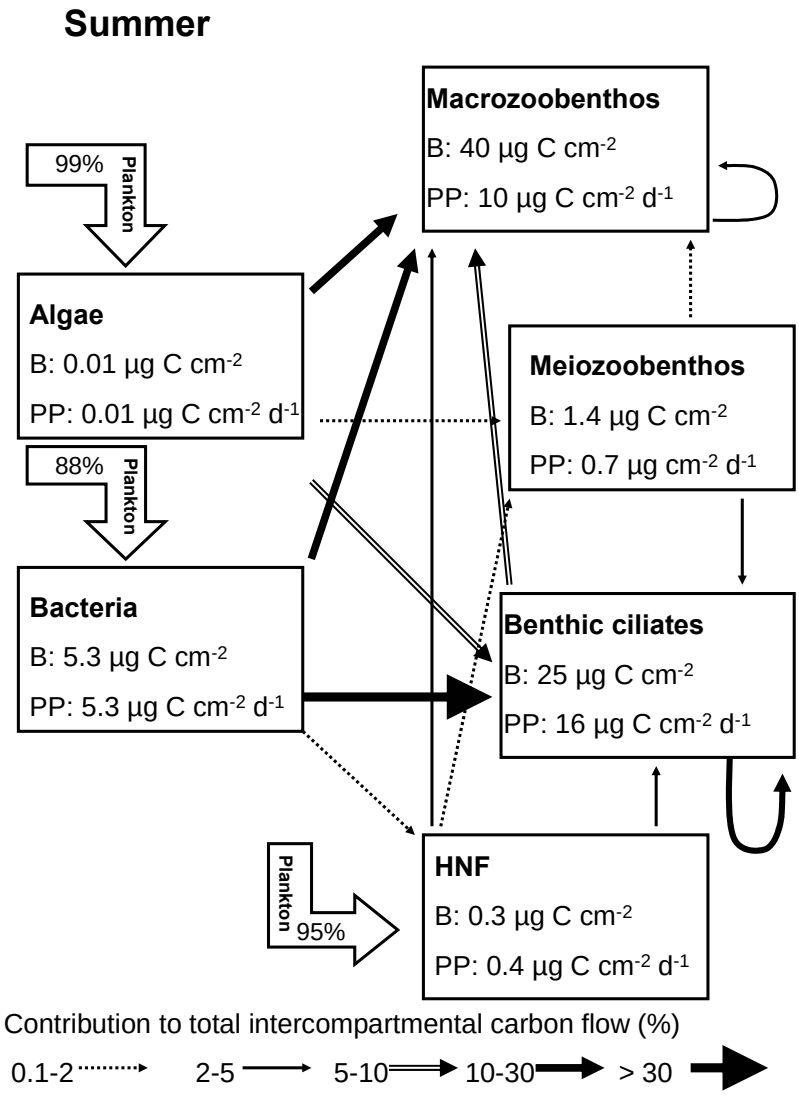

Figure 9 Carbon flow chart for summer (2 July to 12 August). The arrows indicate the relative contribution of the individual flow to the total intercompartmental carbon flow (91 µg C cm-²d-1 for summer) within the biofilm food web. Biomass (B) and potential production (PP) of the biofilm groups are indicated in the respective boxes.

Potential carbon flow during autumn (Figure 10)

During autumn, the calculated production by algae was 0.1 µg C cm^{-2} d^{-1}. The bacterial potential production was slightly lower than in summer (4.8 µg C cm^{-2} d^{-1}). Similar to spring and summer, the highest potential production among the biofilm groups was found for ciliates (18 µg C cm^{-2} d^{-1}), followed by macrozoobenthos (3 µg C cm^{-2} d^{-1}). In contrast to the situation in spring and summer, the potential production of HNF (1.7 µg C cm^{-2} d^{-1}) was higher than the production of the meiofauna (0.3 µg C cm^{-2} d^{-1}). The calculations indicated that the complete phytobenthos and bacterioplankton production covered less than 7% of the potential consumption of predators within the biofilm. The calculated production of the heterotrophic flagellates within the biofilm in autumn represented almost 70% of the calculated potential consumption of specific predators (i.e. ciliates, meio- and macrozoobenthos). According to the estimates, ciliates were the most dominant consumers of bacteria, algae and HNF. Metazoans were the second dominant consumers within the biofilm.

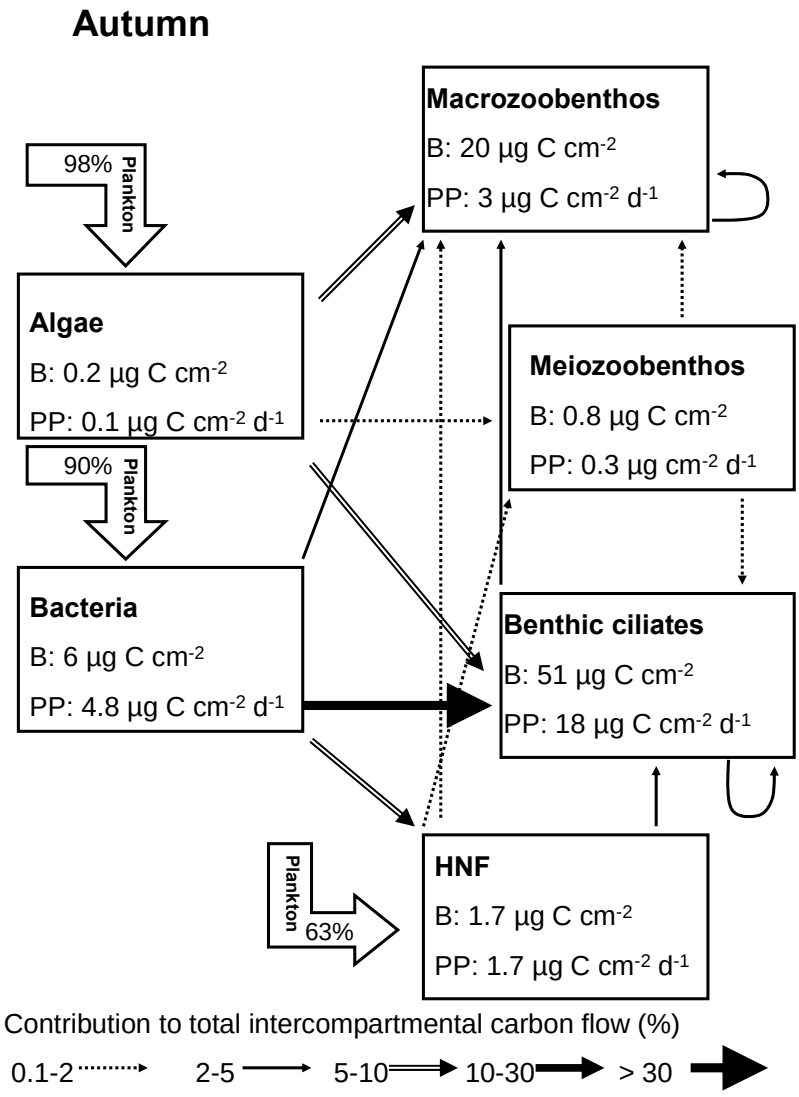

Figure 10 Carbon flow chart for autumn (22 September to 21 December). The arrows indicate the relative contribution of the individual flow to the total intercompartmental carbon flow (71 µg C cm-²d-1 for autumn) within the biofilm food web. Biomass (B) and potential production (PP) of the biofilm groups are indicated in the respective boxes.

Potential carbon flow during winter (Figure 11)

During winter, the calculated production by algae was similarly low as in summer (0.03 µg C cm^{-2} d^{-1}). The bacterial potential production was lower than in spring, summer and autumn (2.7 µg C cm^{-2} d^{-1}). Similar to spring, summer and autumn, the highest potential production among the biofilm groups was found for ciliates (3.1 µg C cm^{-2} d^{-1}), followed by macrozoobenthos (1.2 µg C cm^{-2} d^{-1}). The potential production of HNF (0.2 g C cm^{-2} d^{-1}) and the potential production of meiofauna (0.2) were in a similar order of magnitude. The calculations indicated that the overall phytobenthos and bacterioplankton production covered less than 17% of the potential consumption of predators within the biofilm. The calculated production of heterotrophic flagellates within the biofilm in winter covered almost 100% of the calculated potential consumption of specific predators (ciliates, meio- and macrozoobenthos). According to the estimates, ciliates were the most dominant consumers of bacteria. Macrozoans were the most dominant consumers of algae, and meiofauna organisms were the most dominant consumers of HNF within the biofilm.

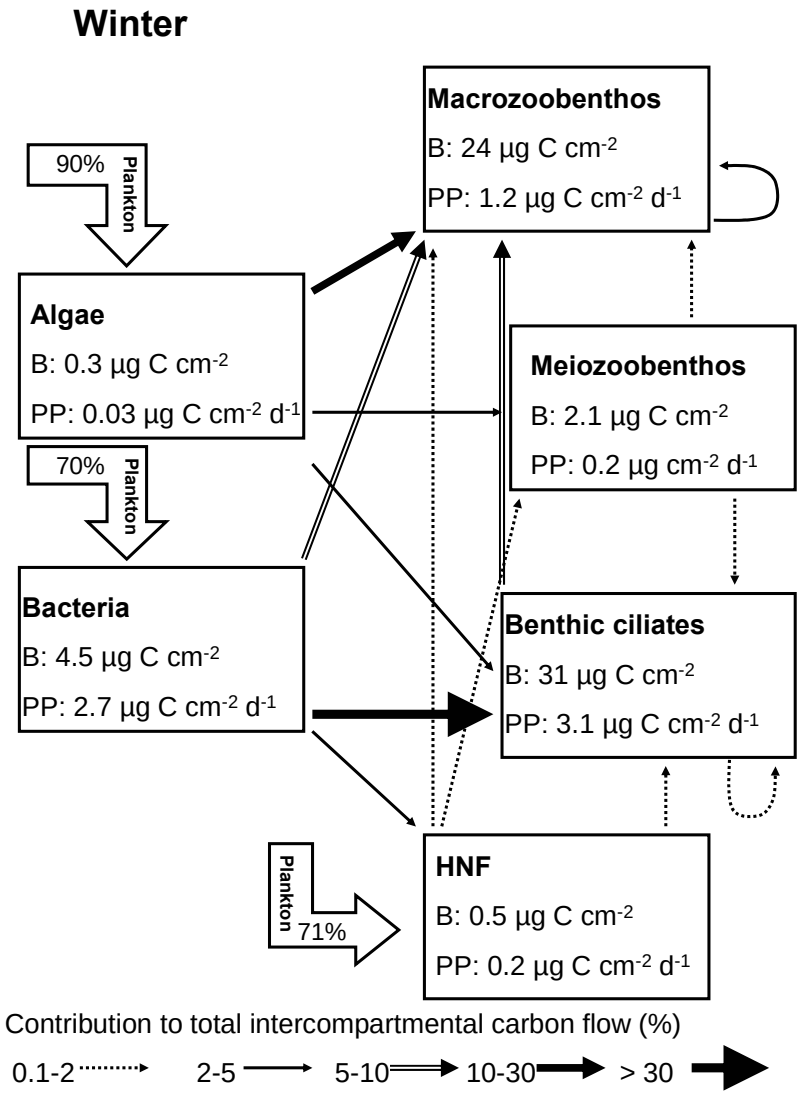

Figure 11 Carbon flow chart for winter (3 January to 18 February). The arrows indicate the relative contribution of the individual flow to the total intercompartmental carbon flow (16 µg C cm-²d-1 for autumn) within the biofilm food web. Biomass (B) and potential production (PP) of the biofilm groups are indicated in the respective boxes.

Discussion

The principal aim of the study was to compare the role of all major groups of organisms within the biofilm community of a river, a topic seldomly addressed in studies of river food webs. Our studies were stimulated by earlier studies of the planktonic food web of the Rhine indicating a significant loss of organisms to the benthic compartment (Weitere et al., 2005) which could not be fully explained by the filtration activity of benthic macrofauna (Weitere et al., 2003). The connection between microbial food web functioning and the classical studies of macrofauna activity has seldomly been carried out. Generally, such analyses were restricted to parts of the food web, e.g. meiofaunal grazing on microbes (Borchardt and Bott, 1995), bacteria in microbial biofilms (Battin et. al, 2003).

Methodological considerations

Biofilms are perceived hot spots for biotic interactions, genetic exchange and the biogeochemical cycling of elements (Krumbein et al., 2003; Parry, 2004). Only minor autochtonous production of the primary producers could be established in the model biofilm. This is in agreement with the general situation found in large parts of the benthic community in the river Rhine. As mentioned above, the mean value determined in the box containing the slides was 4.18 µmol/ s^{-1} /m^{-2} (n=5), meaning a reduction to about 2% of the surface light intensity, being in agreement with values determined directly above ground (4 m depth). The rationale of this experimental design was to simulate the conditions near the ground of the river. This approach severely limited autotrophous production; the annual mean of the contribution of algae carbon to total biofilm carbon production was only 0.2%. A mean annual production of 0.09 µg C cm^{-2} d^{-1} of algae contrasted with a demand of algivorous consumers of about 31 µg c cm^{-2} d^{-1}. Algal growth rate in the river Rhine is mainly controlled by available light (Ieetswaart et a., 1999). In a study of Schöl et al. (2002), the seasonal mean

depth of the productive layer was determined to be 2.1 m compared to a mean water depth of 3.6 m at km 550. The principal mass of microbial biofilms in the river Rhine occurred below the compensation layer. Several studies of epilithic biofilms concluded that algal exudates are a major carbon source for bacteria (Geesey et al., 1978; Haack and McFeters, 1982; Kaplan and Bott, 1989). This explains, why the potential production of bacteria in the biofilms in our study contributed only about 2% to the overall carbon flow in the biofilm.

Comparisons between biofilms on glass slides investigated in this study and biofilms on the natural substrate on stones from the river bottom demonstrated similar community structures (see Chapter 1). This is in agreement with comparative studies published in literature (Foissner et al., 1992; Schönborn, 1998; Schmidt-Denter, 1999; Weitere et al., 2003). Slides were considered a suitable model system, because they can be conveniently handled and allow sampling of all biofilm components, a prerequisite for the present study. For a long time, artificial substrates such as glass slides have successfully been used to monitor the water quality of rivers and to study benthic microbial food webs (e.g. Wilbert, 1969; Cairns et al., 1974; Stössel, 1979).

Abundances and biomasses of the different groups of biofilms organisms are in the range of values published by other authors for riverine biofilms communities (see Chapters 1 and 2 for literature and discussion). The loss of the delicate protozoans during the sampling procedure could be minimized, since samples were investigated directly after slide removal from the flow channel in the Ecological Rhine Station. The same should apply to the other constituents of the biofilm community (algae and metazoans). The determination of bacterial abundance by counting DAPI-stained samples under the epifluorescence microscope might have resulted in an underestimate of bacterial abundance due to the necessity to apply ultrasonification. Therefore, the three-dimensional structure of bacterial communities was studied partly in parallel by confocal laser-scanning microscopy (Budde, 2005). The results

point to the existence of classical morphologies of bacteria (Costerton et al., 1995) including holes, mushroom like structures etc. (Budde, 2005).

Estimated major matter flow through the biofilm components

Bacteria.

The present study demonstrated that bacteria on biofilms are subject to a high grazing pressure. The mean annual bacterial production of 3.98 µg C cm^{-2} d^{-1} contrasted with a demand of the predators of 117 µg c cm^{-2} d^{-1}. The highest grazing pressure originates from protozoans. Heterotrophic nanoflagellates accounted only for about 2% of the grazing pressure, ciliates for about 90 %.

Heterotrophic nanoflagellates (HNF).

About 99% of the HNF detected in the present study in biofilm belonged to bacterivorous forms. This is in agreement with earlier studies (Schmidt-Denter, 1999) who detected a correlation between bacteria and HNF biomass and postulated that HNF in biofilms of the Rhine are bottom-up controlled. A bacteria:HNF ratio of about 220:1 was found in streambed sediments (Bott and Kaplan, 1989). In addition, Bott and Kaplan (1990) calculated that a significant amount of the annual streambed bacteria production was consumed by HNF (52-119%). The mean bacteria:HNF ratio in the present biofilm study was 4324:1; 28% of the potential bacterial production of the biofilm may have been consumed by benthic HNF (see also Chapter 1).

The relationship between ciliate and flagellate biomass in the present study was 6.2:1 indicating top down control of the flagellates by ciliates. The potential consumption of nanophagous ciliates is about 10 times the potential production of HNF. Even if the growth rates of the HNF would be higher than the assumed conservative figures, the HNF would be exposed to an enormous grazing pressure which may have resulted in a specific species

spectrum in biofilm characterized by dominance of smaller HNF with high growth rates. Other predators of HNF are macro- and meiofauna. The mean annual HNF production of 1.94 µg C cm^{-2} d^{-1} contrasted with a total demand of predators of 7.35 µg c cm^{-2} d^{-1}.

Ciliates.

In the pelagic zone, flagellates have an advantage compared to ciliates due to their higher growth rates, resulting in an approximate biomass ratio of 9:1 in the river Rhine (Weitere et al., 2005). In biofilms, ciliates are not exposed to the high grazing pressure of filter feeders (especially mussels; see Weitere et al., 2003) and therefore can build up higher population densities. The relationships are even more drastic if one considers the relationship of abundances. In the pelagic zone, a ratio of 1860:1 was established between HNF and ciliates abundance (Weitere et al., 2005), while the ratio was 4.7:1 on biofilms (Schmidt-Denter, 1999) in 1999, which was in the same range as data from the present study (6.2:1).

The large abundance of ciliates generated a high grazing pressure on bacteria, HNF and algae. The mean annual production of 45 µg C cm^{-2} d^{-1} of ciliates contrasted with a demand of predators of only 17 µg c cm^{-2} d^{-1}. This demonstrates that only a minor part of the ciliate biomass in biofilm itself was utilized by benthic predators. Therefore, ciliate biomass can be exported from biofilm to the pelagial. This is in agreement with the results of Scherwass (2001) on the structure of the ciliate fauna in the potamoplankton of the river Rhine at Cologne. A large part of the ciliate community (in some cases > 50%) in the potamoplankton of the river Rhine was composed of typical sessile groups of ciliates (e.g. *Vorticella, Epistylis*) (Scherwass, 2001). Data in the study of Scherwass (2001) suggest that Peritrichia were torn off from the ground or other substrates due to changed hydraulic conditions in the beginning of flood events.

Meiofauna.

The potential consumption rate of meiofauna on slides almost corresponded to the cumulated production of bacteria, nanofauna and algae. It should be noted here that biofilms represent an open system interacting with the surrounding media. This suggests that a substantial portion of the food demand of meiofauna is supplied by the surrounding water. On the other hand, meiofaunal organisms were subject to a tremendous predation pressure by the macrofauna. Rough estimates based on the above mentioned assumptions suggest that the complete potential production of the meiofauna would only cover about 25% of the food biomass requirements of the predatory macrofauna. The mean annual meiofauna production of 1.8 µg C cm^{-2} d^{-1} contrasted with a total demand of potential predators on meiofauna of about 4.1 µg c cm^{-2} d^{-1}. This considerable grazing pressure may have induced structuring of the meiofauna, resulting in a low meiofauna diversity and only allowing the colonization of meiobenthos organisms capable of protecting themselves against grazing by embedding into the structures of the biofilm. The dominating meiofauna organisms were well adapted to the life in biofilms. The largest portion of the meiofauna biomass was formed by chromadorid nematodes. *Punctodora ratzeburgensis* and *Chromadorina bioculata* possess a caudal gland, its mucus secretions is extruded through a spinneret at the tip of the tail and enables it to attach itself temporarily to the substratum (Traunspurger, 2000). The impact of macrograzers on meiofauna has rarely been assessed in a small river (Bott, 1996).

Macrofauna.

Based on the estimates of production and consumption, macrofauna (in particular *Ancylus, Dikerogammarus, Corophium)* had a high grazing effect on bacteria, flagellates and algae, despite its low potential turnover rates. In addition to the grazing effect, macrofauna also certainly exerted a structure-forming effect. Important were the colonies of *Cordylophora caspia*; their stalks were colonized by peritrichous ciliates *Vorticella, Zoothamnium,*

Carchesium, Epistylis) and choanoflagellates (*Monosiga*, *Codonosiga, Salpingoeca*). *Corophium* tubes multiplied the biofilm surface area and significantly increased the local abundance of nematodes and rotifers (see Chapter 2). Snails (esp. *Ancylus fluviatilis*) have a very strong impact on biofilms removing the entire superstructure and essentially reducing it to a relatively thin layer of bacterial cells (Lawrence et al., 2002; Ackermann, unpubl.). Macrofauna may alter the architecture of biofilm completely by grazing or disrupting structures by its movements.

Interseasonal comparison

The results of this study demonstrate large differences between the seasons in the densities of the biofilm groups. The highest values of algae, ciliates, meiozoobenthos and macrozoobenthos were found in spring. The highest heterotrophic production of bacteria was found in summer after an increase in mean water temperature. The highest values of HNF were found in autumn; in summer and winter, we detected high densities of colonial choanoflagellates. This is in agreement with the typical expected seasonal pattern for riverine communities in the temperate region (Sommer et al., 1986). In spring, a phytoplankton peak of 1210 µg C l^{-1} was detected in the pelagial, stimulating heterotrophic production. Consequently, the production of bacteria increased in summer due to the algal exudates.

The main objective of this study was to identify the general matter flow throughout the biofilm in the river Rhine near Cologne. Through the course of the year, the dominating matter flow was stable. Ciliates represented the most important predators in biofilm. The major portion of the matter flow in all 4 seasons runs from bacteria to ciliates. The largest ciliate biomass was detected in spring; it was stimulated by a phytoplankton peak. In spring and summer, high macrofauna abundances were detected. In spring, biofilm is populated by juvenile macrofauna organisms and utilized as a nursery (e.g. gammarids). The pattern of

carbon flow through biofilm is independent from season and estimated growth rate. The major pathway for the matter flow through biofilms would be dominated by protozoans, even if the assumed growth rate would change.

It is assumed that seaonal effects are overridden by the effect of succession of the biofilm-community. This becomes evident when comparing the biofilm biomass on the slides between starting phase and the end of the exposition period. In June 2003, the biofilm-community had formed a biomass of $156x10^6$ µm cm^{-2}, in June 2004, it was actually 7522 x 10^6 µm cm^{-2}. This means that in June 2003, the biofilm mass was only 2% of the biofilm mass in June 2004. One reason which could be demonstrated for the tremendous increase in biomass that at the end of the exposition period, successions occurred due to factors like secondary structures generated by metazoans. However, this does not alter the general matter flow in biofilm in the river Rhine near Cologne. A comparison of the portions of the components comprising biofilm communities during the investigation period demonstrates that the relative portions and consequently the matter flow values are stable (Fig. 12). Although the relative portions of the components varied throughout the study period, the main portion of the potential production in all 4 seasons was represented by protozoans (in particular ciliates.

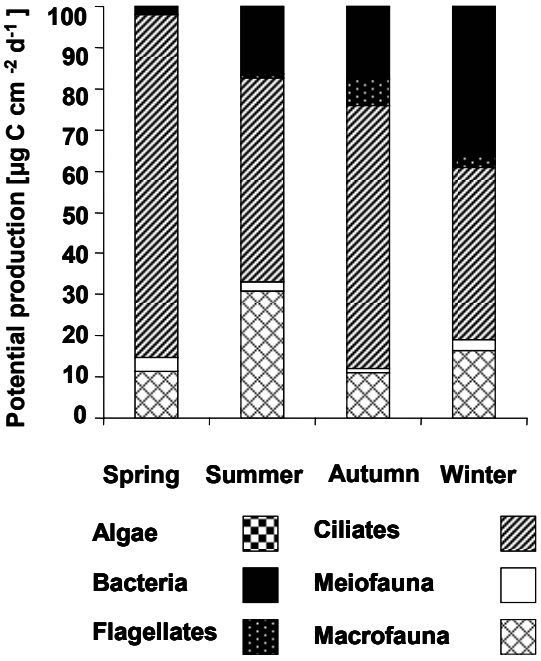

Figure 12 Seasonal changes in relative contribution of the potential production of the components of the biofilm community.

Benthic pelagic coupling

The data presented demonstrate that the biofilm community should significantly degrade plankton organisms. Most earliers studies focused on benthic macrofauna organisms and underestimated the importance of macrofauna in the top-down control of riverine potamoplankton.

In our study, protozoa contributed up to 64% to the annual carbon production in biofilm on slides. In contrast, metazoobenthos contributed 30% of the annual carbon production in biofilm. Bergtold and Traunspurger (2004) investigated the share of benthic production by micro-, meio- and macrobenthos in the profundal of an oligotrophic lake; in their study, they found a share of protozoans of about 39% to benthic carbon production, while the contribution of macrobenthos to the annual carbon production was about 11%. This means that also this study confirms the important role of protozoa in energy flow within the benthos.

As discussed above, the mean potential production of algae throughout the year covered only less than 1% of the potential consumption of benthic predators. The potential production of benthic bacteria covered only less than 4% of the potential consumption of benthic predators, and the potential production of HNF only covered less than 8%. It appears that the food supply of the biofilm biomass is much too small to represent the only food source for biofilm organisms. The autochthonous biofilm biomass was not sufficient to support the growth of nano-, micro, -meio- and macrofauna. However, the current study indicates that biofilm is an open system. On one hand, a significant portion of the biofilm community is planctivorous and directly feeds from pelagial, on the other hand, also exogeneous algae, bacteria and flagellates are required from the drift in the pelagial to continuously renew the biofilm community by colonization. Quantitative studies have shown that the biofilm community at the investigated site of the River Rhine is intensively renewed by colonization from drifting organisms (Arndt et al., 2003). At the early stages of colonization, an immigration rate of about 250 HNF cm^{-2} d^{-1} was determined for heterotrophic flagellates on glass slide biofilms (Arndt et al., 2003). In the present study, a maximum growth rate of 1.7 d^{-1} was assumed for HNF, which is exceeded by a factor of about 150 by the above immigration rate. The study of Schmidt-Denter (1999) determined a theoretical growth rate of about 21 d^{-1} .on slides for heterotrophic flagellates of biofilms of the river Rhine.

The understanding of mass flow in biofilm required the consideration of planktonic carbon flux. While the mean primary carbon production was only 4 µg C cm^{-2} d^{-1}, carbon requirement was approximately 150 µg C cm^{-2} d^{-1}. It can only be covered, if 99% of the carbon of algae and 96 % of the carbon of bacteria are imported from the pelagial.

These data demonstrate that benthic biofilmes in the river Rhine represent an important parameter in the regulation of potamoplankton. In particular the role of prozoans

contributing more than 60% to the total intercompartmental carbon flow in biofilm, is stressed by the present study.

Earlier studies demonstrated top down control due to benthic pelagic coupling to reduce planktonic biomass in rivers, where high bottom surface area to volume ratios and high water mixing rates enhance the exploitation of pelagic resources by benthic reducers (Welker and Walz, 1998). Benthic filter feeders can strongly reduce plankton in rivers (Alpine and Cloern, 1992; Köhler, 1995; Basu and Pick, 1997; Caraco et al., 1997; Pace et al., 1998; Welker and Walz, 1998; Schöl et al., 1999; Weitere and Arndt, 2002a). De Ruyter van Steveninck et al. (1992) postulated a potential important role of benthic macrofauna in trophic relations with the Rhine plankton. Ietswaart et al. (1999) calculated from earlier published data the potential impact of benthic filter feeders and postulated that the loss rate of zooplankton detected in the river Rhine determined by the authors could be explained by the potential consumption by benthic filter feeders.

However, the role of benthic macrofauna alone cannot explain the plankton losses in running waters in all cases (e.g. Monaghan et al., 2001; Weitere et al., 2003). In addition to the benthic macrofauna, benthic protozoans and metazoans can be abundant on stones and other hard substrates in rivers (e.g Foissner et al., 1992; Franco et al., 1998; Duggan, 2001; Arndt et al., 2003). Microbes living in matrix-enclosed biofilms contribute substantially to energy flow and nutrient recycling in aquatic ecosystems (Pusch et al., 1998; Battin et al., 2003). Among the periphytic protozoans and metazoans, several species can efficiently feed on planktonic organisms (e.g. Foissner et al., 1992; Arndt, 1993; Franco et al., 1998). In rivers covered with suitable substrates such as the river Rhine, the biofilm community can colonize a large surface area. As a major transfer of carbon in large river food webs, the flow between phytoplankton and planktonic and benthic consumers becomes a relevant issue (Schöl et al., 2002).

A comparison of the mean carbon flow investigated for the plankton community of the river Rhine at Cologne (Weitere et al., 2005) and the mean carbon flux established by this study (Fig. 13) illustrates benthic pelagic coupling in the river Rhine. Carbon flux calculations for plankton indicated only a minor role of planktonic metazoans and ciliates in controlling phytoplankton. The consideration of the absolute fate of the riverine plankton production shows that only a small amount of the production of algae and protozoans was utilized by planktonic predators (Weitere et al., 2005). Figure 13 shows that a large part of the carbon production (algae 79%, bacteria 54%, HNF 81%, ciliates 82% and metazoans 81%) was not metabolized in the planctonic food web but was exported into the benthic system and downstream drift. This tremendous input from the pelagial drives the mass flow in the benthic biofilms of the river Rhine at Cologne.

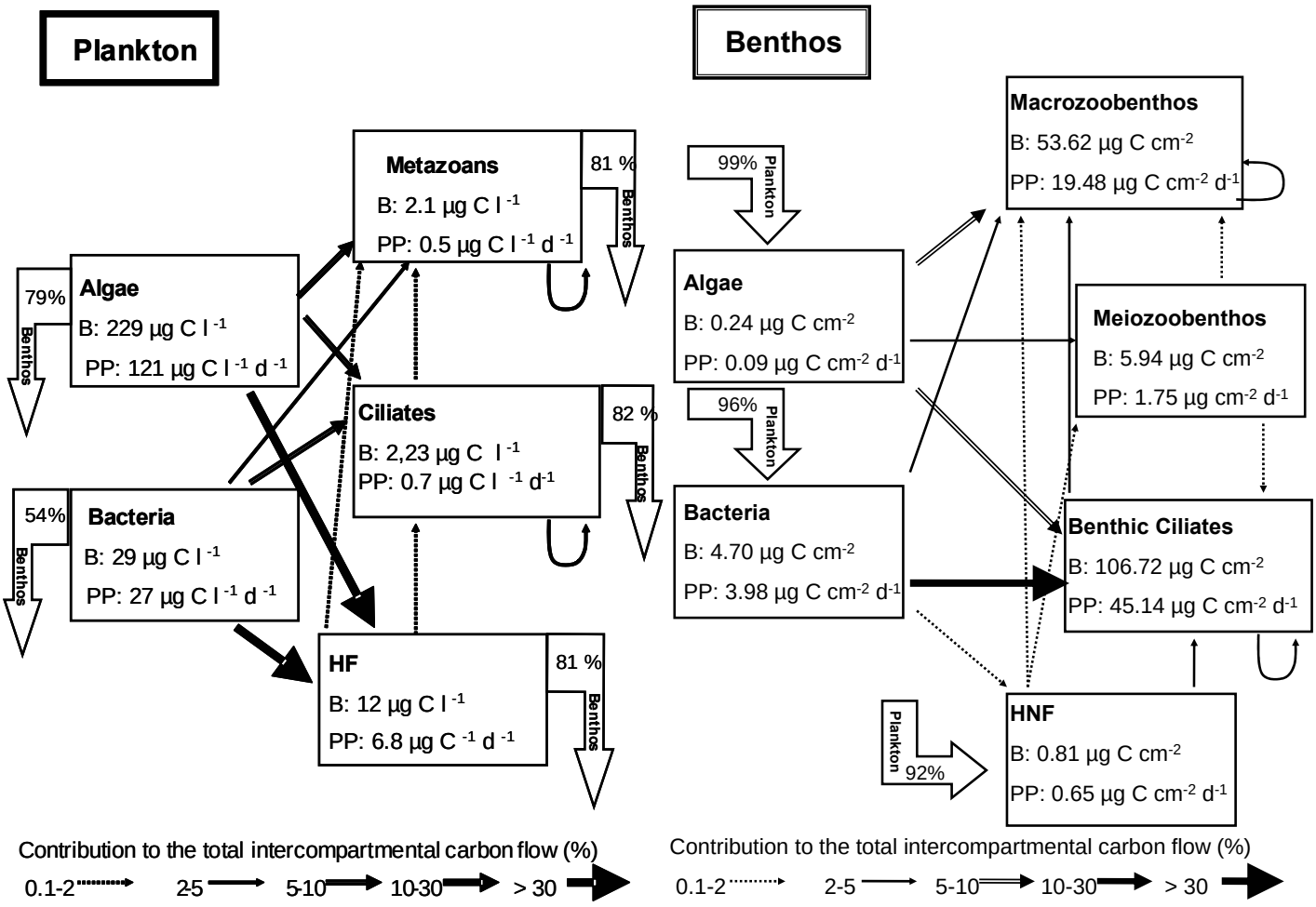

Figure 13 Left panel: Mean annual carbon flow chart for the plankton community of the river Rhine at Cologne (data from Weitere et al., 2005). Right panel: Mean annual carbon flow chart for the biofilm community of the river Rhine at Cologne.

Conclusion

The consideration of the fate of the riverine plankton production shows that only a small amount of the production of algae and protozoans was utilized by planktonic predators (Weitere et al., 2005). In biofilm, ciliates are not exposed to the high grazing pressure of filter feeders and therefore can build up a higher biomass. Ciliates were the dominating component regarding the functioning of the biofilm. The share of protozoa of the potential production (µg C cm^{-2} d^{-1}) in biofilm was about 78%. Biofilm communities of bacteria, protozoa and microalgae bridge the gap between the microbial loop and the algal grazer pathway. In this way, the use of planktonic organisms from pelagial by the benthic subsystem is possible. Only

2% of the total intercompartmental carbon flow has been autochthonously produced in the biofilm, the residual carbon requirement should be covered as an input from the pelagial. The import of planktonic organisms into the benthal microbial food web drives the mass flow in the investigated benthic riverine community.

The importance of the microbial biofilm community in the control of potamoplankton as a link between planktonic and benthic food web is possibly of much higher general importance in rivers than currently believed.

Table 1 Mean assumed growth rates (d^{-1}) of the different groups of biofilm organisms. The growth rates are based on measurements in the Rhine for HNF (Weitere and Arndt, 2002a,b) as well as on assumptions for the other groups according to de Ruyter van Steveninck et al. (1992) for Rhine bacteria, Schöl et al. (2002) for Rhine algae, Müller and Geller (1993) and Scherwass (2001) for ciliates, Stemberger and Gilbert (1985) and Stelzer (1998) for metazoans.

Season	Algae	Bacteria	HNF	Ciliates	Meiozoobenthos	Makrozoobenthos
Spring	0.50	1.05	0.43	0.45	0.30	0.15
Summer	0.70	1.00	1.17	0.63	0.50	0.25
Autumn	0.50	0.80	1.02	0.35	0.10	0.05
Winter	0.10	0.60	0.10	0.10	0.10	0.05

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Kapitel 4

Colonization of heterotrophic nanoflagellates on biofilms of a large Antarctic ice floe during the Austral summer

Introduction

To improve the understanding of the role of early summer physical and biological atmosphere-ice-ocean interactions in the Western Weddell Sea, a 40-day drift station was established during Austral summer. R.V. Polarstern was anchored at a large ice floe which was followed for a distance of about 111 km during Austral summer, until the size of the floe was less than one quarter of its original size. The sea-ice region in the Weddel Sea contains the largest perennial ice zone of the Southern Ocean and has therefore a significant impact on the whole life in this region. This drift experiment offered a unique chance to analyze the sea ice community of the melting ice pack.

The overall importance of the so-called microbial loop has originally been described by Azam et al. (1983; see also Fenchel, 1987; Gasol and Vaqué1993) for marine waters and has also been found to play a fundamental role in Antarctic waters (e.g. Anderson and Rivkin, 2001). Sea ice can possess hot spots of biological activity in the Antarctic ocean. Dense populations of algae, bacteria and protozoans form an active and very abundant biofilm community ((e.g. Legendre, et al., 1992; Spindler, 1994; Petz et al., 1995; Garrison et al., 2005). The sea ice biota may also influence the pelagic systems under the ice cover and at ice edges due to the high concentrations of organisms living in the ice compared to abundances in the surrounding pelagic waters (e.g. Legendre et al., 1992; Petz et al., 1995). Observations of a diverse microbial assemblage in sea ice have suggested that the sea ice communities are similar to those of the water column with respect to an active microbial food web (see reviews by Legendre et al., 1992; Garrison and Mathot, 1996). A significant fraction of the carbon fixed by microalgae and transferred partly to other microbial components such as bacteria, heterotrophic flagellates and ciliates should be exported out of the production zone during summer. This particulate production by the sea ice community is assumed to be passed on to the pelagic and benthic food webs. The ice biota in Antarctic waters should provide a

tremendous surface for biofilms forming one of the earth´s most important biofilm communities.

Heterotrophic nanoflagellates in a size range of 2-15 µm are considered potentially important members of the microbial sea ice community (e.g. Archer et al., 1996; Garrison et al., 2005) being able to control the bacterial production and making recycled inorganic nutrients available for the algae (Azam et al., 1983; Delille et al., 2002). Though heterotrophic flagellates are known to be a typical component of sea ice fauna (e.g. Garrison et al., 2005), its composition has seldomly been quantified (e.g. Ikävalko and Thomsen, 1997; Ikävalko and Gradinger, 1997; Sime-Ngando et al., 1999). One major reason is that such studies require the use of live-counting, since group-specific fixation artifacts make it impossible to compare the abundance of the different groups of flagellates (e.g. Sonntag et al., 2000; Arndt et al. 2000). On board R/V Polarstern, field samples could be analyzed within 30 minutes after sampling which offered the opportunity to carry out comparative studies of the sea ice nanobiota.

Our studies was intended to contribute to improve our understanding of the seasonal interaction between biota and sea ice in the perennial ice region in the Southern ocean. The following questions were to be answered by the study: 1) Is there a nanofauna typical for Antarctic ice biofims? 2) What is the temporal variation in the flagellate community abundance and composition in the course of the melting process? 3) What is the difference in brine and ice slush nanofauna communities?

Materials and Methods

Collection of samples.

Ice samples were taken from an ice floe during the interdisciplinary expedition called ISPOL (Ice Station Polarstern) carried out from November 2004 until January 2005. For a 40-day drift station in the western Weddell Sea, R.V. Polarstern was anchored at an ice floe which had an original area of about 16 km² and less than about 4 km² at the end of the experiment. The drift of the ice floe was followed for a distance of about 111 km from November 27th, 2004 until January 2nd, 2005 (Fig. 1). Ice samples were taken from three different habitats, i.e. ice slush, ice core and brine. Ice slush was a dynamic habitat developing in the course of melting of the ice floe, when the ice surface of the floe was covered by sea water (see Haas et al., 2001). Ice slush was siphoned off from the floe surface using a clear polycarbonate container (3 liter) and immediately (within 30 minutes) analyzed by live-counting onboard. For live-counting of heterotrophic flagellates, slush water droplets of 20µl were transferred to a miniaturized version of a Sedgewick-Rafter chamber with a height of about 0.2 mm. Samples were analyzed not longer than 5 minutes on a cooled microscopic table by means of a phase contrast microscope (Zeiss Axiovert) equipped with a video camera. 200x and 400x magnifications were used for quantitative counts. Video recordings were used to support later determinations of flagellate morphospecies. Some choanoflagellates were determined to the genotype level (see below). Ice core samples were obtained from drilled ice cores. The length depended on the thickness of the ice floe. All cores were taken in the same undisturbed region of the floe (12*12m) in a distance of about 2 m from each other. Samples of the melted ice core were investigated by live-counting (see above). In addition, brine water flowing into the ice hole after drilling was investigated by live-counting. At least three replicate samples were analysed for each sampling which means samples from different ice cores or different slush areas on the floe. In addition, slush samples were stored at 4°C in tissue culture flasks for later morphological and molecular studies at the laboratory in Cologne.

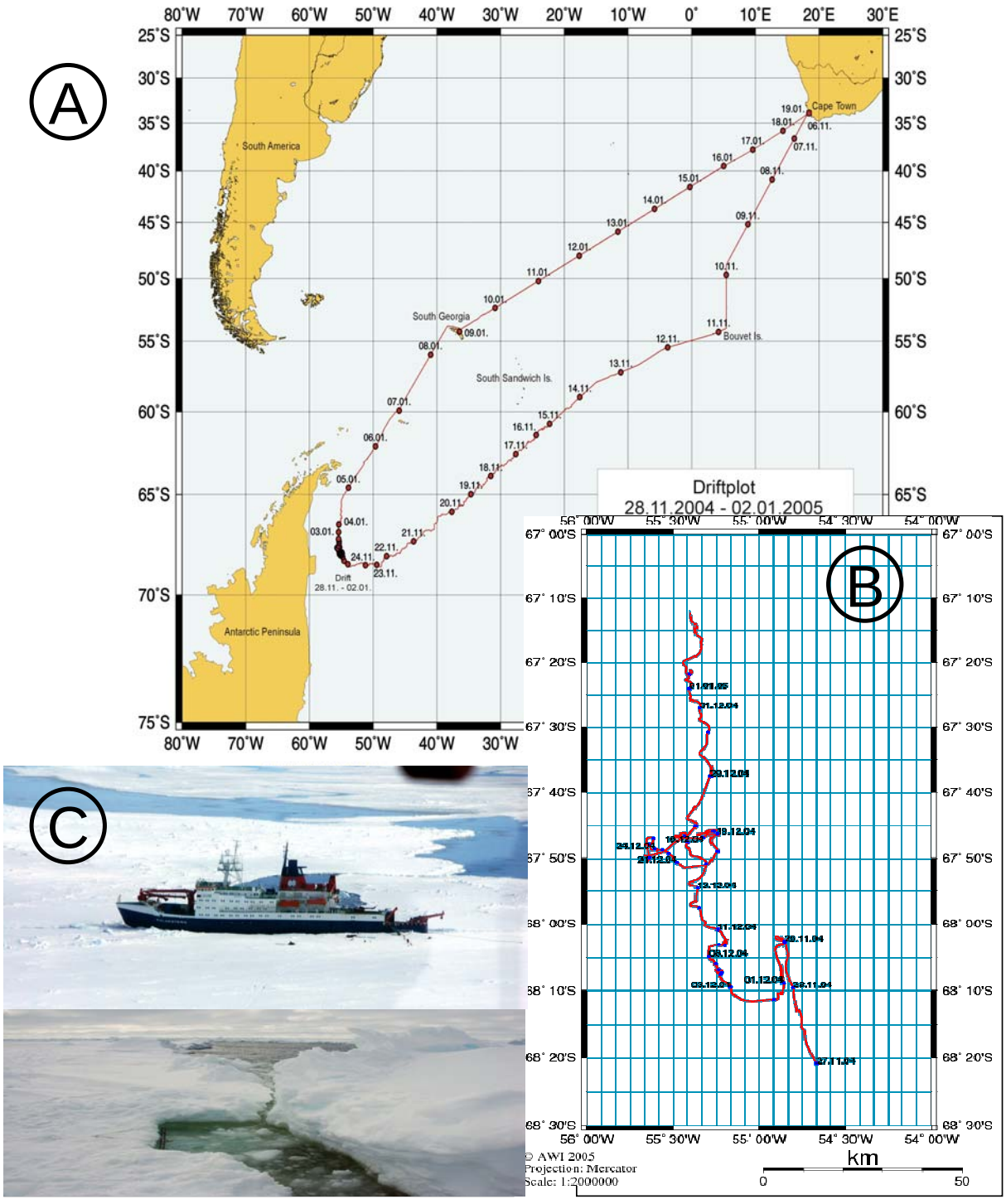

Figure 1 Cruise of the ISPOL expedition (A), study area of the drift IceStationPolarstern (B), and RV Polarstern at the drifting floe with the slush ice layer at the edge of the floe (C).

Determination of choanoflagellates.

Additional molecular studies were carried out by Nitsche (unpubl.).

Upon arrival of water samples in Cologne, samples were partitioned into tissue culture flasks containing 30 ml of water. After sufficient growth of species, the samples were divided for morphological and molecular-biological examinations.

For scanning electron microscopy (SEM) preparation, samples were fixed with 50:50 Bouin solution (75% picric acid [saturated], 25% formaldehyde [38%] and 2% acetic acid [100%] and 0.2% glutaraldehyde [38%]) for 45 minutes at 4°C. Samples were placed on a polycarbonate (PC) filter with a pore size of 2 µm and filtered using a vacuum lower then 150 mbar. Followed by a dehydration series of ethanol with 30%, 50%, 60%, 70%, 80%, 90%, 96% and pure ethanol (each step was performed 3 times and left for 10 minutes) and then a 50:50 hexamethyldisilazane (HMDS)-ethanol solution for 30 minutes followed by pure HMDS for 30 minutes. Afterwards, the samples were allowed to dry. SEM samples were sputtered with a 120Å layer of gold and then transferred to SEM. To gain rRNA, single cell PCR was done. A micromanipulator was used to collect cells which were transferred then to 27 µl of ddH_2O and frozen at -20°C for 3hours. For PCR, a 18sFor (AACCTGGTTGATCCTGCCAGT) and 18sRev (GTAGGTGAACCTGCGGAAGGATCA) Primer were used at a concentration of 1.6 nMol. For more details see Scheckenbach et al. (2005).

Determined sequence fragments were assembled manually. The determined sequences were aligned together with other sequences retrieved from GenBank/EMBL using the ClustalX multiple alignment program (Thompson et al., 1994). Uncorrected genetic distances (*p* distances) were calculated using the program PAUP v4.0b (Swofford, 2000). Phylogenetic analyses were carried out involving the minimum evolution (ME) method (Nei and Kumar 2000). The Kimura 2-parameter distance (K2P) model (Kimura, 1980) of nucleotide

substitution was chosen for the ME analyses. The reliability of internal branches was assessed by bootstrapping (Felsenstein, 1985) with 1000 resamplings.

Results

The ice nanofauna of the open Weddell Sea was investigated in the course of melting of one and the same ice floe. The nanofauna of ice cores was dominated by colourless euglenids (e.g. *Petalomonas*-like) and chrysomonads (e.g. *Spumella*-like) representing more than 90% of heterotrophic flagellate abundance (Fig. 2, 3). The composition of brine samples was similar with the exception that choanoflagellates occurred in low abundances (Fig. 1, 3). The flagellate community of the ice slush was very different (Fig. 2, 3). It was dominated by choanoflagellates (e.g. *Acanthocorbis, Diaphanoeca, Sphaeroeca, Monosiga, Salpingoeca*) followed by chrysomonads (*Spumella* and *Paraphysomonas*-like), bodonids (e.g. *Bodo, Neobodo, Rhynchomonas*), pedinellids (*Actinomonas/Pteridomonas*) and bicosoecids. In addition, ancyromonads (*Ancyromonas*) and *Cryothecomonas* were regularly recorded.

The abundances of heterotrophic flagellates were 878 $\pm$ 398 cells/ml for the ice cores, 1167 $\pm$ 369 cells/ml for brine samples, and 1418 $\pm$ 369 cells/ml for ice slush samples (Fig. 2, n=6). The dynamics of abundances in the slush could be followed for a period of 2 weeks (Fig. 4, 5). The temperatures of the slush (thickness 0.3-0.7m) ranged between -2.0 and -1.1 °C (mean: -1.6°C) at salinities of 27.2-30.5 (mean: 29.1) PSU, chlorophyll *a* values of 10.8-14.5 (mean: 13.0) µg Chl *a*/l. Maximum abundances were up to 4200 heterotrophic flagellates/ml. Toward the end of the expedition, numbers of heterotrophic flagellates decreased to values of about 500 cells/ml. The composition of the flagellate community was characterized by varying contributions of choanoflagellates stemming from pelagic waters and bodonids and chrysomonads stemming mainly from the ice core.

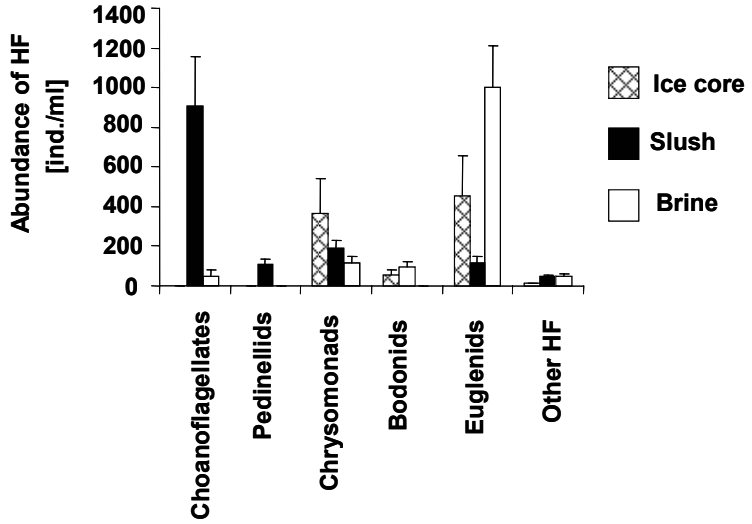

Figure 2 Comparison of the abundance of heterotrophic nanoflagellates (HF) in samples from ice core, slush and brine water from a drifting ice floe (n=6).

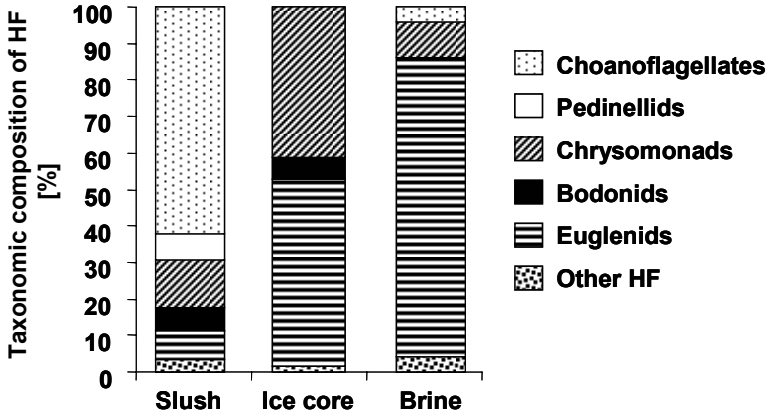

Figure 3 Comparison of the composition of heterotrophic nanoflagellates (HF) in samples from ice core, slush and brine water from a drifting ice floe (n=6).

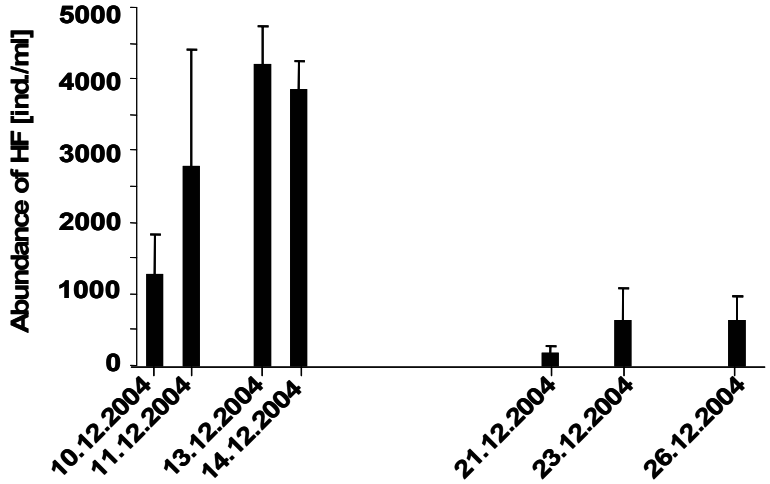

Figure 4 Temporal changes of the abundance of HF in the slush ice layer (n=3) of an antarctic ice floe during drift.

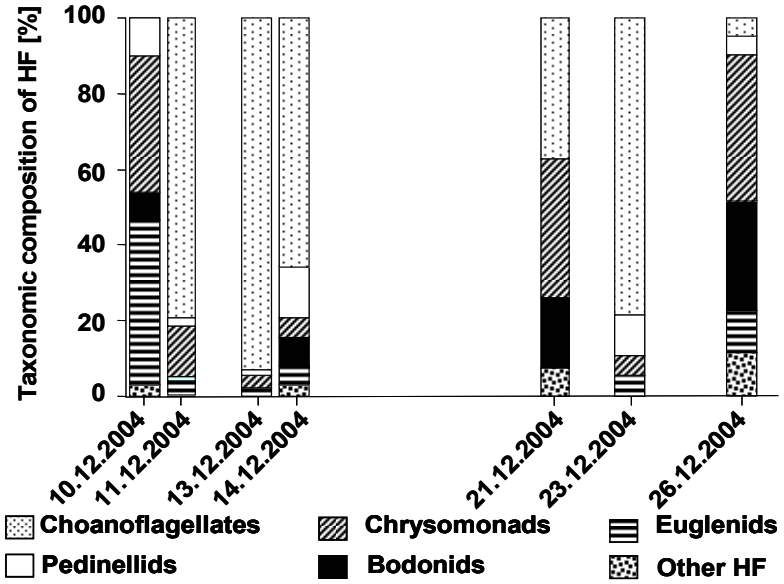

Figure 5 Temporal changes of the taxonomic composition of HF in the slush ice layer (n=3) of an Antarctic ice floe during drift.

Two choanoflagellate species found in the slush samples were analyzed morphologically by SEM and by sequencing the SSU rDNA (bp 590-1300). SEM examinations indicated that the specimens belonged to *Diaphanoeca grandis* and *Acanthocorbis unguiculata* compared with published reference specimen from arctic sites (Fig. 6). Both species are known from pelagic waters but were found during the present investigation also in close contact to surfaces of ice (Eßer, unpubl.) and substrate (Nitsche, unpubl.). The SSU rDNA sequences from the Antarctic isolates showed a 100% similarity compared with the available data in global gene databases for the two species.

Further species of choanoflagellates were found in the samples from Antarctica and are about to be cultivated for morphological and molecular biological studies in the near future.

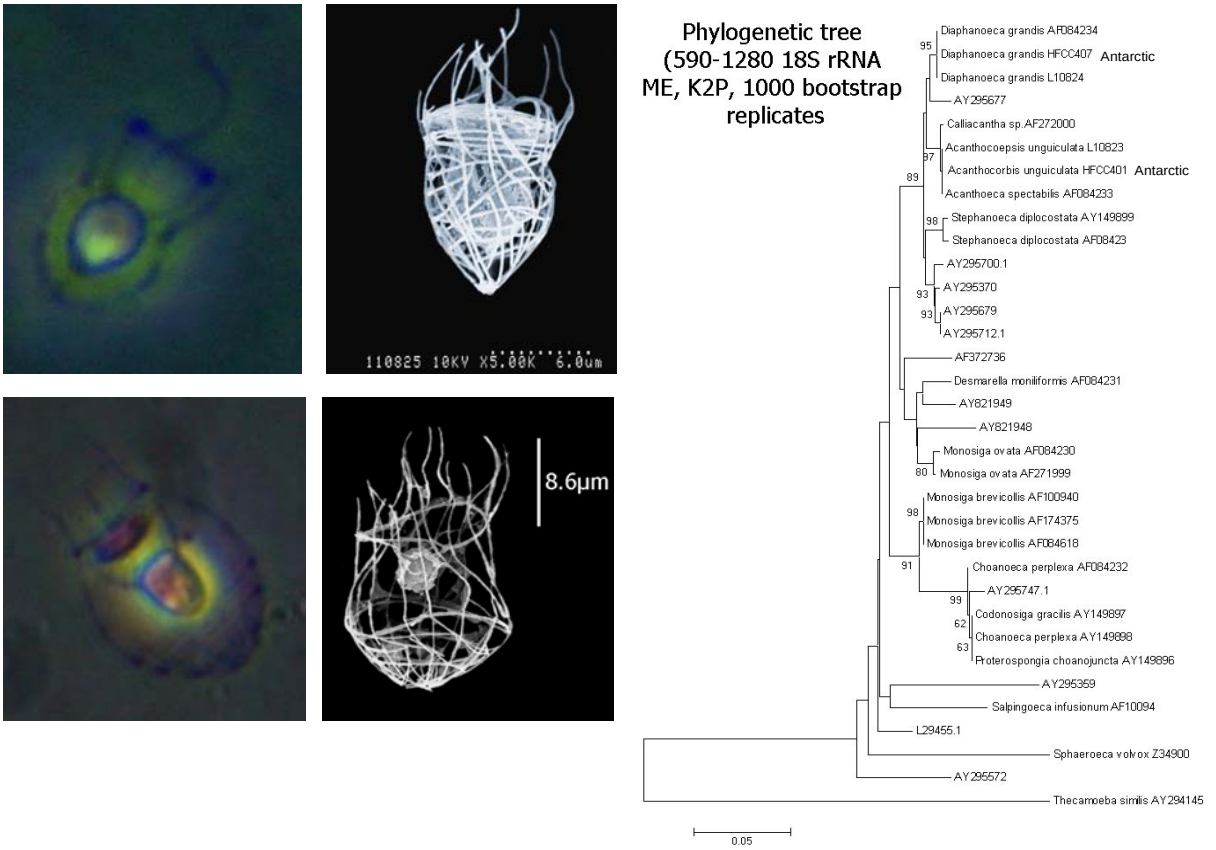

Figure 6 *Acanthocorbis unguiculata* and *Diaphanoeca grandis* identified from the Antarctic waters. Photomicrographs from light microscopy (left) and SEM (middle) of *Acanthocorbis* (upper panels) and *Diaphanoeca* (lower panels). Right panel compares the position of both strains (indicated by „Antarctic“) with regard to published sequences in the SSU rDNA phylogenic tree.

Discussion

The recorded abundances of heterotrophic flagellates were about one to three orders of magnitude higher than values known from Antarctic pelagic waters (e.g. Hewes et al., 1990; Dietrich 1999). Our results support the reports from previous expeditions concluding that microorganisms are concentrated in the pack ice in arctic habitats and contribute significantly to the matter flux in arctic pelagic ecosystems. Only a very few live-count data of heterotrophic nanoflagellates from Antarctic sea ice are available. *Cryothecomonas* is generally found as a dominant component of nanoflagellate communities (Archer et al., 1996; Garrison et al., 2005). In our studies, *Cryothecomonas* did not significantly contribute to the nanoflagellate biomass, instead other components were found to be of significant importance such as small chrysomonads (<5µm), small bodonids and small euglenids. Euglenids have also been reported by Archer et al. (1996) as significant components.

The differences we found regarding the HF community structure of ice core and slush samples agree with observations by Archer et al. (1996) from McMurdo Sound, Antarctica. They found also high abundances of choanoflagellates in slush samples while euglenids were of higher importance in brine samples. Chrysomonads have seldomly been reported as important components of ice nanofauna. Chrysomonads are very delicate forms and easily disrupt upon fixation. This might explain why this important nanoflagellate group might have been ignored by earlier studies.

The differences in the community structure of ice core and slush samples might well be explained by the close contact of the slush to the surrounding pelagic waters which harbor a large variety of choanoflagellates species (e.g. Hewes et al., 1990) and their continuous settlement on the ice. Since abundances of HF in the slush with its high biomass of algae (up to 14.5 µg Chl a/l during the present investigation) are considerably higher than in the pelagial, it has to be assumed that choanoflagellates grew efficiently on the ice surfaces taking advantage of the high bacterial production. Growth rates of ice flagellates of about 0.1/d were

estimated by Archer et al. (1996), which might explain the significant changes in flagellate abundances we observed in slush samples. Another factor that most probably influenced the flagellate community structure in the ice core and the slush is the significant difference in salinity. While salinities in the slush ranged between 27.2 and 30.5 PSU, the salinity in the ice cores were much lower (3.3-11.6 PSU). Though several heterotrophic flagellates are known to tolerate a wide range of salinities (Arndt et al. 2000), the occurrence of many species seems to be restricted to a small range of salinities. While most acanthoecid choanoflagellates are known to occur under marine conditions, genera of bodonids and euglenids recorded by us in the brine water are also typical for brackish and freshwaters. Unfortunately only little is known about the influence of salinity on ice nanofauna.

The morphotypes of the two isolated choanoflagellate species Diaphanoeca grandis and Acanthocorbis unguiculata were identical with the genotype (on the SSU rDNA level). And the sequences were also identical to known sequences from the GenBank which probably were obtained from specimens from the northern hemisphere (unfortunately not mentioned in Genbank). At least these findings indicate an ubiquitous distribution of these two species.

There is increasing evidence that the sea-ice biota plays an ecologically important role in the Antarctic ice-covered regions. Garrison et al. (2005) concluded from their multidimensional scaling plots that sea ice does not constitute a unique habitat in the Antarctic pelagic system. Relatively few studies have been sufficiently comprehensive especially regarding the taxonomic composition of ice organisms to give a general picture of community structures of nano-sized organisms. Our present study does not support the conclusion of Garrison. Though most nanoflagellate taxa we found in the sea ice should also occur in very low numbers in the pelagial (e.g. on marine snow), the numbers of typical benthic forms in the ice (such as euglenids and bodonids) are considerably higher (4 to 5 orders of magnitude!) than in the water column below, indicating the importance of studies of the structure of nanofauna communities tu understand the functioning of the most productive

part of the ice fauna (e.g. Archer et al., 1996). For instance, euglenids should have the same abundance in the 1 m Sea ice layer as in the total 4000 m water column below. And it is very probable that the abundance of deep-sea benthic nanoflagellate communities will not significantly contribute to overall abundances (e.g. Arndt et al., 2003). We think that these considerations throw quite another light on the importance of sea-ice biota. For several months, microorganisms produce a significant amount of carbon in a refuge from predation which is supplied to the pelagic region upon melting at the end of Austral summer.

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Zusammenfassung

Der Stoffkreislauf und die Bedeutung der bentho-pelagischen Kopplung von großen Fließgewässern sind trotz der besonderen Relevanz dieser Gewässer für die Funktion der Landschaft bisher nur sehr unzureichend aufgeklärt. Die Entwicklung von Modellen für das Benthal war bisher durch die geringe Anzahl von Daten über benthische Mikroorganismen und die Zusammensetzung von benthischen Lebensgemeinschaften (inkl. aller wichtigen Organismengruppen) beschränkt (Silvert, 1991; Weitere et al., 2003; Junk, 2005). Es gab Anhaltspunkte, dass insbesondere die auf großen Oberflächen am Gewässerboden und im angeschlossenen Interstitialraum angesiedelten Biofilme diesen Stofftransfer erheblich mit bestimmen (Pusch et al., 1998; Schmidt-Denter, 1999; Reiss, 2002; Arndt et al., 2003; Weitere et al., 2003). Durch diese Arbeit sollte erstmalig modellhaft der Stoffffluss in einem Biofilm in einem großen Fluss analysiert werden und geklärt werden, welches die wichtigsten Interaktionen innerhalb des Systems Biofilm sind. Eine Besonderheit der vorliegenden Studie bestand darin, dass versucht wurde, alle wichtigen Organismengruppen im Biofilm parallel auf dem Modellsubstrat zu untersuchen. Hierzu wurden Objektträger über einen Zeitraum von mehr als einem Jahr in einer Fließrinne an der Ökologischen Rheinstation der Universität zu Köln direkt im Rhein exponiert und alle 3 Wochen mittels direkter Lebendzählung (Arndt et al., 2000) auf ihre taxonomische Zusammensetzung und die Abundanz der nachgewiesenen Arten untersucht.

In Rahmen dieser Studie ist es erstmals gelungen, die Sukzession aller wichtigen Organismengruppen im System Biofilm über einen Zeitraum von 14 Monaten zu beschreiben. Dies ist deshalb hervorzuheben, da die Organismengruppen, die den Biofilm besiedeln (Bakterien, Flagellaten, Meiofauna), teilweise schwer zu untersuchen sind.

Die Zusammensetzung des Biofilms wurde über den gesamten Zeitraum von Ciliaten dominiert, hierbei waren vor allem filtrierende peritriche Ciliaten am Aufbau der Biomasse beteiligt. Darüber hinaus konnte gezeigt werden, dass die Makrofauna die zweite wichtige steuernde Größe des Biofilms darstellt. Neben den sich über den Biofilm erstreckenden Kolonien von *Cordylophora caspia* und *Corophium curvispinum* waren teilweise hohe Abundanzen von Gruppen anzutreffen, die den Biofilm nur temporär besiedeln, wie beispielsweise Gammariden, die den Biofilm als Kinderstube nutzen. Es konnte gezeigt werden, dass es neben direkten Effekten zwischen den trophischen Ebenen auch indirekte Einflüsse gibt. Am Beispiel der Wohnröhren des Schlickkrebses *Corophium curvispinum* konnte gezeigt werden, dass durch die Veränderung des Substrates und die Schaffung von neuen Mikrohabitaten ein signifikanter Einfluss auf die Besiedlung durch die Meiofauna ausgeübt wurde. In den Bereichen mit Wohnröhren des Schlickkrebses wurden signifikant mehr Rotatorien und Nematoden nachgewiesen.

Die vorliegende Untersuchung sollte Hinweise erbringen, ob die Modellvorstellung vom „microbial loop“ von Azam et al. (1983) auf den Biofilm übertragen werden kann. Die Bedeutung der heterotrophen Flagellaten (HF) als Komponente von Biofilmen des Rheins sollte im Rahmen dieser Langzeitstudie aufgeklärt werden. Bisher hatten nur wenige Untersuchungen die HF als Komponente der Biofilmgemeinschaft untersucht (Railkin et al., 1990; Zolotarev, 1995; Widera, 1997). Taxonomische Arbeiten zu HF in Biofilmen lagen bisher nur sehr wenige vor. Es konnte gezeigt werden, dass die HF im Biofilm sehr hohe Abundanzen ausgebildet haben. Vor allem sessile Choanoflagellaten haben Kolonien mit bis zu 100.000 Individuen/cm² entwickelt. Filtrierende Flagellaten (Choanoflagellida und Chrysomonadida) dominierten die Biomasse der HF über den gesamten Versuchszeitraum mit durchschnittlich 84%. Bodonea und Ancyromonadida waren über den Untersuchungszeitraum

die wichtigsten benthischen Nanofaunagruppen, ihr Anteil an der Biomasse betrug über den Versuchszeitraum durchschnittlich 5%. Ein weiteres interessantes Ergebnis ist, dass die Ancyromonadida, die bisher in Lehrbüchern keine Erwähnung finden, mit einer durchschnittlichen Individuenzahl von 817 Ind./cm² im System Biofilm eine wichtige Rolle eingenommen haben. Genetische Studien haben gezeigt, dass sich hinter dem Morphotyp *Ancyromonas sigmoides* verschiedene Genotypen verbergen (Scheckenbach et al., in preparation).

Diese Arbeit sollte die Frage beantworten, wodurch die Abundanz und Sukzession der HF im System Biofilm gesteuert wird. Während die HF im Pelagial des Rheins die dominierende Gruppe im Stoffumsatz darstellen (Weitere et al., 2005), sind die HF auf dem Biofilm einem starken Fraßdruck durch die Ciliaten ausgesetzt, die ihrerseits durch die Strukturen des Biofilms einem nicht so starken Fraßdruck durch ihre Prädatoren ausgesetzt sind wie im Pelagial. Durch die extrem hohen Abundanzen der Ciliaten bestand ein großer Fraßdruck auf die HF, hierdurch hat bei den HF eine Selektion zu kleineren Formen stattgefunden, die sich räumlich perfekt in die Strukturen des Biofilms einnischen konnten. Die im Rahmen dieser Dissertation gewonnenen Daten zeigen ein Verhältnis von Bakterien zu HF von 4321:1. Dieses Verhältnis zwischen Bakterien und HF verbunden mit dem hohen Fraßdruck der picophagen Mikro-, Meio- und Makrofauna zeigt, dass durch die Konkurrenz um die Ressource bakterielle Biomasse eine bottom-up Kontrolle der HF im Biofilm denkbar ist. Durch den enormen Fraßdruck, der auf die HF durch die nanophage Mikro-, Meio- und Makrofauna ausgeübt wurde, scheint dieser mögliche bottom-up Effekt jedoch eindeutig durch einen top-down Effekt überlagert zu werden.

Ein weiterer wichtiger Schwerpunkt dieser Arbeit war es, die Bedeutung der Meiofauna als Komponente von Biofilmen des Rheins im Rahmen einer Langzeitstudie aufzuzeigen. Eine taxonomische Untersuchung zur Bedeutung der Meiofauna war bisher nicht bekannt. Anhand

der im Rahmen dieser Studie gewonnenen Daten kann ihre tatsächliche Relevanz innerhalb des benthischen Nahrungsgewebes abgeschätzt werden. In der vorliegenden Arbeit ist die taxonomische Zusammensetzung der Meiofauna in Biofilmen des Rheins über einen Zeitraum von 14 Monaten beschrieben worden. Es konnte gezeigt werden, dass das System Biofilm durch seine variablen Strukturen auch für die Meiofauna einen Lebensraum bietet, der die Entwicklung von sehr hohen Abundanzen ermöglicht. Der enorme Fraßdruck durch die Makrofauna hat auch bei der Meiofauna zu einer Selektion von kleineren Arten mit hohen Wachstumsraten geführt. So sind wenige Arten nachgewiesen worden, die dafür hohe Abundanzen entwickelt haben. Primär wurden bdelloide Rotatorien und chromadoride Nematoden nachgewiesen, die auf die Individuenzahlen bezogen zusammen über den gesamten Versuchszeitraum mehr als 90% der Metazoen stellten. Dabei haben die chromadoriden Nematoden Individuendichten von bis zu 600 Ind./cm² ausgebildet. Dieser Wert liegt deutlich über bisher publizierten Werten. Darüber hinaus wurden mit Vertretern der beiden im Rahmen dieser Untersuchung dominierenden Meiofaunataxa (chromadoride Nematoden und bdelloide Rotatorien) Fraßexperimente in Miniaturfließkammern durchgeführt. Die Ergebnisse dieser Fraßexperimente und die hierbei ermittelten Fressraten wurden genutzt, um den Effekt dieser beiden dominanten Meiofaunagruppen auf das System Biofilm abschätzen zu können. In Biofilmen ist die Meiofauna eine abundante Komponente und es wurde angenommen, dass die Meiofauna einen starken Effekt auf die Mikrofauna und die Mikroalgen in Biofilmen besitzt (Schmid-Araya und Schmid, 2000). Diese Studie konnte durch die Fraßexperimente mit einer Fressrate von 151 Algen/Nematode/Tag einen starken Einfluss der chromadoriden Nematoden (*Punctodora ratzeburgensis* und *Chromadorina bioculata*) auf die Mikroalgen im Biofilm nachweisen. Dieser experimentelle Effekt wurde durch die Korrelation von Algenabundanz und Nematodenabundanz von den Daten der Langzeitbeprobung der Objektträger gestützt. Der zweite Versuchsansatz mit Vertretern der bdelloiden Rotatorien (*Rotaria rotatoria*) mit Fressraten von 1780 HF/Rotatorie/Tag zeigte

einen starken Effekt auf die Nanofauna im Biofilm. So konnte nachgewiesen werden, dass beide Meiofaunataxa einen höchst selektiven Einfluss auf die Biofilmgemeinschaft ausüben. Dies widerspricht bisher veröffentlichten Ergebnissen, denen zufolge Nematoden eher ein opportunistisches Fraßverhalten zeigen (Moens and Vincx, 1997). Berechnet man aus ermittelten Fressraten und nachgewiesenen Abundanzen der Meiofauna die potentiellen Konsumptionsraten, ergibt sich, dass die beiden Gruppen einen hohen Fraßdruck auf ihre Nahrungsorganismen ausüben können und damit einen stark strukturierenden Einfluss auf das System Biofilm besitzen. Dies impliziert, dass die Meiofauna eine bedeutende Rolle in der Steuerung von mikrobiellen Biofilmen der Gewässersohle großer Fließgewässer spielt, wobei beachtet werden muss, dass das Modellsystem unter den lichtlimitierten Bedingungen exponiert worden ist, die auch auf dem Grund des Rheins vorherrschen.

Ein weiterer zentraler Aspekt dieser Dissertation war die erstmalige Entwicklung einer Modellvorstellung für das Nahrungsnetz von Biofilmen im Rhein. Bisher war die Entwicklung von Modellen für das Benthal durch die geringe Anzahl an Daten über benthische Organismen und die Zusammensetzung von benthischen Lebensgemeinschaften beschränkt (Silvert, 1991; Weitere et al., 2003; Junk, 2005). In der vorliegenden Arbeit wurde eine modellhafte Vorstellung für den Stofffluss durch das System Biofilm entwickelt. In dieses modellhafte Schema sind die Ergebnisse der quantitativen und qualitativen Erhebung der Biofilmflora und -fauna im Rhein über einen Zeitraum von 14 Monaten eingeflossen. Die Produktion und Biomasse der verschiedenen Gruppen im Biofilm wurden untereinander verglichen und der mögliche Stofffluss zwischen den Organismengruppen des Biofilms wurde abgeschätzt, um den prinzipiellen Verlauf des Kohlenstoffes durch den Biofilm im Rhein zu charakterisieren. Bei der Kalkulation des Stoffflusses wurde auch die offenbar wichtige Kopplung zwischen pelagischem und benthischem Nahrungsgewebe in Betracht gezogen, die bis zum heutigen Tage in der Literatur nicht vollständig verstanden ist (Hershey et al., 2005).

Hierbei wurde versucht, mit den von Weitere et al. (2005) publizierten Ergebnissen für den Stoffffluss im Pelagial des Rheins bei Köln eine Verbindung herzustellen, um weitere Erkenntnisse zu gewinnen, die zu einer besseren Beschreibung des Gesamtsystems im Rhein beitragen können. Die in dieser Studie vorgenommene Abschätzung kann hierbei wichtige Grundlagen für das Verständnis von Stoffflüssen in großen Fließgewässern liefern, wobei die immer tiefgreifendere Kenntnis von den Stoffkreisläufen in den verschiedenen Ökosystemen von immenser Bedeutung ist. Durch Umstrukturierung der Gewässersohle im ohnehin stark vom Menschen überprägten Fließgewässer Rhein könnte so beispielsweise das Selbstreinigungspotential gefördert werden.

Es konnte gezeigt werden, dass der Stoffffluss im Biofilm des Rheins in unserem Modellsystem von Protozoen dominiert wird, die im Jahresmittel mit ungefähr 78% zum Stoffumsatz beitragen. Die Kalkulationen suggerieren, dass die Protozoen die gesamte Bakterien- und Algenproduktion durch den Jahresgang konsumieren. Neben den Ciliaten ist die Makrofauna hinsichtlich Abundanz und Fressraten von großer Bedeutung für das System Biofilm. Eine weitere wichtige Erkenntnis der vorliegenden Studie ist, dass nur ungefähr 2% der im Biofilm umgesetzten Biomasse autochthon im Biofilm gebildet werden, die restlichen 98% werden direkt aus dem Pelagial importiert. Ein Teil dieser importierten Biomasse wird von den die Zusammensetzung der Protozoen dominierenden Filtrierern direkt aus dem Pelagial konsumiert, der andere Teil wird durch die ständige Wiederbesiedlung des Biofilms aus dem Plankton dem benthischen Lebensraum zugeführt. Diese Daten harmonieren mit dem für das Plankton des Rheins von Weitere et al. (2005) veröffentlichten Stoffffluss, der beschreibt, dass nur ein geringer Teil der im Pelagial produzierten Biomasse auch im Pelagial umgesetzt wird. Der größte Teil wird aus dem Pelagial ins Benthal exportiert. Im Fall der Ciliaten auf dem Biofilm verläuft der Stoffffluss umgekehrt. Die von den Cilaten im Biofilm aufgebaute Biomasse wird nur zu einem geringen Teil im Benthal auf dem Biofilm umgesetzt, der überwiegende Teil kann in das Pelagial exportiert werden. Dies stimmt mit

Untersuchungen von Scherwaß (2001) im Rhein überein, die nachgewiesen hat, dass ein großer Anteil der im Plankton des Rheins nachgewiesenen Ciliatenarten verdriftete benthische Arten sind.

Im Rahmen der vergleichenden Untersuchung zu der Zusammensetzung von Flagellatengemeinschaften in den Eishabitaten der Antarktis sollte die Frage geklärt werden, inwieweit sich in diesen speziellen Habitaten von den Flagellatengemeinschaften des Pelagials abgrenzbare Zusammensetzungen nachweisen lassen. Bisherige Untersuchungen haben beschrieben, dass die Zusammensetzung des „microbial food webs“ in den Eishabitaten vergleichbar mit der Zusammensetzung im Pelagial ist (Legendre et al., 1992; Garrison und Mathot, 1996). Insgesamt sind heterotrophe Flagellaten als typische Komponente der Eisfauna bekannt, bisher wurde ihre Zusammensetzung in den Eishabitaten jedoch nur wenig untersucht (Garrison et al., 2005). In dieser Studie konnte gezeigt werden, dass sich die Flagellatengemeinschaft in den untersuchten Lebensräumen des Eises vorwiegend aus benthischen Formen zusammengesetzt hat. Im Wasser-Eis-Gemisch (slush) im Bereich der lichtgesättigten Schollenränder konnte ein deutlich höherer Anteil an Choanoflagellaten als in den beiden anderen untersuchten Eishabitaten (Eiskern, Porenwasser) nachgewiesen werden. Der Biofilm im slush ist, wie die untersuchten Biofilme des Rheins von Filtrierern (Chonoflagellaten und Chrysomonaden) dominiert (im Mittel > 70%). Des Weiteren wurden Pedinellids, Bodonids und Euglenids nachgewiesen.

Durch die Ergebnisse dieser Dissertation konnte ein wesentlicher Beitrag zum Verständnis von Stoffflüssen in großen Fließgewässern geleistet werden. Zusammen mit den von Weitere et al. (2005) veröffentlichten Daten konnte die starke bentho-pelagische Kopplung und die wesentliche zehrende Rolle der von Protozoen dominierten Biofilme im Rhein nachgewiesen werden. Es konnte gezeigt werden, dass der hohe Import von planktischen Organismen den

benthischen mikrobiellen Stoffkreislauf im untersuchten System Biofilm im Rhein steuert. Die Studie unterstreicht die Rolle der benthischen Prädatoren als wichtigen strukturierenden Faktor für das planktische Nahrungsnetz. Die Daten belegen, dass die Biofilmgemeinschaften aus Bakterien, Algen und Protozoen die Lücke zwischen mikrobiellem und makrobiellem Nahrungsgewebe schließen.

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Abstract

Composition and succession of all major organism groups in microfilm in a flow channel in the river Rhine were examined in parallel over a period of 14 months. In addition, abiotic parameters were monitored (e.g. temperature, water level, chlorophyll content). Based on these data an additional laboratory experiments involving chromadorid nematodes and bdelloid rotatoria, the first model ever of matter flow in the biofilm of a large river could be developed, possibly representing an important contribution to the identification of relevant interactions in the biofilm system.

It could be demonstrated that the matter flow in biofilm of the river Rhine was dominated by protozoans - mostly filtrating species - in the investigated model system; they contributed around 78% to the mean annual metabolism. The most important representatives of heterotrophic flagellates were sessile choanoflagellates with abundances up to 100,000 individuals/cm². Throughout the year, ciliates were mostly dominated by peritrichous ciliates. With respect to individual count, metazoans were dominated by bdelloid rotatoria and chromadorid nematodes, representing together more than 90% of the metazoans during the study period. The densities of chromadorid nematodes were high, even up to 600 ind./cm².

Another important result of the current study was the proof of the strong benthic-pelagic coupling in the Rhine. It could be demonstrated that only approximately 2% of the biomass metabolized in biofilm was autochthonously synthesized in the biofilm itself; 98% were imported directly from the pelagial. This high import portion of planctonic organisms controls the benthic microbial metabolic cycling in the investigated system, i.e. biofilm in the Rhine, and therefore also represents an important structuring factor for the planctonic food web.

Sampling of ice habitats in the Antarctic demonstrated that also the communities of heterotrophic flagellates in Antarctic were primarily composed of benthic species. Biofilm in slush was dominated by filtrating species (choanoflagellates and chrysomonads) at the floe margins, being in agreement with the biofilm samples of the Rhine. Compared to individual counts in the pelagial, the individual density of protozoans in ice-related biofilms was significantly higher. It is suggested that the accumulation of organisms in the biofilms of ice-related habitats also in Arctic habitats plays an important role in the control of nutrient cycling.

Kurzzusammenfassung

Im Rahmen der Studie wurden Zusammensetzung und Sukzession aller wichtigen Organismengruppen im Biofilm (Algen, Bakterien, Flagellaten, Ciliaten, Meiofauna und Makrofauna) parallel über einen Zeitraum von 14 Monaten in einer Fließrinne im Rhein untersucht. Zusätzlich wurden abiotische Parameter erfasst (z.B. Temperatur, Wasserstand, Chlorophyllgehalt). Auf der Grundlage dieser Daten und der Ergebnisse zusätzlicher Laborexperimente mit chromadoriden Nematoden und bdelloiden Rotatorien konnte erstmalig eine Modellvorstellung für den Stofffluss in einem Biofilm eines großen Flusses entwickelt werden. Das aufgestellte Modell kann einen wichtigen Beitrag zur Identifizierung der wesentlichen Interaktionen innerhalb des Systems Biofilm leisten. Es konnte nachgewiesen werden, dass der Stofffluss im Biofilm des Rheins im untersuchten Modellsystem von Protozoen dominiert wurde, die im Jahresmittel mit ungefähr 78% zum Stoffumsatz beigetragen haben. Die Protozoen wurden hierbei von filtrierenden Formen dominiert. Bei den heterotrophen Flagellaten waren sessile Choanoflagellaten mit Abundanzen von bis zu 100.000 Individuen/cm² die wichtigsten Vertreter. Die Ciliaten wurden im Jahresgang zumeist von peritrichen Ciliaten dominiert. Die Metazoen wurden hinsichtlich Individuenzahl von bdelloiden Rotatorien und chromadoriden Nematoden dominiert, die über den gesamten Versuchszeitraum mehr als 90% der Metazoen stellten. Dabei bildeten die chromadoriden Nematoden Individuendichten von bis zu 600 Ind./cm² aus.

Ein weiteres wichtiges Ergebnis der Studie war der Nachweis der starken bentho-pelagischen Kopplung im Rhein. Es konnte aufgezeigt werden, dass nur ungefähr 2% der im Biofilm umgesetzten Biomasse autochthon im Biofilm gebildet wurden, die restlichen 98% wurden direkt aus dem Pelagial importiert. Dieser hohe Import von planktischen Organismen steuert den benthischen mikrobiellen Stoffkreislauf im untersuchten System Biofilm im Rhein und wird somit auch für das planktische Nahrungsnetz zu einem wichtigen strukturierenden Faktor. Die Beprobung von Eishabitaten in der Antarktis ergab, dass sich auch die Gemeinschaften der heterotrophen Flagellaten in den dortigen Biofilmen vorwiegend aus benthischen Formen zusammensetzt haben. Der Biofilm im Wasser-Eis-Gemisch (slush) an den Schollenrändern war, wie die untersuchten Biofilme des Rheins, von Filtrierern (Chonoflagellaten und Chrysomonaden) dominiert. Die Individuendichte der Protozoen in den eisangebundenen Biofilmen war im Vergleich zu den Individuenzahlen im Pelagial deutlich erhöht. Es kann unterstellt werden, dass die Akkumulation von Organismen in den Biofilmen der eisangebundenen Habitate auch in den arktischen Lebensräumen eine wichtige steuernde Größe innerhalb der Stoffkreisläufe einnimmt.

Erklärung

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

Markus Eßer