Retention Effects of Biofilms in the River Rhine

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“The river has great wisdom and whispers its secrets to the hearts of men.” - *Mark Twain*

for Mom and Dad

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Zusammenfassung

Der Rhein ist eine der wichtigsten Schifffahrtsstraßen in Europa und war in der Vergangenheit von Veränderungen des Flussbettes in Form von Kanalisierungen und Begradigungen betroffen. Hierdurch wurden Habitate für Flora und Fauna des Flusses verringert bzw. verändert, so auch die Oberflächen auf denen Biofilme wachsen. Biofilme sind in allen aquatischen Ökosystemen zu finden und bevölkern die Kontaktschicht zwischen Wasser und verschiedenen Substraten. Letztere können anorganisch sowie organisch sein und werden primär von Bakterien besiedelt, welche die Bildung von Biofilmen initiieren. Durch die Ausscheidung von extrazellulären polymere Substanzen (EPS) der Bakterien können sich andere Organismen an die Biofilme anheften - in Flüssen sind dies Algen, Flagellaten, Ciliaten und andere Organismen, die sich dann vom Biofilm und seinen Bewohnern, aber auch den Organismen und Partikeln des umgebenden Wassers ernähren. Biofilme bilden als Lebensraum ein Bindeglied zwischen Pelagial und Benthos und spielen daher in Flüssen eine wichtige Rolle.

In einer Modelluntersuchung über ein Jahr wurde der Effekt der Biofilme und deren Organismen auf die Abundanzen verschiedener Organismen des Pelagials im Rhein in Köln als Beispiel eines großen europäischen Flusses der Potamalregion untersucht. Durch das Design des Versuchsaufbaus konnte ein Einfluss der Makrofauna eliminiert werden. Dieser wurde bisher die größte Bedeutung auf die Reduktion von planktischen Organismen in Flüssen zugesprochen. Die Biofilme konnten die Bakterienabundanz im durchströmenden Wasser um 22 - 63 % (durchschnittlich 47 %) reduzieren und somit durchschnittlich $10{,}9 * 10^3$ Liter Wasser pro Quadratmeter Biofilm am Tag von Bakterien bereinigen. Darüber hinaus betrug der Rückhalt von Phytoplankton 30 % und von planktische heterotrophe Flagellaten 26 % im Jahresmittel, woraus sich eine Eliminationsrate von $6{,}7 * 10^3$ L m^{-2} d^{-1} für das Phytoplankton und $5{,}8 * 10^3$ L m^{-2} d^{-1} für planktische heterotrophe Flagellaten ergab. Bei der Untersuchung der Biofilme stellte sich heraus, dass vor allem peritriche und heterotriche Ciliaten für die Reduktion von Bakterien aus dem Pelagial verantwortlich sein sollten. Ein weiterer wichtiger Bestandteil des Biofilmbiovolumens stellten zentrische und pennate Diatomeen dar, die sich aus dem Pelagial des Rheins an den Biofilm angeheftet haben und so die komplexe dreidimensionale Struktur der Biofilme förderten.

In einem weiteren Teil der Arbeit wurde untersucht, ob die dreidimensionale Struktur sowie die Organismen des Biofilms auch einen Einfluss auf Mikroplastikpartikel in Fließgewässern haben können und ob ein Transfer von Mikroplastikpartikeln über das Nahrungsnetz in höhere trophische Ebenen möglich ist. Die Ergebnisse der Untersuchungen zeigten, dass Biofilme eine signifikant höhere Abundanz von Mikroplastikpartikel gegenüber anderen getesteten Substraten aufwiesen und bis zu 10.000 Partikel pro Quadratzentimeter Biofilm eingebettet werden können. Es zeigte sich auch, dass unter den verschiedenen getesteten Größen von Mikroplastikpartikeln (1 µm, 6 µm und 10 µm) die Abundanz in den Biofilmen mit der Größe der Mikroplastikpartikel anstieg. Aufnahmeexperimente mit dem Ciliaten *Stentor coeruleus* als im Rhein dominierenden Modellorganimus ergaben eine hohe Aufnahmerate der größeren Partikel. Es konnte weiterhin gezeigt werden, dass Ciliaten, die Mikroplastikpartikel enthielten, durch Gammariden bzw. Fischlarven konsumiert werden und so eine Weitergabe von Mikroplastikpartikeln in der Nahrungskette erfolgt.

Die Ergebnisse der vorliegendenden Arbeit unterstreichen die Rolle von Biofilmen im Ökosystem Fluss. Sie sind eine sehr wichtige Senke für Bakterien (incl. Krankheitserreger). Die Untersuchungen unterstreichen die Notwendigkeit, im Management von Flussökosystemen besonderes Augenmerk auf den Erhalt bzw. die Vergrößerung von Oberflächen in Flüssen zu legen. Auf der anderen Seite werden in den Biofilmen Plastikpartikel akkumuliert deren Auswirkung in künftigen Untersuchungen weitere Aufmerksamkeit geschenkt werden muss.

Abstract

The River Rhine is one of the most important navigation routes in Europe and has been affected by changes of the riverbed, in the form of canalization and straightening in the past. These changes have reduced and changed habitats for the river's flora and fauna, including the surfaces on which biofilms grow. Biofilms are found in all aquatic ecosystems and populate the contact layer between water and various substrates. These can be inorganic or organic substrates and are first colonized by bacteria, which initiate the formation of biofilms. The bacteria's excretion of extracellular polymeric substances (EPS) allows other organisms to attach to biofilms; in rivers, these are algae, flagellates, ciliates and various other organisms, which then feed on the biofilm and its inhabitants but also on the organisms and particles of the surrounding water. Biofilms as a habitat thus form a link between pelagic and benthic systems and therefore play an essential role in rivers.

In a model study over a period of one year, the effect of biofilms and their organisms on the abundances of various pelagic organisms was investigated using the River Rhine at Cologne as an example for a large European river in the potamal region. The design of the experimental setup eliminated the influence of macrofauna, which was previously thought to have the most significant impact on the reduction of planktonic organisms in rivers. The biofilms reduced the bacterial abundance in the water flowing through by 22 - 63 % (47 % on average) and thus cleaned an average of $10.9 * 10^3$ liters of water per square meter of biofilm per day of bacteria. In addition, the retention of phytoplankton was 30 % and of planktonic heterotrophic flagellates 26 % on an annual average, resulting in an elimination rate of $6.7 * 10^3$ L m^{-2} d^{-1} for phytoplankton and $5.8 * 10^3$ L m^{-2} d^{-1} for planktonic heterotrophic flagellates, respectively. Examinations of the biofilms revealed that especially peritrich and heterotrich ciliates were most likely responsible for reducing bacteria from the pelagic. Centric and pennate diatoms represented another significant component of the biofilm biovolume attaching to the biofilm from the pelagic of the River Rhine and promoting the complex three-dimensional structure of the biofilms.

In a different part of the study, it was investigated whether the three-dimensional structure and the organisms of the biofilms can affect the retention or accumulation of microplastic particles and whether a transfer of microplastic particles via the food web to

higher trophic levels is possible. The results of the experiments showd that biofilms had a significantly higher abundance of microplastic particles incorporated than other tested substrates, and up to 10,000 particles per square centimeter of biofilm could be embedded. Among the different size classes of microplastic particles tested (1 µm, 6 µm and 10 µm), the abundance in biofilms increased with the size of microplastic particles. Feeding experiments with the model ciliate *Stentor coeruleus* dominating in the River Rhine showed high rates of uptake for larger particles. Further, it was shown that ciliates containing microplastic particles were ingested by gammarids and fish larvae resulting in the transfer of microplastic particles up the food chain.

The findings of the study highlight the role of biofilms in the river ecosystem. They serve as important sinks for bacteria (including pathogens). Further, they underline the necessity to preserve and enlarge surfaces for biofilm growth in river systems. On the other hand, microplastic particles can accumulate in biofilms, the consequences of this process still needs further investigations.

1. Introduction

River Biofilms

"A river is water in its loveliest form, rivers have life and sound and movement and infinity of variation, rivers are veins of the earth through which the lifeblood returns to the heart." – Roderick Haig-Brown.

From the earliest times, rivers played an essential role for humanity. They are sources of food and water, protective barriers and transport routes; therefore, many people settled and formed villages near rivers, which are the basis for many cities today. Documentations from the 13th century show that humans impacted the river ecosystem and its inhabitants, here in the form of the disappearance of beavers on the Odra River (Mielzarek, 2019). With time and the introduction of navigation to transport various goods, rivers became more and more canalized to be passable for large transport ships (Ackermann et al., 2011, Weitere et al., 2003). European rivers connect the mainland with the Atlantic, North and Baltic Seas, as well as the Black Sea and the Mediterranean (Wollenberg et al., 2019). Therefore, the River Rhine is one of the most essential and largest waterways in Europe and is affected in many areas by reconstruction and canalization. These changes of the riverbed increase the flow velocity and lead to losses in the ecosystem and surfaces, which contribute to the change and reduction of the biodiversity of fauna and flora (Kinzelbach, 1983, 1987).

Due to the reduction of habitats by canalization and the resulting higher flow velocity, the introduction of invasive species, and climate change, flora and fauna of the River Rhine constantly have to adapt to the new conditions. However, this adaptation is not always possible and can lead to the decimation of biodiversity. One example for the River Rhine near Cologne is the introduction of the mussel *Corbicula* spp. in the 1980s, which displaced the until-then dominant species *Dreissena polymorpha*, and the introduction of the amphipod *Corophium curvispinum*, which has no natural enemies in the River Rhine and can cope with rising water temperatures (Den Hartog et al., 1992; Karatayev et al., 2005; Vohmann et al., 2010). Climate change is another factor, and increasingly frequent extreme weather events, such as droughts or floods, can impact river flora and fauna. The mentioned mussels, for example, stop their filtering function at high water temperatures (>28 °C) and may also die (Matthews and McMahon, 1999; Belz et al., 2004; Vohmann et al., 2009; Kathol et al., 2009). So far, Bivalvia have been considered the main link between pelagic and benthos of rivers and the main consumer of pelagic bacteria (Welker and Walz, 1998; Caraco

et al., 2006). In this study, we want to show that biofilms can play an essential role in the benthic-pelagic coupling as Bivalvia do and that biofilm organisms can adapt to changing water conditions faster, stay active and consume bacteria also during periods of difficult conditions as high water temperatures.

The Rhine originates in the mountains of Switzerland and reaches the North Sea in the Netherlands in several estuaries. Along the way, the Rhine has several tributaries, such as the Moselle, which, through their inflow, increase the discharge of the Rhine and bring in organisms, nutrients, pollutants and waste (Den Hartog et al., 1992). The above mentioned alterations of the riverbed are also present at the study site in Cologne, leading to a mean flow velocity of 1.5 m s^{-1} and the mean discharge of 2,000 m^3 s^{-1} (Ackermann et al., 2011; Weitere et al., 2003) making it a typical model river that is used for navigation. The canalization also reduces the surfaces for biofilm growth. Previous studies have shown how important biofilms can be for the water quality of large rivers (Monaghan et al., 2001; Arndt et al., 2003; Weitere et al., 2003; Kathol et al., 2009; Ackermann et al., 2011; Weitere et al., 2018). We conducted our experiments on the float of the Ecological Rhine Station of the University of Cologne (see Fig. 2.1. in chapter 1), which is located in the main stream of the River Rhine and offers the possibility to conduct experiments *in situ* under natural conditions. The experimental setup is filled with glass spheres and mimics a natural habitat like the riprap. Artificial substrates have proven to be suitable for biofilm growth (e.g., Norf et al., 2007; Kathol et al., 2009). The riprap is exposed to large water level fluctuations, which could be circumvented in the experimental setup, as the Ecological Rhine Station follows the water level and thus ensures more constant conditions for the biofilms (Brabender et al., 2016; Brauns et al., 2019).

Biofilms can form whenever a substrate (e.g. rocks, plants, wood, plastic, larger organisms) is in contact with water. Thus, they are found in all aquatic ecosystems and also play a significant role in rivers. For the formation of biofilms, attachments of bacteria from the pelagial are fundamental. They can attach to various substrates and secrete extracellular polymeric substances (EPS), serving also as adhesives for other organisms from the pelagial. Thus, algae, heterotrophic flagellates, ciliates, and meiofauna are gradually colonizing the biofilm and forming the biofilm's typical three-dimensional structure (see Fig. 1.1. from Egan et al., 2013; Costerton et al., 1987; Vasudevan, 2014). The three-dimensional structure

consists of complex pits, burrows and tunnels, which serve as microhabitats for various organisms and sinks for different organic and inorganic materials. Water flows through the microhabitats, providing a constant supply of nutrients, but strong water flow can also cause parts of the biofilm to tear off, allowing it to reattach and re-colonize elsewhere in the river. Further, they are part of the energy flux of the river by being and providing food sources for higher trophic levels in the food web of rivers (Arndt et al., 2003; Battin et al., 2003; Kathol et al., 2011; Weitere et al., 2018). The biofilm organisms can feed on the biofilm components and take up bacteria, algae or heterotrophic flagellates from the pelagic (Weitere et al., 2003; Ackermann et al., 2011). For example, various ciliates of the biofilm filter bacteria from the pelagic and can contribute to the self-purification of the river (Kathol et al., 2011). This role was previously attributed primarily to Bivalvia, although ciliates may have an equal or even higher suspension-feeding activity due to their high abundance and high contribution to the biovolume of the biofilm (Arndt et al., 2003; Weitere et al., 2003, 2005).

Not only can the canalization of rivers have an impact on its water quality and its self-purification potential, but so can the introduction of bacteria and pathogens via effluents from wastewater treatment plants and nonpoint sources as agriculture (Mallin et al., 2000; Jokinen et al., 2010; Wu and Chen, 2013; Prasad et al., 2015; Tillburg et al., 2015). In addition, due to human-induced climate change, extreme events, such as floods or draughts, occur more frequently, which can lead to high and low water levels but also change water temperature conditions (Belz et al., 2004; Huang et al., 2015; Wolff et al., 2021). These changes can impact the activity of various organisms in the river, especially bivalves (Matthews and McMahon, 1999; Belz et al., 2004; Vohmann et al., 2009). However, the biofilm organisms can adapt and remain active even under extreme conditions, thus contributing to the self-purification of the river (Kathol et al., 2009, 2011; Viergutz et al., 2007). To quantify this potential effect of biofilms was one of the major aims of the present study.

Thinking on the ability of biofilms to retain bacteria from the pelagial, it can be assumed that biofilms and their organisms also have an impact on microplastic particles from the pelagic, as they are similar in size to bacteria and smaller protozoa.

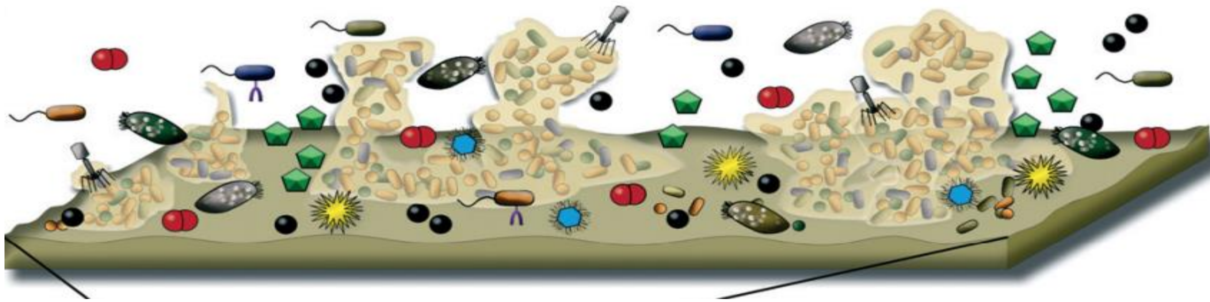

Fig. 1.1. Schematic drawing of a biofilm with different inhabitants (from Egan et al., 2012)

Microplastics

Plastic in the environment has been a big problem for some decades, and it is getting bigger, although people's attention is increasingly drawn to this problem. The media are full of reports on plastic and microplastic pollution of the oceans, and also research is focused on effects of plastic pollution. However, plastic and microplastics in particular can be found in a wide variety of ecosystems, for example, in the oceans, in soils, air and rivers, but also in artic ice (Woodall et al., 2014; Van Cauwenberghe et al., 2015; Horton et al., 2017; Peeken et al., 2018; Evangeliou et al., 2020). According to the currently valid definition, microplastic is smaller than 5 mm (Arthur et al., 2009); however, the definition of microplastic is still under discussion, and some scientists suggest that only particles smaller than 1 mm should be considered microplastic (Van Cauwenberghe et al., 2015; Dekiff et al., 2014); in this study the focus will be on these small microplastic particles and their fate in a large riverine system. Microplastics enter rivers in three ways: 1) as microplastic particles from e.g. sewage treatment plants, 2) as part of macroplastic, e.g. tire abrasions via precipitation and runoff or 3) as macroplastic, which fragments into microplastic due to the river currents or substrates (see Fig. 1.2. from Bertling et al., 2018; Auta et al., 2017).

The fate and distribution of microplastics in rivers are poorly understood. Rivers can transport microplastics to the sea, and along the way, they can also settle in sediments, but it is unknown which effects microplastics have on the ecosystem river and its organisms (Dris et al., 2015; Besseling et al., 2017; Lebreton et al., 2017). In this study, we want to find out if biofilms influence the retention of microplastic particles. Because of their size, which is

similar to bacteria and protozoans, microplastic particles might be embedded in grooves or channels of the complex three-dimensional structures of biofilms and might stay there for some time. Thus, the structure of the biofilm might change and protective areas for bacteria or heterotrophic flagellates, for example, might be occupied for a longer period of time until the microplastic particles get detached from the biofilms. Further, the organisms of the biofilms might ingest microplastic particles attached to the biofilm or from the surrounding river water. Due to the embedding and ingestion, microplastic particles might be retained and therefore not transported further ending up in oceans (Besseling et al., 2017). Another aspect of the impact of biofilms on microplastics is that attachment to the biofilm and uptake by organisms can enable the transport within the food web of the river and microplastic particles can thus enter higher trophic levels as fish and ultimately be ingested by humans (Yokota et al., 2017). To investigate these possible pathways of microplastics in rivers, it was intended to grow biofilms *in situ* under natural conditions on the float of the Ecological Rhine Station. Furthermore, feeding experiments with model ciliates of the River Rhine should indicate the transfer of microplastics to higher trophic levels.

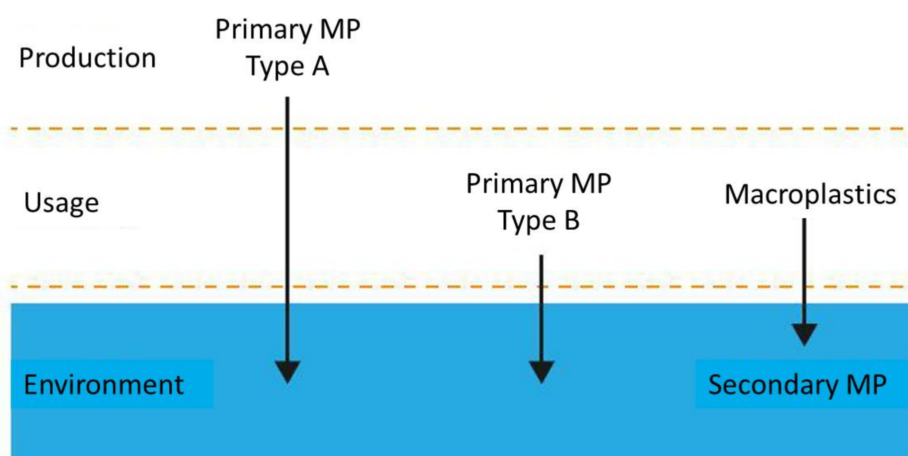

Fig. 1.2. Description of three types of development of microplastics (MP; edited from Bertling et al., 2018)

Aims

The main purpose for this study was the investigation of biofilms, their organisms and their effects on the quality of the surrounding river water. For this aim, biofilms grown *in situ*

were studied and the first part of the study addressed the following questions. Which effects do biofilms and their organisms have on the bacteria, algae and heterotrophic flagellates of the pelagic when they pass the biofilms? Do the changes in the water conditions during the year influence the effects of the biofilm?

Furthermore, the ever increasing problem of microplastics in rivers required investigations of the effect of biofilms on microplastics in the second part of this study. Here the questions were asked if the complex three-dimensional structures of biofilms play a role for the retention of microplastic particles of the pelagial? Is the heterotrich ciliate *Stentor* sp., which occurs frequently on the biofilms of the River Rhine, able to take up microplastic particles? And is a transfer of microplastic particles to higher trophic levels via the river food web possible? In addition, a literature review was conducted on the topic of microplastics in relation to Protozoa.

Summary

This dissertation is dealing with the effects of biofilms on planktonic organisms of the River Rhine and the effects of biofilms and its organisms on the retention of microplastics. The experimental approach is quite dissimilar, *in-situ* and laboratory studies, so that the effects on planktonic organisms and the effects on microplastics by biofilms are treated separately.

The studies on effects of biofilms and protozoa on microplastics were carried out in close cooperation with Leandra Hamann (supervised by Prof. A. Blanke). Felicia Haase and Massimo Thiel assisted in carrying out the experiments as part of their final theses.

For an easier assignment of the references, these are provided respectively for effects of biofilms on planktonic organisms, the effects of biofilms and its organisms on the retention of microplastics and for the general introduction and conclusion. Further, the numbering of the figures and tables has been adjusted to the numbering of the chapters, so that that of the tables starts at 2.

2. Retention effects of biofilms on planktonic organisms in the River Rhine

2.1 Abstract

River biofilms are communities of bacteria, algae, protozoans, and metazoans. The extracellular polymeric structures secreted by bacteria and algae create a three-dimensional structure that serves as a habitat for various microbial organisms. It is well known that many eukaryotes inhabiting biofilms are grazers of bacteria. In the present study we show that this bacteria consumption reaches quantitatively important values. In a seasonal study of the River Rhine at Cologne, we could show that the biofilm showed an annual average retention effect of about 22 – 63 % (average 47 %) regarding the elimination of the surrounding planktonic bacteria. Moreover, peritrich ciliates were estimated to be by far the most important consumers of bacteria. In addition, protists on the biofilm were able to consume significant amounts of phytoplankton (average 30%) and other small protists as e.g. planktonic heterotrophic flagellates (average 26%). Up to now, mostly filter-feeding macrofauna (e.g. bivalves) were considered to be important consumers of planktonic microbes in rivers. During our study, estimates of the clearance rate of the biofilm of the River Rhine at Cologne resulted in in average values of $10.9 * 10^3$ L m^{-2} d^{-1} for bacteria, $6.7 * 10^3$ L m^{-2} d^{-1} for phytoplankton and $5.8 * 10^3$ L m^{-2} d^{-1} for planktonic heterotrophic flagellates. The results of the present study emphasize the role of biofilm microbes in the benthic-pelagic coupling and the potential importance in reducing bacteria in rivers, which may also lead to the reduction of pathogenic bacteria. Thereby, an increase of the surface structures of rivers can contribute significantly to the self-purification potential of rivers.

2.2 Introduction

'House of biofilm cells', 'Biofilm, city of microbes' or 'Microbial landscape' are just some descriptive names for the complexity of biofilms (Watnick and Kolter, 2000; Battin et al., 2007; Flemming et al., 2007). Biofilms are ubiquitous and can be found in almost any aquatic ecosystem. They can form on surfaces of various substrata (e.g. stones, wood, plants, etc.) by the colonization of bacteria, which form colonies and excrete extracellular polymeric substances also referred to as EPS (formerly called 'glycocalyx' e.g. in Costerton et al., 1981). The EPS support the settlement of a variety of organisms including algae, amoebae,

heterotrophic flagellates, ciliates, rotifers, nematodes, oligochaetes, insect larvae, and small crustaceans (Costerton et al., 1987; Vasudevan, 2014). The interacting resident organisms of biofilms form food sources for other trophic levels, thus playing an important role for the entire ecosystem. Biofilms also play a fundamental part in the energy flux of aquatic environments (Arndt et al., 2003; Battin et al., 2003; Kathol et al., 2011; Weitere et al., 2018). Organisms on biofilms not only feed on benthic but also on the pelagic matter and thus can influence organisms and their abundances in the open water of rivers (e.g. Weitere et al., 2003; Ackermann et al., 2011). Previously, most of the participation in the benthic-pelagic coupling was attributed to bivalves (Welker and Walz, 1998; Caraco et al., 2006). However, consecutive studies showed that ciliates living in biofilms (especially peritrichs and heterotrichs) also have a significant impact on the pelagic community (e.g. Kathol et al., 2011). In terms of their heterotrophic biomass, ciliates often dominate biofilms and their suspension-feeding activity can exceed that of macroinvertebrates or may also have an even greater influence on the benthic-pelagic coupling (Arndt et al., 2003; Weitere et al., 2003, 2005)

The important role of biofilms in the material loop in river ecosystems, indicate their importance for the self-purification of rivers (Monaghan et al., 2001; Weitere et al., 2003; Kathol et al., 2009). Anthropogenic influences, such as straightening of river beds or effluents from wastewater treatment plants and non-point sources from agriculture, have limited the rivers' self-purification potential (Mallin et al., 2000; Jokinen et al., 2010). Furthermore, these sources can introduce bacteria and pathogens whose abundances can occasionally reach critical levels (e.g., Jokinen et al., 2010; Wu and Chen, 2013; Prasad et al., 2015; Tillburg et al., 2015). Additionally, the anthropogenically induced global changes have increased the occurrence of flood events as well as draught events with extremely low water levels (Belz et al., 2004; Huang et al., 2015; Wolff et al., 2021). These extreme events can limit the activity of the organisms in rivers and can also reduce the abundance of certain species due to the drastic abiotic changes in river conditions under these circumstances (Matthews and McMahon, 1999; Belz et al., 2004). Some macrozoobenthic organisms (e.g. bivalves) cannot cope with such exceptional conditions, resulting in reduced or even stopped filtration activity, e.g. when the water temperature reaches critical values (Matthews and

McMahon, 1999; Belz et al., 2004; Vohmann et al., 2009; Kathol et al., 2009). Interestingly, this is not the case for biofilm organisms, as they can continue their activity even at comparatively higher temperatures (Kathol et al., 2009; Viergutz et al., 2007) and can moreover adapt more flexibly to changed conditions (Kathol et al., 2011).

The aim of the present study was to analyze the influence of the protozoan and meiofauna community in biofilms on the reduction of the potamoplankton of the River Rhine. The River Rhine is one of the largest European rivers and functions as an important waterway serving as a model system for river systems that are intensively influenced by anthropogenic activities such as straightening of the river bed, the influence of a high numbers of water treatment plants associated with the river as well as warming effects by power plants (Weitere and Arndt, 2002; Ackermann et al., 2011; Kathol et al., 2011). This influence is reflected e.g. by the comparatively high flow velocity at Cologne (which may reach up to 2 m s^{-1}), significant changes of the water level which can reach 9 m within a year, and water temperatures which may be as high as 29 °C and fall not below 2 °C (Belz et al., 2004).

The in-situ methodical approach presented here was intended to answer the following questions: 1) Is there a retention effect by defined biofilms on the abundance of plank-tonic bacteria, on heterotrophic protists, and algae passing the biofilm under field conditions? 2) Are there long-term and seasonal changes in the retention effect?

To answer these questions, we conducted long-term in-situ studies from a platform exposed in the main flow of the River Rhine, allowing the establishment of natural biofilm communities under field conditions. We used a flow-through system that allowed the quantification of organisms suspended in the water and passing the biofilm to estimate the clearance of organisms passing a certain area of biofilm.

2.3 Material and methods

2.3.1 Study site and experimental setup

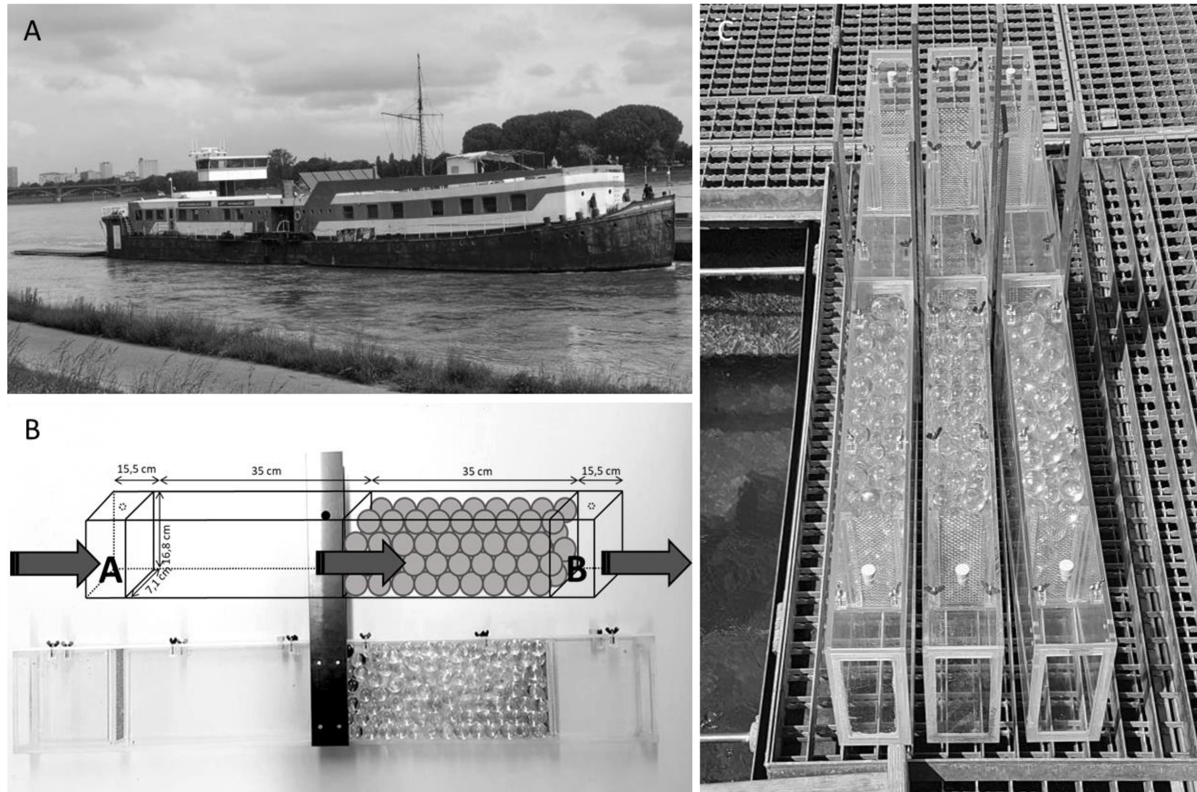

Fig. 2.1. Ecological Rhine Station of the University of Cologne with attached raft (Rhine km 684.5, Cologne, Germany) (A) and experimental setup of Plexiglas channels each filled with 200 glass spheres (diameter 25 mm) and a 2 mm by 2 mm mesh behind sample spot A in the first half of the Plexiglas channels (B, C).

The River Rhine is about 1,230 km long and has an average discharge of about 2,000 $m^3 s^{-1}$ and a mean flow velocity of 1.5 $m s^{-1}$ at Cologne. The study took place at the Ecological Rhine Station of the University of Cologne (Rhine kilometer 684.5, Fig. 2.1. A) from April 2018 to May 2019. The station floats in the main stream (outer bank) of the river and holds a raft with two differently sized channels, which lie in the water of the river and enables experiments under field conditions (Fig. 2.1. A).

The experimental setup consisted of three identical Plexiglas channels of 101 x 16.8 x 7.1 cm with open ends (Fig. 2.1. B, C). For sampling, the lid of the Plexiglas channels could be opened and closed via 6 screws. The entire setup was covered with an opaque PVC plate to reduce algae growth and prevent clogging of the channels. A 2 * 2 mm

mesh at both ends of the channels ensures that larger organisms (e.g. *Dreissena* sp., *Corbicula* sp., insect larvae, amphipods, etc.), do not interfere with the biofilm. The second halves of the channels are filled with 200 glass spheres of a diameter of 25 mm. One month prior to sampling the he channels were exposed to the river to ensure biofilm growth. Glass spheres with a diameter of 25 mm were chosen as substrates for the biofilms in the experimental setup (Fig. 2.1. B). The experimental setup was intended to mimic the situation of the river bottom. A long-term study conducted by Esser (2006) at the same sampling site showed that the community structure on glass surfaces was comparable to the nearby river bottom community. Furthermore, the setup was covered with an opaque PVC sheet to represent the by far prevailing darker parts of the river (deeper than 0.2 m).

2.3.2 Sampling procedure

Once a week, bacteria plankton samples were taken from sample spot A, before the glass spheres, and from sample spot B, behind the glass spheres, from each of the three Plexiglas channels weekly between June 28th, 2018 and May 23rd, 2019 (Fig. 2.1. B). Samples of 50 ml were immediately fixed after sampling with formaldehyde (end concentration 2 %) and, after 2 - 3 days of storage at 4 °C, stained with DAPI (0.01 µg/ml end concentration; Porter & Feig, 1980). 4 ml of the stained samples were filtered onto black membrane filters (0.2 µm pore size, Nucleopore: Whatman, Newton, TX, USA) and stored at -20 °C until the quantifications of bacteria abundances. Quantifications were conducted with an epifluorescence microscope (Zeiss Axio Lab.A1, 1,000x magnification). For each sample, approximately 400 bacteria were measured and assigned to six size classes (cocci: 0.5 * 0.5 µm, 0.25 * 0.25 µm; rods: 0.5 * 0.25 µm, 1 * 0.5 µm, 2 * 0.5 µm, > 2 * 0.5 µm).

For the analysis of heterotrophic flagellates and algae, five replicate plankton samples were taken at the sample spots A and B of the three Plexiglas channels, sampling started on May 24th, 2018. Live-counting of protists was carried out in 5 µl subsamples immediately after sampling on board the Ecological Rhine Station using microchambers and the live-counting method (Arndt et al., 2000; Jeuck et al. 2017) and a Zeiss Axiostar Microscope (phase contrast; 200 - 400x magnification). Heterotrophic flagellates, algae, ciliates,

amoebae and actinophryid heliozoans were classified into taxonomic groups (mostly to the genus level according to Foissner et al., 1991; Foissner et al., 1992; Foissner et al., 1994; Foissner et al., 1995; Foissner and Berger, 1996; Foissner et al., 1999; Jeuck and Arndt, 2013; Linne von Berg et al., 2004).

Biofilm samples were taken in weekly intervals from May 24th, 2018 until May 23rd, 2019. Glass spheres were removed from each of the three channels (and replaced with a new ones) and the biofilm was gently detached using a toothbrush and immediately diluted in 20 - 50 ml filtered (< 0.2 µm) river water. Three subsamples of every glass sphere were microscopically analyzed in microchambers by live-counting as described above.

Information on the water levels were provided by the "Wasserstraßen- und Schifffahrtsverwaltung des Bundes" (https://www.elwis.de), information on temperature, conductivity, oxygen content, and flow velocity were obtained from "Bundesanstalt für Gewässerkunde" (http://undine.bafg.de).

2.3.3 Calculation of clearance rate

For the measurement of the width of the River Rhine, 14 points were measured in a GIS map (at the average water level). For the flow velocity (*FV*, m s^{-1}), the water discharge (*WD*, m^3 s^{-1}) was divided by the water level (*WL*, m) and the mean River Rhine width of the 14 measured points (*RW*, m).

$$FV = WD / WL / RW \quad (1)$$

Retention (*R*, %) is defined here as the consumption of planktonic organisms by biofilm-dwelling organisms and is estimated by subtracting the abundance of plankton at sample spot *A* before the biofilm from that at sample spot *B* behind the biofilm (Fig. 2.1. B).

$$R = 100 - (B / A \times 100) \quad (2)$$

Volume of water (*V*, L m^2 d^{-1}) in contact with biofilm (*BF*, here 0.73 m^2) per day was determined at 14th of June, 2018 using Uranin. Since the flow velocity in the river did not dramatically change in the course of the experimental period, a linear relationship between flow velocity (*FV*) and contact volume was assumed as a rough estimate.

$$V = (FV_{14th\ of\ June,\ 2018} / FV_{sampling\ day\ x}) \times L \times BF / d \quad (3)$$

The clearance rate (*CR*) is the volume of water which is cleared of plankton organisms by the biofilm and calculated by the amount of water flowing through the channels each day (*V*) multiplied with the retention effect (*R*)

$$CR = V \times R \qquad (4)$$

2.3.4 Statistical evaluations

Statistical analyses were carried out using R 3.5.0 for Windows (https://cran.r-project.org/bin/windows/base/old/3.5.0/). Retention effects were calculated using the difference of the mean values between sampling spots A and B. An ANOVA was used to evaluate the differences (package “lawstat”; $*p < 0.05$). Shapiro test and Levene tests were previously performed for analysis of normally distributed values and homogenous variances. Correlation (Spearman) analyses were conducted between the retentions of algae, flagellates, and bacteria and abiotic parameters of the river water and the abundances of certain organisms on the biofilm (package “ggplot”; $*p < 0.05$, $**p < 0.01$).

2.4 Results

2.4.1 Water parameters

The summer and late autumn in 2018 were characterized by extreme conditions such as a water temperature maximum of 27.2 °C in August and a minimum water level of 71 cm in October 2018. This water level was the lowest ever recorded at Cologne since beginning of the official measurements. During the investigation period, the minimum temperature of 4.1 °C occurred in January 2019 and the highest water level of 562 cm in March 2019 (mean water temperature 15.4 ± 6.9 °C; mean water level 236 ± 123 cm; Fig. 2.2. A, B, Tab. 4.1. in supplementary material). The minimum water discharge occurred at the time of the lowest water level (569 $m^3 s^{-1}$ in October 2018); the maximum water discharge occurred at one of the high-water level events during investigation period exceeding to 3700 $m^3 s^{-1}$ in March

2019 (mean water discharge 1483 ± 750 $m^3 s^{-1}$; Fig. 2.2. A, Tab. 4.1.). In the course of the experimental period, the conductivity fluctuated between 385 µS cm^{-1} (March 2019) and 710 µS cm^{-1} (February 2019) with a mean conductivity of 501 ± 72 µS cm^{-1} (Fig. 2 B). The oxygen content varied between 7 mg l^{-1} (August 2018) and 12.1 mg l^{-1} (January 2019; mean oxygen content 9.6 ± 1.4 mg l^{-1}; Fig. 2.2. B, Table 4.1.). The lowest oxygen saturation was 79.7 % (December 2018) and the highest 127.2 % (August 2018; mean oxygen saturation 97.3 ± 7.6 %; Fig. 2.2. B, Tab. 4.1.).

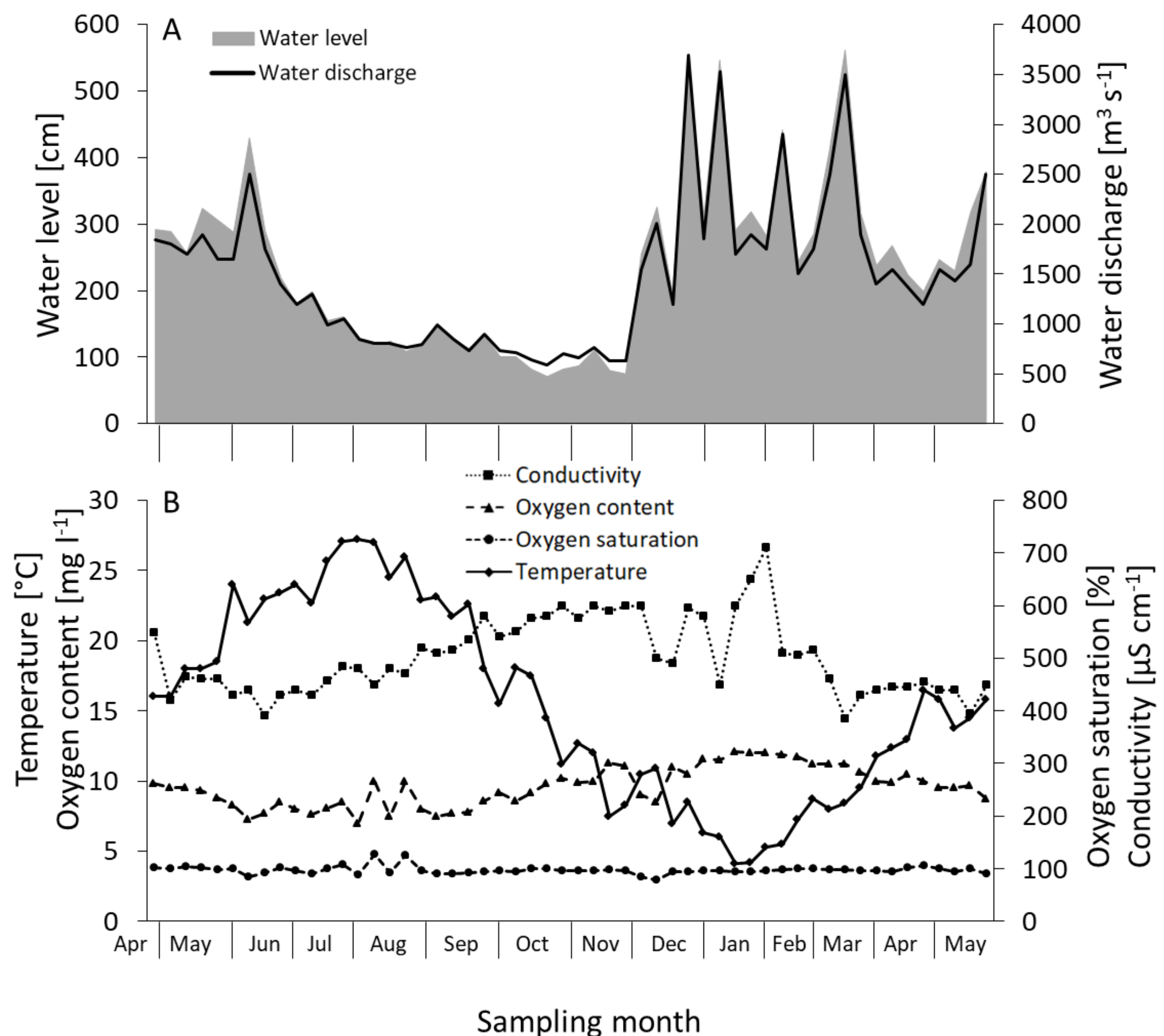

Fig. 2.2. Water parameters for the period April 2018 until May 2019: Water level (cm) and flow velocity ($m^3 s^{-1}$) (A); Temperature (C°), oxygen content (mg l^{-1}), oxygen saturation (%) and conductivity (µS cm^{-1}) (B). Data obtained from https://www.elwis.de and http://undine.bafg.de, detailed data in 6. Supplementary material Table 4.1.

2.4.2 Biofilm composition

The most abundant **ciliate** group regarding biovolume were oligohymenophoreans (mainly scuticociliates, vorticelliid ciliates, zoothamniid ciliates, and peniculids), especially during the winter months, when zoothamniid ciliates in large colonies were the most dominant ciliates (Fig. 2.3. A). Vorticelliid ciliates occurred mainly in spring and summer months. Another abundant group was heterotrichs, mainly represented by stentorid ciliates which occurred especially in the spring and summer. Due to their large size, they contributed a large part to the biovolume on the biofilm (Fig. 2.4. A). Other less abundant groups were spirotrichs (e.g. *Aspidisca*, *Euplotes, Holosticha,* and seldom also planktonic oligotrichs), suctorians (e.g. *Metacineta*) as well as litostomatids (e.g. *Lacrymaria, Litonotus*). These groups contributed only small portions to the biovolume of ciliates on the biofilm at some sampling dates (Fig. 2.3. A).

The **algae** abundance on the biofilm increased with the maturation of the biofilm and was dominated by centric (e.g. *Cyclotella*, *Melosira*, *Stephanodiscus*) (8 %, Fig. 2.4. A) and pennate diatoms (e.g. *Amphora*, *Asterionella*, *Cymbella*, *Gomphonema*, *Gyrosigma*, *Navicula*, *Nitzschia*, *Stauroneis*, *Synedra*) (10.6 %, Fig. 2.4. A) throughout the experimental period. Chlorophytes (e.g. *Ankistrodesmus*, *Chlamydomonas*, *Chlorella*, *Coelastrum*, *Crucigenia*, *Monoraphidium*, *Pediastrum, Scenedesmus*) and xanthophytes (e.g. *Tribonema*) were abundant only on single sampling days. Cryptophytes (*Cryptomonas*) were also present but contributed only a small portion of algae biovolume (Fig. 2.3. B).

Heterotrophic flagellates contributed only a minor part to the biofilm biovolume though abundant in the potamoplankton (Fig. 2.3. C). The most abundant heterotrophic groups regarding the biovolume were kinetoplastids, especially bodonids (e.g. *Bodo, Neobodo, Rhynchomonas*), and cryptomonads (*Goniomonas*). In a few cases, high choanoflagellate biovolumes were registered when large colonies of *Codosiga* sp. were present in the biofilm samples, more seldom salpingoecids occurred. Euglenids (e.g. *Anisonema, Entosiphon*) had high biovolume proportions on only a few sampling dates. Cercozoans, chrysomonads (different *Spumella*-like forms), and kathablepharids (*Kathablepharis*) were present in small proportions concerning the biovolume of the biofilm (Fig. 2.3. C).

Large unidentified **amoebae** occurred on the biofilms in the spring and summer months, while small species (< 10 µm; e.g. vannellids, mayorellids) were also present during the whole study period. Actinophryids (e.g. *Actinophrys*) were part of the biofilm during spring and summer months (Fig. 2.3. D). Metazoans were not always present in significant numbers, nematodes and rotifers (e.g. *Colurella*) contributed the largest part of the metazoan biovolume. Nematodes occurred more often in winter months. Oligochaetes (e.g. *Chaetogaster*) occurred only rarely (Fig. 2.3. E).

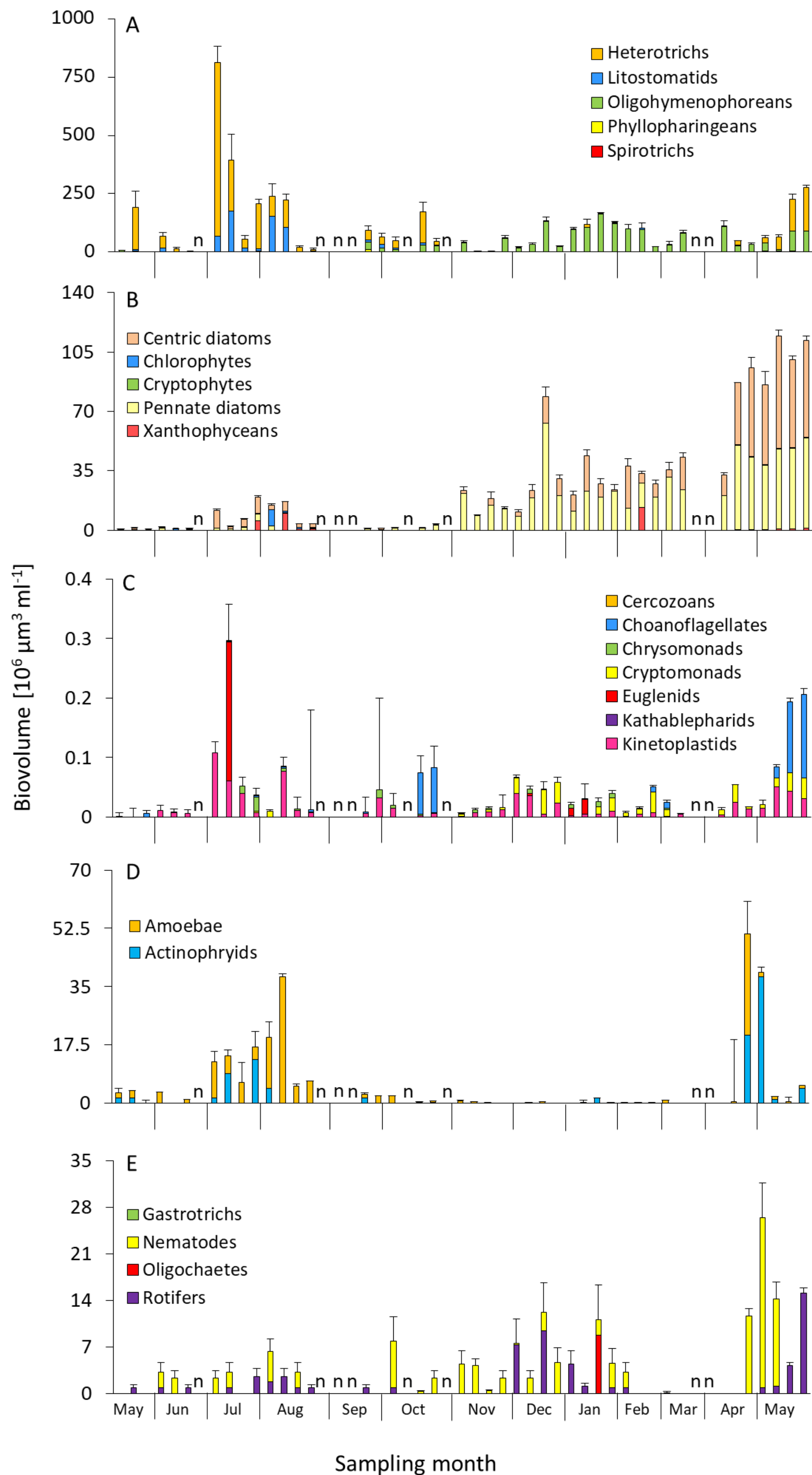

Fig. 2.3. Composition of the biofilm biovolume (µm^3 ml^{-1}) separated for ciliates (A), algae (B), heterotrophic flagellates (C), amoebae and actinophryids (D), and metazoans (E) (x ± SD; 9 replicates) for the sampling period May 2018 until May 2019, n = data not available.

On average, ciliates accounted for 74 % of the mean biovolume, with heterotrichs (38 %) and oligohymenophoreans (26 %) having the largest share (Fig. 2.4. A). The other groups as litostomatids, phyllopharingeans, and spirotrichs made up the remaining part of the ciliate biovolume. The second largest part of the mean biovolume was contributed by algae (19.3 %; centric diatoms with 8 % and pennate diatoms with 10.6 %). Due to their small size, flagellates contributed only 0.03 % of the mean biovolume. And also the rare amoebae, actinophryids, and metazoans were only of low importance regarding their biovolume (2.3 %, 1.6 %, and 2.8 %, respectively; Fig. 2.4. A).

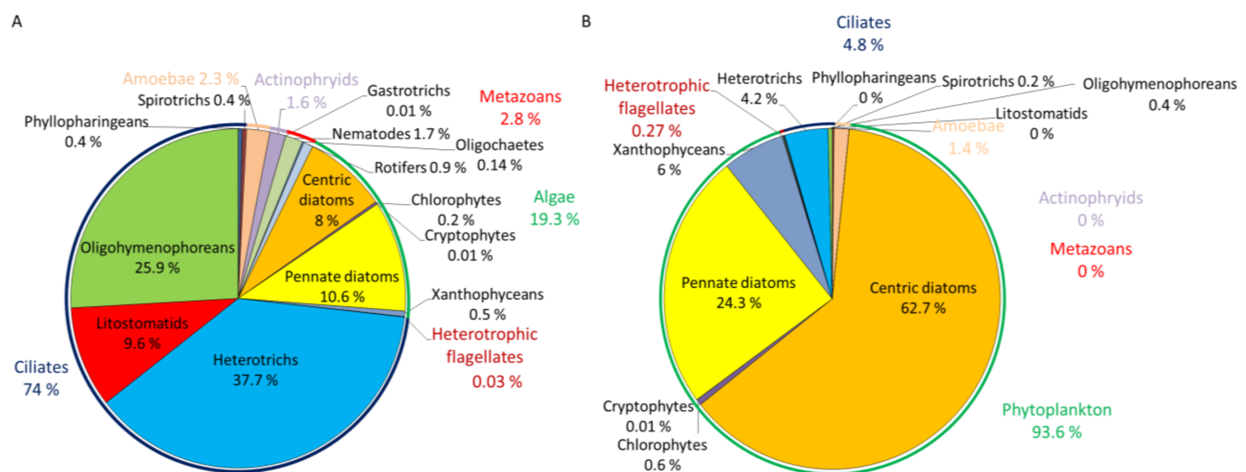

Fig. 2.4. Contribution to mean biovolume (%) of the different taxa of the biofilms (A) and the plankton (B) from May 2018 until May 2019)

2.4.3 Plankton composition

The pelagic bacteria community was mainly composed of small cocci and single-celled rods (diversity not shown in detail, for general abundances see below).

The species composition of eukaryotic plankton organisms changed throughout the experimental period (Fig. 2.5.). The algae abundance increased towards the summer months in both years of the study period (Fig. 2.5. A). The most abundant algae groups in the channels at sample spot A and B were centric and pennate diatoms (Bacillariophyta), especially *Melosira*, *Stephanodiscus*, *Navicula*, and *Synedra*. Additional common diatom genera were *Cyclotella*, *Amphora*, *Asterionella*, *Cymbella*, *Closterium*, *Gomphonema*, *Gyrosigma*, *Nitzschia*, and *Stauroneis*. Another abundant taxon in the summer period was

Tribonema (Xanthophyceae). Not as abundant but occasionally present in the experimental period were Cyptophytes (*Cryptomonas*) and Chlorophytes (*Ankistrodesmus*, *Chlamydomonas*, *Chlorella*, *Coelastrum*, *Crucigenia*, *Monoraphidium*, *Pediastrum*, *Scenedesmus*, *Schroederia*) (Fig. 2.5. A).

Planktonic heterotrophic flagellates, though not as abundant as algae, were present on every sampling date during the experimental period (Fig. 5 B). The most abundant flagellate group were kinetoplastids, mainly the genera *Bodo*, *Neobodo*, and *Rhynchomonas*. Further, *Spumella*-like chrysomonads could be found throughout the year. Other groups of heterotrophic flagellates group occurred occasionally: choanoflagellates (*Monosiga*-like, *Codosiga*, *Salpingoeca*- like), kathablepharids (*Kathablepharis*), euglenids (e.g., *Petalomonas*), cercozoans (cercomonads), cryptomonads (*Goniomonas*) (Fig. 2.5. B).

The relative abundance of ciliates in the plankton was generally not as high as on the biofilm (Fig. 2.5. C compare to Fig. 2.3. A). Most abundant were zoothamniid ciliates (Peritrichia) and stentorid ciliates (Heterotrichia) at the sample spot A, before the water passes the biofilm. At the sample spot B behind the biofilm, the stentorid ciliates were not found (data not shown). Vorticelliid ciliates, *Metacineta* (Suctoria), *Holosticha* (Spirotrichia), *Aspidisca*, *Euplotes* (Hypotrichia) and *Climacostomum* (Heterotrichia) were found at single sampling dates (Fig. 2.5. C).

Small individuals of naked amoebae (e.g. vahlkampfiids) were found on several sampling dates in the plankton (Fig. 2.5. C).

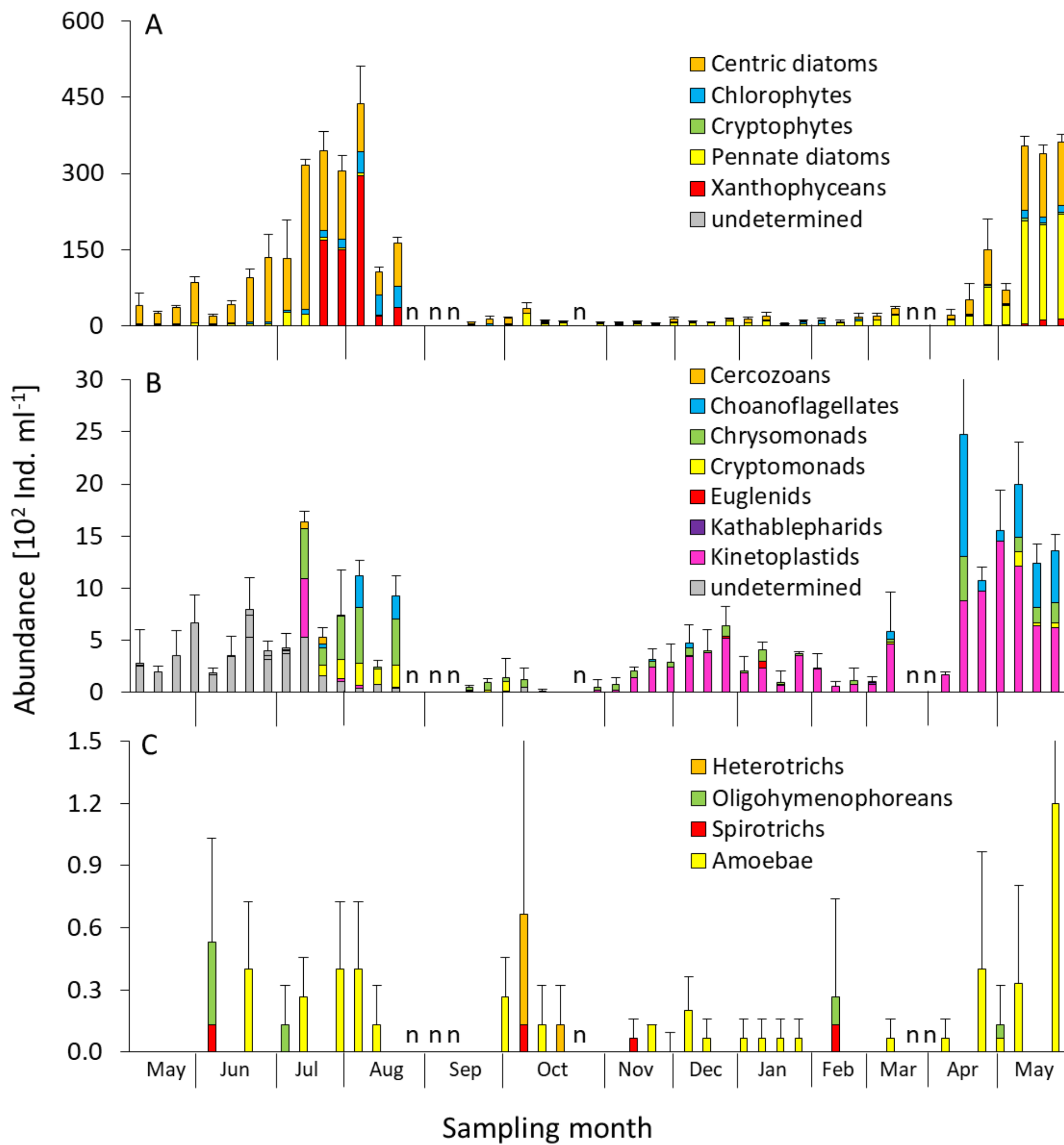

Fig. 2.5. Composition of the plankton abundances (Ind. ml^{-1}) separated for phytoplankton (A), planktonic heterotrophic flagellates (B) and others (C) for the sampling period May 2018 until May 2019 (x ± SD; 15 replicates). n = data not available.

2.4.4 Retention of plankton by the biofilms

The abundance of planktonic bacteria ranged from $1.22 * 10^6$ Ind. ml^{-1} (July 2018) to $4.04 * 10^6$ Ind. ml^{-1} (April 2019) in samples before the biofilm inflow and from

0.43 * 10^6 Ind. ml^{-1} (June 2018) to 2.27 * 10^6 Ind. ml^{-1} (April 2019) in samples behind the biofilm (Fig. 2.6. A). The abundance of planktonic bacteria was significantly reduced after passing the biofilm (Fig. 6 A). The mean retention effect on bacteria in the surrounding water due to the biofilm was 46.8 ± 10 % (n = 44) for the entire period of the experiment and varied between 22.1 % (September 2018) and 63.4 % (February 2019) (Fig. 2.6. A). In 86 % of cases, the retention effect was significant ($p<0.05$). With the age of the biofilm, the retention effects slightly increased.

The phytoplankton abundance ranged from 313 Ind. ml^{-1} (January 2019) to 44.1 * 10^3 Ind. ml^{-1} (August 2018) before the biofilm and from 193 Ind. ml^{-1} (February 2019) to 28.6 * 10^3 Ind. ml^{-1} (July 2018) behind the biofilm (Fig. 2.6. B). The retention effect on algae due to the biofilm varied between 0 % (January 2019) and 80 % (March 2019) and reached a mean value of 30 ± 89 % (n = 43) for the overall experimental period (Fig. 2.6. B). At 7 out of 48 samplings, a detachment of algae from the biofilm was observed under the microscope, which led to negative retention values. In the course of the study, the retention varied from week to week, except for a period with more constant retention values from February until May 2019 (with 54 % to 80 % retention of algae). Though the retention effect was not stable for the period examined, it was significant ($p<0.05$) on 17 % of sampling dates (Fig. 2.6. B).

The flagellate abundance in the plankton passing the channels ranged from 0 Ind. ml^{1} (on several sampling dates) to 5.84 * 10^3 Ind. ml^{-1} (April 2019) in samples before the biofilm inflow and from 0 Ind. ml^{-1} (on several dates) to 2.44 * 10^3 Ind. ml^{-1} (October 2018) in samples behind the biofilm (Fig. 2.6. C). The retention of planktonic heterotrophic flagellates due to the biofilm varied between 0 % (October 25th 2018) and 100 % (October 2018 and April 2019) and reached a mean value of 26 ± 59% (n = 40, excluding the high negative values of September 26th, October 18th, and 25th 2018) for the overall the experimental period (Fig. 2.6. C). Negative retention effects occurred due to the detachment of large flagellate colonies (choanoflagellates of the genus *Codosiga*) from the biofilm. The retention effect was highly variable over the period examined and was significant ($p < 0.05$) only for some sampling dates (mainly in autumn).

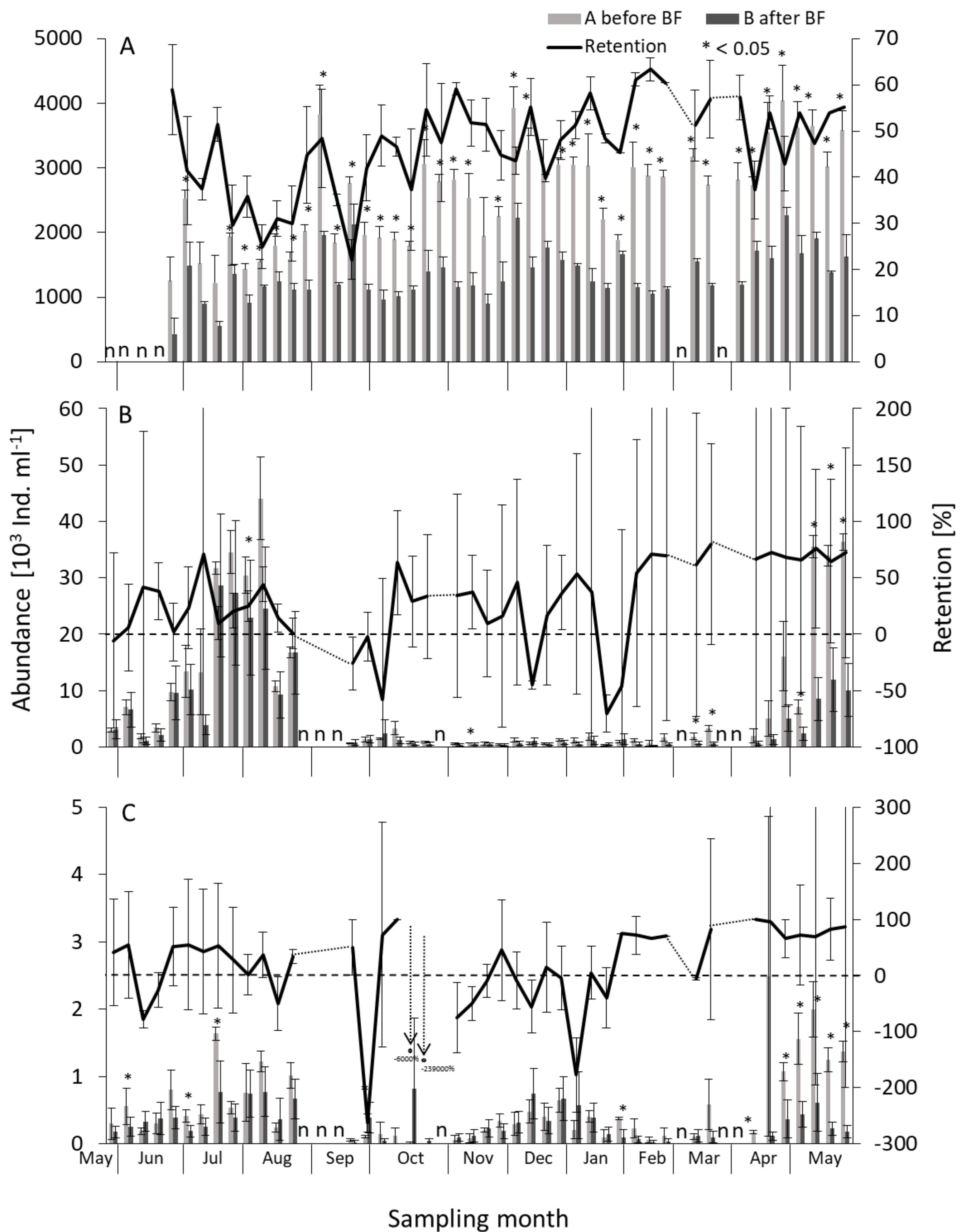

Fig. 2.6. Abundance (10^3 Ind. ml^{-1}; left axis) of planktonic bacteria (A), phytoplankton (B), and planktonic heterotrophic flagellates (C) before and after the impact of biofilm (grey bars: before; black bars: behind; 15 replicates; x ± SD) and retention (%) of planktonic organisms (continuous line, right axis) after passing the biofilm. Significant differences between abundances before and after the impact of biofilms are indicated by *$p < 0.05$, n = data not available.

Spearman rank correlation analyses revealed that the retention of algae was negatively correlated with the conductivity of the river water (-0.38; $p < 0.05$; Tab. 2.1.). Significantly positive correlations were detected regarding the retention of the phytoplankton with the algae biovolume on the biofilms (0.57; $p < 0.01$), as well as the zoothamniid ciliate abundance on the biofilm (0.36; $p < 0.05$; Tab. 2.1.). The retention of flagellates was negatively correlated with the conductivity of the river water (-0.35; $p < 0.05$) and positively correlated with the algae biovolume of the biofilm (0.48; $p < 0.01$; Tab. 2.1.). The retention of bacteria was negatively correlated with the water temperature (-0.54; $p < 0.01$; Tab. 2.1.). Significant positive correlations were detected with the water level (0.41; $p < 0.01$), oxygen content (0.45; $p < 0.01$), as well as with the algae biovolume on the biofilm (0.48; $p < 0.01$), vorticelliid and zoothamniid ciliate abundances on the biofilms (0.46 and 0.044; $p < 0.01$; Tab. 2.1.).

Table 2.1. Correlation between retention of phytoplankton, flagellates and bacteria of plankton and water level, temperature, conductivity, oxygen content of the water column and of algae, flagellate, ciliate and meiofauna biovolume of the biofilm and stentorid, vorticelliid, zoothamniid ciliate and bodonid flagellate abundances of the biofilm. Spearman rank correlation (*$p < 0.05$, **$p < 0.01$).

	Retention of phytoplankton	**Retention of flagellates**	**Retention of bacteria**
Water level	0.31	0.20	**0.41****
Temperature	-0.16	-0.04	**-0.54****
Conductivity	**-0.38***	**-0.35***	-0.04
Oxygen content	0.18	0.14	**0.45****
Algae biovolume	**0.57****	**0.48****	**0.48****
Flagellate biovolume	0.20	0.08	-0.05
Ciliate biovolume	0.13	0.31	-0.25
Meiofauna biovolume	0.03	0.03	-0.19
Stentorid abundance	0.11	0.25	-0.09
Vorticelliid abundance	0.01	-0.12	**0.46****
Zoothamniid abundance	**0.36***	0.28	**0.44****
Bodonid abundance	0.39	0.25	0.25

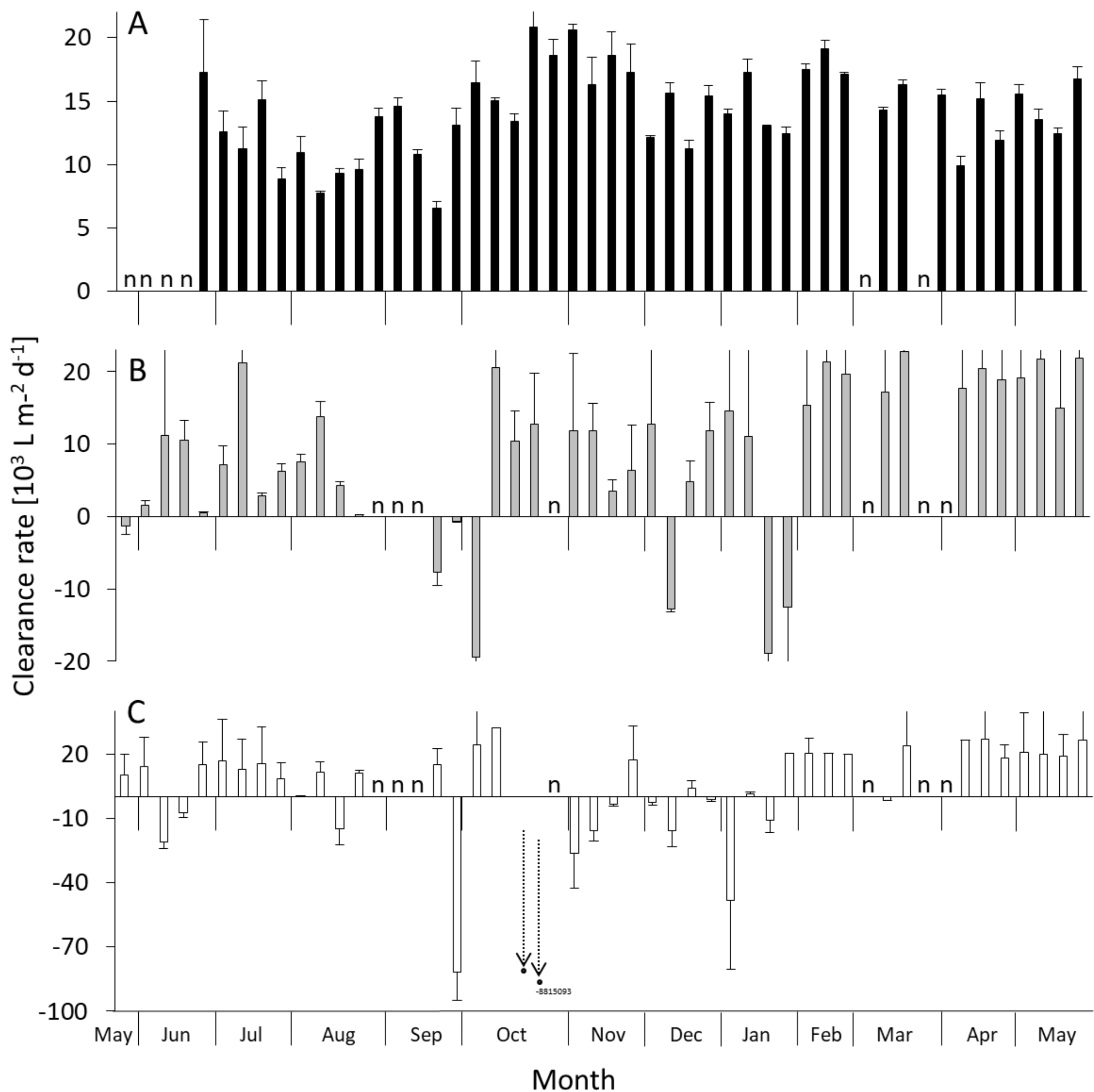

Fig. 2.7. Clearance rate (10^3 L m^{-2} d^{-1}) of planktonic bacteria (A), phytoplankton (B), and planktonic heterotrophic flagellates (C) after passing the biofilm, n = data not available.

Clearance rates were estimated by relating the number of planktonic organisms filtered out of the water to the amount of water that passed the biofilm (Fig. 2.7., see also Fig. 2.1.). The estimated clearance rate of the biofilm for planktonic bacteria ranged between 6.6 * 10^3 L m^{-2} d^{-1} (September 2018) and 20.9 * 10^3 L m^{-2} d^{-1} (October 2018), and a mean clearance rate of about 14.2 * 10^3 L m^{-2} d^{-1} (SD ± 0.9 * 10^3 L m^{-2} d^{-1}, n = 44) was reached between June 2018 until May 2019 (Fig. 2.7. A). The estimated clearance rate for

phytoplankton reached several times negative values (esp. in October 2018 and January 2019, indicating detachment of periphyton), but also reached higher values up to 22.9 * 10^3 L m^{-2} d^{-1} (March 2019). Average clearance rate over the experimental period was estimated as 8.8 * 10^3 L m^{-2} d^{-1} with a high variability (SD ± 11.8 * 10^3 L m^{-2} d^{-1}, n = 43; Fig. 2.7. B). For planktonic heterotrophic flagellates, the clearance rate was even more variable and ranged between -48.4 * 10^3 L m^{-2} d^{-1} (September 2018) and maximum positive values of 32.4 * 10^3 L m^{-2} d^{-1} (October 2018). On average, the clearance rate for flagellates was 7.6 * 10^3 L m^{-2} d^{-1} (SD ± 19 * 10^3 L m^{-2} d^{-1}, n = 40; excluding the high negative values of September 26th, October 18th, and 25th 2018; Fig. 2.7. C).

2.5 Discussion

This study demonstrates the importance of biofilms for the retention of planktonic bacteria in a river system throughout the investigation period, which covered a year's period. Even during the short period of contact of the river water with the biofilms in the experimental setup, the retention effects could show to be substantial: on average, about half of the planktonic bacteria community was eliminated within the time passing the biofilm, which could be e.g. only sixty seconds (this was time needed for 10 L of river water to pass over 7,000 cm^2 of biofilm in the experimental setup at a flow velocity of 1.57 m s^{-1}; e.g. 14th of June, 2018). Resident organisms of the biofilms, in particular ciliates, had not only a substantial effect on the retention of planktonic bacteria, but also on the retention of phytoplankton and planktonic heterotrophic flagellates.

2.5.1 Composition of the biofilm community

At most sampling dates, ciliates were the most important group of organisms regarding its contribution to the biofilm biovolume (Fig. 2.3. A). On average, they amounted for about 74 % of the mean biovolume of the biofilm; here, the ciliate groups stentorid (Heterotrichia),

zoothamniid and vorticelliid ciliates (Peritrichia) dominated throughout the experimental period (Fig. 2.4. A). The peritrich ciliates which were dominant on the biofilm feed primarily on suspended bacteria while the observed heterotrich stentorid ciliates prey also on benthic bacteria (Foissner et al., 1992). The presence of significant numbers of predacious litostomatid ciliates (9.6 % of the mean biovolume) indicates also the importance of predator-prey interactions within the biofilm community itself. Similar to our results, even short-term experiments showed that algae attach to biofilms, especially diatoms which are introduced into the River Rhine upstream Cologne by the River Moselle (Früh et al., 2011, Scherwass et al., 2010).

Algae contributed a large part to the biofilm community concerning its biovolume mainly due to the attachment of diatoms from the plankton from November 2018 on (19.3%; Figs. 2.3. B, 2.4. A). However, the algae abundance of the plankton of the River Rhine already peaked in July and August 2018 and in May 2019. These peaks are similar to phytoplankton peaks of earlier observations (Admiraal et al., 1994; Zwolsman and Bokhoven, 2007; Friedrich and Pohlmann, 2008). Although algae cannot actively influence the retention of bacteria, they provide a substrate for further attachment of other organisms (mainly bacteria) due to the excretion of extracellular polymeric substances and the building of three-dimensional structures. Thus they are part of the formation of the complex structure of the biofilm and can lead to a passive reduction of planktonic bacteria (e.g., Battin et al., 2016; Costerton et al., 1981; Vasudevan, 2014). Due to their minute sizes, heterotrophic flagellates, neither contributed significantly to the biovolume of the biofilm on single sampling dates (Fig. 2.3. C) nor to the mean biovolume of the experimental period (annual average 0.03 %, Fig. 2.4. A). Macrofauna was prevented from entering the set up due to the mesh in the first half of the experimental setup and only small metazoans mainly belonging to meiofauna (rotifers, nematodes, oligochaetes, and gastrotrichs) occurred on several sampling dates (Fig. 2.3. E). Micrometazoan meiofauna only accounted for around 2.8 % of the mean biovolume of the biofilm (Fig. 2.4. A). The composition of the biofilm is usually also influenced by the macrofauna of the pelagic. By excluding these, predators of ciliates were absent and could explain the high biovolume of ciliates and the high losses to the benthos (Weitere et al., 2005).

Similar results regarding the composition of the biofilm community of the River Rhine at Cologne and the dominance of heterotrich and peritrich ciliates were found during earlier studies (Wey et al., 2008; Kathol et al., 2009; Wey et al., 2009; Ackermann et al., 2011; Kathol et al., 2011). As in studies of Ackermann et al. (2011), our present study showed remarkable increases of the biofilm biomass towards the end of exposure time indicating the importance of long-term studies. As the biofilms in our study were exposed at naturally variable conditions including non-linear changes in flow velocity, biofilm may have detached in situ, as consequence of biofilm maturing or changed current velocity. This was the case at the end of August of 2018 after about 3 months of biofilm growth (Fig. 2.3.). Occasionally, disrupted peritrich ciliates were found in plankton samples taken behind the experimental biofilms. This corresponds with observations of Scherwass and Arndt (2005) who found that disrupted benthic peritrich species contributed on annual average to about 39 % of total ciliate plankton abundance in the River Rhine at Cologne. Disruption and dispersal can again lead to the formation of new biofilms (Risse-Buhl and Küsel, 2009) and biofilms can be found on various substrata in rivers (Baldock et al., 1983, 1986).

2.5.2 Bacteria retention by the biofilm community

Our calculations reveal that up to 20 x 10^3 liters of water per m^2 per day could be purified from bacteria by the biofilm (Fig. 2.7. A). The clearance rate of the natural *in-situ* biofilms was about one order of magnitude larger than that of biofilms at the same station which were kept under semi-natural conditions in a flow through system with water from the River Rhine in the lab in a former study (up to 565 L m^{-2} d^{-1}, Kathol et al., 2011). This underlines the importance of *in-situ* studies. Thus, our studies illustrate the potential elimination capacity of biofilms in rivers and the necessity of using them for the purification of streams. Throughout the experimental period of the present study, the retention effect on pelagic bacteria by the biofilm was always positive (22.1 % - 63.4 %, Fig. 2.6. A). A stabilizing retention effect between 37 % and 57 % occurred towards the end of the experiment period, but even during the different seasons with changing water parameters throughout the whole study, the retention never dropped below 20 %.

It is very likely, that bacteria have been mainly consumed by suspension-feeding ciliates of the biofilm (Fig. 2.3. A), as these have shown to be important in bacterial elimination (see below). Positive Spearman rank correlations of 0.46 ($p < 0.01$) between the retention of bacteria and the abundance of vorticelliid ciliates on the biofilms and of 0.44 ($p < 0.01$) between the retention of bacteria and abundance of zoothamniid ciliates on the biofilms support this assumption (Tab. 2.1.). In particular, importance of peritrich ciliates for the reduction of pelagic bacteria was also recorded by Kathol et al. (2011) in lab experiments on the River Rhine and this phenomenon could also be shown to be typical for other river systems (Weitere et al., 2018). For example, peritrich vorticelliids can consume 7,200 up to 25,000 bacteria per individual per day and clear over 4,000 nl of water per individual and day (Schönborn, 1982; Sanders et al., 1989). Due to the exclusion of macrofauna, ciliates may not have had any predators resulting in a high abundance and biovolume of ciliates and thus in a high loss of bacteria and flagellates to the ciliates of the benthos (Weitere et al., 2005) However, in our study, the retention effect of ciliates could not only be attributed to suspension-feeding ciliates. According to Esser (2006), biofilm dwelling ciliates in the River Rhine at Cologne haven been shown to substantially contribute to the reduction of benthic bacteria (> 30 %) whereas heterotrophic flagellates seemed to play only a minor role in the consumption of bacteria (0.1 - 2 %).

Potentially, choanoflagellates can be voracious bacteria feeders (Carrias et al., 1996; Boenigk and Arndt, 2000; Weitere et al., 2018). In the present study, colonies of choanoflagellates were occasionally important among the bacterivorous filter feeders and (mainly *Codosiga*) occurred in significant amounts in October of 2018 and May of 2019 (Fig. 2.3. C). They can feed on up to 1,000 bacteria per individual per day and show a clearance rate between 200 to 1,300 nl per individual per day (Sanders et al., 1989). Other phylogenetic groups of heterotrophic bacterivorous flagellates were present in our studies (kinetoplastids, chrysomonads) but contributed only a minor part to the biofilm community and could occasionally contribute to the grazing on planktonic bacteria when bacteria got attached to the biofilm.

The algae biovolume of the biofilm had a positive effect on the retention of bacteria (0.48, $p < 0.001$; Tab. 2.1.). Algae could not directly contribute to the elimination of

planktonic bacteria; however, they contribute to the three-dimensional structure of the biofilm. The filamentous algae *Melosira* contributed to the complex structure of the biofilm. The large surface and amount of EPS matrix produced by algae may support attachment of bacteria which can be subsequently grazed by biofilm dwelling protists.

2.5.3 Phytoplankton retention by the biofilm community

The retention effect by the biofilm on the phytoplankton was positive on most of the sampling dates and ranged between -70.2 % and 79.8 % (ø 29.8 %; Fig. 2.6. B). Although the clearance for phytoplankton by the biofilm was very variable and showed high standard deviations, it was mostly positive. This can be explained not only by the consumption of organisms, but might mainly be caused by the attachment of phytoplankton to the biofilms (Risse-Buhl and Küsel, 2009; Früh et al., 2011). Especially during spring, the clearance rate of phytoplankton was equal to that of bacteria and even exceeded it on certain days (up to 22 * 10^3 liters of water per m^2 per day; Fig. 2.7. B). Still, the organisms of the biofilms might have consumed a certain amount of phytoplankton. The abundance of stentorids in July and August of 2018 and in Mai 2019 can have contributed to the retention of phytoplankton during these periods because stentorid ciliate individuals are able to consume 50 to 800 algae of different sizes during one day (Kim et al., 2007). In the cases of negative clearance rates, the detachment of algae from the biofilm let to dispersal, which in turn may promote the growth of new biofilms (in these cases, phytoplankton appeared at higher numbers in samples taken after the passage of the experimental biofilms, at sampling spot B; data not shown).

In contrast to the retention of bacteria, there were pronounced seasonal changes in the retention of phytoplankton. Especially at the beginning of the study period during the early growth phase of the biofilm, centric and pennate diatoms attached to the biofilm and contributed significantly to the formation and growth of the biofilm (Figs. 2.3. B, 2.4. A). This observation is supported by the highly positive and significant Spearman rank correlation between the algal biovolume of the biofilm and the retention of phytoplankton (0.57; $p < 0.01$; Tab. 2.1.). On the other hand, phytoplankton (e.g. filaments of Melosira) could also

detach and which may have been the reason for the negative retention effects found in October and December of 2018, January of 2019 e. g. (Fig. 2.6. B). This is supported by the spearman rank correlation which was significantly positive for the relationship between retention of planktonic algae and the abundance of predominantly bacterivorous zoothamniid ciliates (0.36; $p < 0.05$; Tab. 2.1.). Here, the branching habit of zoothamniid ciliates may be of importance, offering a large surface for the attachment of algae.

2.5.4 Planktonic heterotrophic flagellate retention by the biofilm community

The retention of heterotrophic flagellates from the pelagic by the biofilm fluctuated during the whole experimental period except for the last sampling dates, where it was stable at a positive level (66 - 95 %, Fig. 2.6. C). The latter could be explained by the complex three-dimensional structure of the biofilm towards the end of the experimental period providing shelter for heterotrophic flagellates. This is supported by a highly positive and significant correlation between the retention of flagellates and the algae biovolume of the biofilm (0.48; $p < 0.01$; Tab. 2.1.). A higher algae biovolume on the biofilm provides grooves, burrows, and loops for flagellates to settle down and ensure shelter from water currents and hiding places from larger predators (Reynolds and Carling, 1991). Raptorial feeders among ciliates (litostomatids) and suspension feeding stentorids may feed on planktonic heterotrophic flagellates (Foissner et al., 1992, 1995) and might have contributed to the variable retention of flagellates during summer (Figs. 2.3. A, 2.6. C). During winter, the retention of planktonic heterotrophic flagellates reached negative values at several sampling dates due to the detachment of large choanoflagellate colonies. Although various influences affect the retention of heterotrophic flagellates, the biofilm had a general positive influence on the clearance rate of pelagic heterotrophic flagellates with a mean value of $8 * 10^3$ liters of water per m^2 per day (Fig. 2.7. C).

2.5.5 Conclusion

The experimental set-up shows that biofilms in situ can filter and purify a large amount of water in river systems. Though our estimates regarding the clearance rate are very rough, the biofilm of the River Rhine at Cologne can eliminate bacteria from a volume of more than 10 m^3 per square meter on each day all the year round. This remarkable retention underlines the necessity to increase surfaces to support self-purification processes in rivers.

While the overwhelming importance of biofilms for the elimination of bacteria was obvious, the clearance rate regarding heterotrophic flagellates and algae was moderate and very variable. Nevertheless, regarding their elimination, there was a net reduction in the course of the year of several cubic meters per square meter and day. The reduction of bacteria could be attributed to direct grazing by suspension feeding ciliates or to an indirect loss via attachment to the biofilm and subsequent grazing by biofilm-dwelling ciliates. The three-dimensional structure seems to play an important role in this process. For the elimination of phytoplankton and heterotrophic flagellates the underlying processes were not as obvious. Here, the complex trophic interactions between the different components of the biofilm have to be considered. The results fit to the analyses of plankton loss rates obtained from the discrepancy between production and population increase observed for plankton organisms in the River Rhine at Cologne (Weitere et al., 2005). Laboratory experiments in the past have shown that abiotic factors certainly play an important role for the processes on the biofilm (e.g. Norf et al., 2007, 2009; Willkomm et al., 2007). Disentangling the influence of abiotic and biotic conditions was difficult during this one-year study. To clarify this, multi-annual studies are necessary. Already in the course of our long-term seasonal study, a stabilizing retention effect could have been recorded. The strong reduction of plankton by bivalves (e.g. mussels) is a long-known phenomenon (see Welker and Walz, 1998; Caraco et al., 2006), however, due to our findings it might well be, that the effect of biofilms which are built to a large part of microbes is of similar or even larger importance in this respect.

Large river systems such as the River Rhine are faced with the inflow of wastewater contaminated with bacteria and microplastics from wastewater treatment plants, non-point

sources, and urban runoff (Mallin et al., 2000; Jokinen et al., 2010; Wu and Chen, 2013; Prasad et al., 2015; Tillburg et al., 2015) and thus there will be a continued need to improve stream water quality. One option could be the increase of surfaces to support natural biofilm communities and similarly increase planktonic retention.

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3. Retention effects on microplastics by biofilms and its ingestion by micro- and macrofauna

3.1 Abstract

Plastic introduction into different ecosystems has become a major problem within the last decades. Though there are many studies on larger plastic particles in marine habitats, we still have to investigate the role of rivers transporting plastic particles into seas. Due to different sources, such as tire abrasion, sewage treatment plants and grinding of larger plastic particles, microplastic particles (< 5 mm) are introduced into rivers. These smaller particles might have an effect on river organisms such as fish and mussels, but also microzoobenthos living on biofilms. Biofilms are present in all aquatic ecosystems and play an important role in being a habitat for bacteria, flagellates, ciliates and other protists, but also in being a food source for macrozoobenthic organisms. In this study, we want to show that biofilms and its residential organisms interact with microplastic particles.

We added medium enriched with different microplastic particle concentrations into endless flow channels with biofilms and determined the amount of microplastic particles within the biofilms. Further, we added different microplastic particle concentration to *Stentor coeruleus* cultures and analyzed the microplastics uptake by the ciliate.

We found that the abundance of microplastic particles in biofilms is significantly higher than in other tested surfaces. Furthermore, we could show that with increasing microplastic particle size, the abundance in biofilms also increases. The experiments with *Stentor* individuals showed that no saturation point could be reached for the uptake of microplastic particles by the ciliate.

In our study, we want to present the roles of biofilms and their organisms in the retention of microplastics from river waters and a possible solution for the reduction of microplastics in aquatic ecosystems. In the last decades, plastic pollution has become a major problem in different ecosystems. Though first experiments for solutions to remove macroplastics, especially in the world’s oceans, are already present, solutions for the reduction of microplastics are rare.

3.2 Introduction

Small plastic particles, so called microplastics, are released into the environment due to a broad range of human activities (Auta et al., 2017). Originally, microplastics result from sources such as cosmetics, abrasion from synthetic clothing, loss of virgin plastic pellets, tire wear or fragmentation from larger objects (Auta et al., 2017; Boucher and Friot, 2017; Falco et al., 2019), and they are found in the different environmental compartments such as air (Evangeliou et al., 2020), soil (Horton et al., 2017), and various aquatic environments, such as sediments (Van Cauwenberghe et al., 2015), deep sea (Woodall et al., 2014) and arctic ice (Peeken et al., 2018). Microplastic particles enter riverine systems via point sources, e.g., sewage treatment plants, factories, and non-point sources, e.g., precipitation, land runoff, but little is known about the fate and effects of microplastics in freshwater systems compared to that in marine ecosystems (Wagner et al., 2014; Horton et al., 2017; Li et al., 2018a).

Riverine ecosystems play a major role in transporting and distributing microplastics and plastic litter from inlands to shores and oceans (Lebreton et al., 2017). Highest concentrations were measured in the river Yangtze (Asia) with 4137 particles per m^3 with 90 % in the size range of 0.5 to 5 mm (Zhao et al., 2014). In the River Rhine (Europe) only 0.5 up to over 20 particles per m^3 were measured in the size range of 300 µm to 5 mm at 11 locations (Mani et al., 2016). Sediments from the shore of the River Rhine, concentrations between 228 to 3763 particles per kg sediment were reported (Klein et al., 2015). This leads to the conclusion that during the transport to and within rivers particles are retained in sediments (Dris et al., 2015; Nizzetto et al., 2016; Besseling et al., 2017; Hoellein et al., 2019). Beyond these general findings, details of fate and distribution as well as effects on organisms and ecosystems within rivers remain still unclear.

One neglected aspect of microplastic retention in rivers is the role of biofilms. These microbial communities of bacteria, algae and protists are living in a matrix consisting of extracellular polymeric substances (EPS) on different organic and inorganic substrates (Branda et al., 2005; Böhme, Risse-Buhl & Küsel, 2009). They are found on stones, leaf litter, macrophytes, animals, and artificial substrates (Arndt et al., 2003). Biofilm formation is

initiated by bacteria of the surrounding water that attach and excrete EPS, which in turn supports further attachment of algae and protozoans. With ongoing growth and the entrapping of different organisms, biofilms form a three-dimensional structure with varying thickness (Costerton et al., 1981; Vasudevan, 2014).

Models of riverine transport of microplastics shows that biofilms on the surface of plastic particles influence the settling behavior (Besseling et al., 2017; Hoellein et al., 2019), similar to marine environments (Lobelle and Cunliffe, 2011; Zettler et al., 2013; Yokota et al., 2017). However, the retention of microplastics within biofilms is nearly unknown and has not been considered in retention models. Yet, biofilms might act as a sink for microplastics as it has been observed for other suspended matter in streams (Arnon et al., 2009; Böhme et al., 2009; Battin et al., 2016), and thereby foster the incorporation of particles into microbial and macrobial food webs. Effects of microplastics on biofilm-associated microorganisms have seldomly been studied. Reported effects of microplastics on primary producers include reduction in photosynthetic activity and growth rate after the retention on the surface of macroalgae (Yokota et al., 2017). Since protozoans living in biofilms such as amoebae, heliozoans, heterotrophic flagellates, and ciliates retain food particles through suspension and raptorial feeding, their importance as consumers of microplastics and their potential enrichment should be even more important than that of algae. Only few studies have investigated the uptake by protists, most were carried out with marine species such as dinoflagellates (Cole et al., 2013), ciliates (Setälä et al., 2014), and foraminiferans (Langlet et al., 2020). Additionally, little is known about the indirect uptake of microplastics by larger organisms, i.e. through the ingestion of organisms which have previously consumed microplastics, and the role for the trophic transfer, even for metazoans (Farrell and Nelson, 2013; Au et al., 2017; Nelms et al., 2018). We assume that the position of biofilms within food webs might be an entry path of retained microplastics into higher trophic levels, even for very small particles.

In this study, we want to find out to what extent biofilms can retain microplastic particles from the surrounding water (1), if and how much a common model ciliate of the River Rhine can take up microplastic particles (2), and if microplastic particles can reach higher trophic levels via ciliates (3).

3.3 Material and methods

3.3.1 Study site and study species

The River Rhine flows from its origin in Switzerland northwards through the west side of Germany and ends into the Dutch North Sea. The section of the River Rhine at Cologne, Germany, is characterized as the Lower Rhine (Preusser, 2008) with a mean flow velocity of about 1.5 m s^{-1}. The study was conducted on board the Ecological Rhine Station and boathouse of the University of Cologne, which is permanently anchored at Cologne-Bayenthal (Rhine kilometer 684.5, see Fig. 2.1. A). Behind the boathouse in downstream direction, a float with channels is attached, which allows the growth of biofilms on different substrates in situ under natural conditions. Before experiments of particle retention were started, biofilms were grown there for 4.5 weeks on clay tiles (4.9 x 4.9 x 0.5 cm) attached to concrete holders in December 2018 and March 2019.

As model species to study the uptake of microplastic particles the biofilm-associated ciliate *Stentor coeruleus* (Stentoridae) was used. *Stentor coeruleus* was chosen for the experiments because of it is a common ciliate on biofilms of the River Rhine at Cologne and its size of 250 µm up to 1.5 mm facilitates the analysis (Ackermann et al., 2011). Cultures of *S. coeruleus* were obtained from Helbig (Prien am Chiemsee, Germany) and stored at 20 °C. The cultures were fed with algae, *Chlorococcum* sp. (Chlorococcaceae; "Lebendkulturen Helbig", Prien am Chiemsee, Germany) and Chlamydomonas asymmetrica (Chlamydomonadaceae; Culture collection of algae of the University of Cologne (CCAC)) every third to fourth day. The gammarid *Dikerogammarus villosus* was collected close to the Ecological Rhine Station. The individuals were kept at 13 °C in River Rhine water in culture bottles (FALCON, Darmstadt Germany) and were provided with dried leaves as food source from deciduous trees. Oxygen supply was generated using a pump (optimal, SCHEGO).

3.3.2 Microplastic model particles

For all experiments, Fluoresbrite® polystyrene microspheres (Polysciences Europe GmbH, Hirschberg an der Bergstrasse, Germany) stained with phyco-erythrin ('polychromatic red', 525 - 565 nm excitation, Ø 1 µm and Ø 6 µm) and fluorescein ('yellow-green', 441 - 485 nm

excitation, Ø 10 µm) produced by Polysciences, Inc. were used as tracer microplastic particles (MP). In both experiments, MP particles were added to each channel, with a calculated start concentration of 500 particles ml^{-1} of 6 µm-sized particles in the first experiment and 1 µm, 6 µm, and 10 µm-sized particles in the second experiment. In the absence of data on the abundance of microplastic particles, this concentration was chosen since the natural microplastic concentration in the River Rhine is about ten 500 µm-particles per milliliter (conservative estimate based on Mani et al., 2015) and assuming that a degradation of those particles to spheres of the sizes 1 µm, 6 µm, and 10 µm would reveal concentrations of about 1258.8 particles ml^{-1}, 5.77 particles ml^{-1}, and 1.25 particles ml^{-1}, respectively. The concentration of microplastics used in the experiments was chosen (500 particles ml^{-1}) to be lay in the range of these values similar to previous laboratory experiments (GESAMP, 2016).

3.3.3 Experimental setup

3.3.3.1 Experiments in endless flow channels

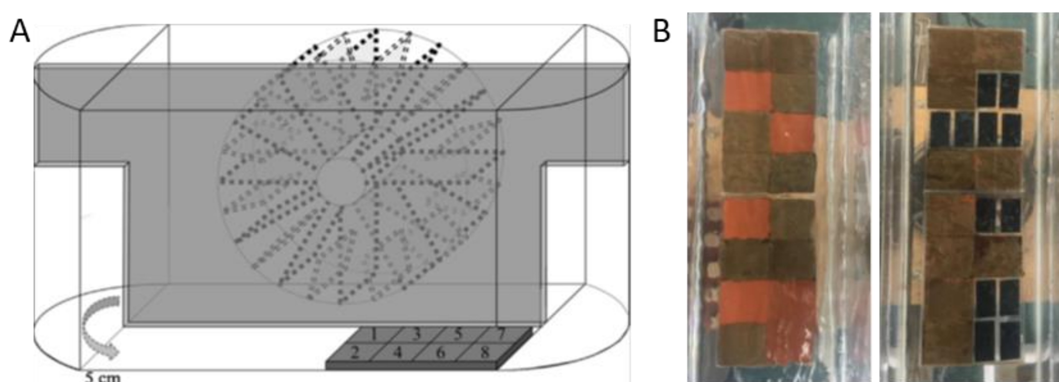

Fig. 3.1. (A) Endless flow channel with a paddle wheel to induce a current and a frame for tiles in the opposite side (figure adjusted from Schössow et al., 2016). (B) Clay tiles with biofilms, either in combination with empty clay tiles or empty Plexiglas tiles (Exp. 1).

The retention of microplastics by biofilms compared to other surfaces without biofilms was investigated using endless flow channels (Schössow et al., 2016). The flow channels

were equipped with paddle wheels which were connected to an electromotor by which the flow velocity could be adjusted manually for two channels at a time (Fig. 3.1. A). For the experiments, the channels were filled with 20 litres of filtered water using Whatman® GF/C filters (pore size about 1.2 µm, Whatman plc, Maidstone, Kent, United Kingdom). Frames with different substrata were installed on the opposite side of the paddle wheels.

The biofilms previously grew for 4.5 weeks in the River Rhine (see above) were inserted 24 hours before the experiment in two of the four channels, so the biofilms could pre-adjust to the conditions of the setup, e.g. water temperature and light conditions and flow velocities. In the first experiment, the effect of rough and smooth Plexiglass tiles without biofilms were compared with that of smooth clay tiles containing natural biofilms in the first channel to determine the influence of the wall of the flow channels consisting of smooth Plexiglas tiles. All tiles used in experiments had a size of 4.9 x 4.9 x 0.5 cm. In the second channel, rough and smooth clay tiles were exposed to check for the effect of surface roughness on particle retention. Channels 3 and 4 had the same setup of tiles, but while the first two channels were run at a flow velocity of 0.1 m s^{-1} the latter two were run at 0.2 m s^{-1} to check for the effect of flow velocity on particle retention (Tab. 3.1.).

Experiment 2 was designed to check for the effect of plastic particle size on the retention; only biofilm containing or fresh smooth clay tiles were exposed for comparison. While in the first experiment only 6µm-particles were offered, experiment 2 received 1 µm, 6 µm and 10 µm particles at flow velocities of 0.1 m s^{-1} in the first two channels and again 0.2 m s^{-1} in the latter two channels (Tab. 3.1.).

Table 3.1. Setups of experiment 1 (December 2018) and experiment 2 (March 2019) with the combination of substrata (biofilm on clay tiles, smooth and rough Plexiglas tiles, smooth and rough clay tiles), microplastic particle size (1 µm, 6 µm, 10 µm), Microplastic particle concentration (500 p ml^{-1}), and flow velocity (0.1 m s^{-1}, 0.2 m s^{-1}) for endless flow channels 1 - 4; MP = Microplastic particle, PG = Plexiglas.

Exp. 1 (Dec 2018)	Channel 1	Channel 2	Channel 3	Channel 4
Substrata	Biofilm on clay tiles (n=10) smooth PG (n=3) rough PG (n=3)	Smooth clay (n=4) Rough clay (n=4)	Biofilm on clay tiles (n=10) Smooth clay (n=3) Rough clay (n=3)	Smooth clay (n=4) Rough clay (n=4)
MP Size	6 µm	6 µm	6 µm	6 µm
MP Concentration	500 p ml^{-1}	500 p ml^{-1}	500 p ml^{-1}	500 p ml^{-1}
Flow velocity	0.1 m s^{-1}	0.1 m s^{-1}	0.2 m s^{-1}	0.2 m s^{-1}
Exp. 2 (Mar 2019)	**Channel 1**	**Channel 2**	**Channel 3**	**Channel 4**
Substrata	Biofilm on clay tiles (n=10)	Smooth clay (n=10)	Biofilm on clay tiles (n=10)	Smooth clay (n=10)
MP Size	1 µm, 6 µm, 10 µm	1 µm, 6 µm, 10 µm	1 µm, 6 µm, 10 µm	1 µm, 6 µm, 10 µm
MP Concentration	500 p ml^{-1}	500 p ml^{-1}	500 p ml^{-1}	500 p ml^{-1}
Flow velocity	0.1 m s^{-1}	0.1 m s^{-1}	0.2 m s^{-1}	0.2 m s^{-1}

The substrata were exposed for 24 hours to the microplastic particles. Afterwards, samples (10 ml for tiles without biofilm or 25 ml for tiles with biofilm with filtered River Rhine water) of the substrata surfaces were taken using a brush and transferred into 50 ml screw cab tubes (Sarstedt, Germany) (see Tab. 3.1. for number of replicates). Water samples were taken with three replicates per sample spot in front, above, and behind the frames with different substrata using a syringe and were filled into 15 ml graduated centrifuge tubes (Sarstedt, Germany). All samples were stored in the dark at 4 °C.

Microplastic concentrations of samples were quantitatively analyzed using an Axiostar Plus Fluorescence (FL) microscope (Carl Zeiss Jena GmbH, 07740 Jena, Germany), which was connected to an external lightning unit (HXP-120, Zeiss, Germany) for fluorescence excitation and excitation light of the filter set 38 HE (excitation BP470/40, beam Splitter FT 495, emission BP 525/50, Zeiss, Germany). Particle concentrations in biofilms and the water column were determined by quantitative counting of the fluorescent microplastic particles in a defined sample volume (2 µl for biofilms and 5 µl for water samples) placed in a droplet on glass objectives with 3 to 4 replicates. Particle counts per cm² were calculated by the means of the replicates multiplied with the dilution divided by the surface area of the tiles. For the

tiles without biofilms, it was assumed that a height of 100 µm water was transferred the surface of the tiles.

3.3.3.2 Feeding experiments

Two fixation methods were tested: formalin (4 % formaldehyde solution) and Lugol's solution to count ingested microplastic particles in *Stentor coeruleus* specimens. They were exposed to 6 µm and 10 µm MP with a concentration of 2500 p ml^{-1} for one hour. To prevent the sedimentation of the microplastic particles, a plankton wheel was used (2.39 rpm). At the end of the experiment, half of the sample was fixed with 4 % formalin and the other half was counted alive. 1 ml of each sample was placed in a Sedgewick-rafter cell and counted under the microscope (Axiophot Fluorescent Microscope, Zeiss, 10 x magnification). Further, the cyclose of *S. coeruleus* was studied to determine the appropriate time span for the ingestion experiment. After one hour, single *S. coeruleus* were separated in a Sedgewick-rafter cell with autoclaved River Rhine water without microplastics in the medium. The microplastic particles ingested by three *S. coeruleus* were counted periodically every seven minutes under the microscope, thus determining the time when particles were egested.

The ingestion of microplastics by *S. coeruleus* was studied for 6 µm and 10 µm particles in varying concentrations of microplastic particles (500 p ml^{-1}, 1,250 p ml^{-1}, 2,500 p ml^{-1}, 5,000 p ml^{-1}). Prior to each experiment, 70 ml of the medium with microplastic particles and 15 ml of the *S. coeruleus* cultures were transferred into a 120 ml tube. The microplastic particle suspension was vortexed prior to the experiment to avoid agglutination. Experiments were run in three replicates for each concentration of 500 p ml^{-1}, 1,250 p ml^{-1}, 2,500 p ml^{-1} and 5,000 p ml^{-1} plus one control without microplastic particles. After one hour 1 ml in the plankton wheel (2.39 rpm) was taken from the 120 ml tubes immediately and transferred to a Sedgewick-rafter cell. The *S. coeruleus* specimens were then examined individually for microplastic particle uptake under the microscope (10x magnification with fluorescent light) (Axiophot Fluorescent Microscope, ZEISS).

To show the transfer of microplastic particles between *S. coeruleus* and *D. villosus*, 15 ml of *S. coeruleus* culture and 70 ml River Rhine water with a microplastic particle concentration

of 10,000 p ml^{-1} were placed in 120 ml tubes and put onto the plankton wheel for two hours. After two hours, 20 individuals of *S. coeruleus* were and transferred into a petri dish (SARSTEDT, Nümbrecht Germany) with one *D. villosus* individual. The feeding behavior by *D. villosus* was filmed with a camcorder (HDR-XR160E, Sony) fixed on the microscope for one hour. Afterwards, it was immediately frozen, the surface of *D. villosus* was cleaned with demineralized water, and the digestive tract was dissected and scrutinized for analysis of ingested microplastic particles. The attempt was reiterated with two more individuals of *D. villosus*. The relevant footage of particle ingestion was analyzed by using the Manual Particle Tracking PlugIn by ImageJ to indicate the path of the ciliate towards the mouth parts. Footage is available in the supplement information.

3.3.4 Statistical analyses

Results were analyzed in the R programming environment (version 3.5.2, R Core Group 2018, packages: ggplot, dunn.test) using the Levene test to test for homogeneity in variance and the Shapiro test to evaluate the normal distribution. Mean concentrations between samples were tested for significance using an ANOVA for parametric data or alternatively Kruskal-Wallis-Test for non-parametric data. The control groups, in which no microplastic particles were used, were zero in all results and thus were not included in the statistical tests.

3.3.5 Literature review

Literature was searched in terms for experimental studies on the retention by biofilms and ingestion of microplastics by protozoans. Since the term ‘microplastics’ has not been defined before 2004 (Hartmann et al., 2019), the search was extended by searching for feeding experiments of protozoans with plastic particles or plastic beads. Here, the focus was on ciliates, flagellates, and amoeba as these are abundant organisms in biofilms (Arndt et al., 2003; Ackermann et al., 2011). Information about particle size, polymer type, exposure

concentration, length of exposure and effect was extracted. An overview of the literature can be found in Tab. 3.3. in the discussion of chapter 2.

3.4 Results

3.4.1 Retention of microplastics by biofilms (Lab)

The temperature in the circular flow tanks was 10.65 °C in the first and 12.3 °C in the second experiment at the start. This was similar to the temperatures of the River Rhine (Tab. 3.2.). The temperature dropped on average by 1.8 °C in the first and 1.4 °C in the second experiment after 24 hours.

Table 3.2. Overview of temperature (T; °C) and electrical conductivity (σ; µS cm^{-1}) measured in experimental channels 1 - 4 at start and end of the experiment 1 (December, 2018) and experiment 2 (March, 2019) (Haase, 2019). Temperature (T; °C) and conductivity (σ; µS cm^{-1}) of the River Rhine were taken from http://undine.bafg.de.

Exp. 1	Channel	Channel 2	Channel 3	Channel 4	River Rhine
start	T=9.4 °C σ=723 µS cm^{-1}	T=10.0 °C σ=711 µS cm^{-1}	T=11.8 °C σ=704 µS cm^{-1}	T=11.4 °C σ=713 µS cm^{-1}	T=10.4 °C σ=490 µS cm^{-1}
end	T=8.9 °C σ=723 µS cm^{-1}	T=8.7 °C σ=726 µS cm^{-1}	T=8.9 °C σ=727 µS cm^{-1}	T=8.9 °C σ=732 µS cm^{-1}	T=10.7 °C σ=495 µS cm^{-1}
Exp. 2					
start	T=12.5 °C σ=744 µS cm^{-1}	T=12.5 °C σ=742 µS cm^{-1}	T=12.2 °C σ=742 µS cm^{-1}	T=12.1 °C σ=746 µS cm^{-1}	T=8.7 °C σ=515 µS cm^{-1}
end	T=11.0 °C σ=753 µS cm^{-1}	T=11.0 °C σ=751 µS cm^{-1}	T=10.8 °C σ=754 µS cm^{-1}	T=10.8 °C σ=757 µS cm^{-1}	T=9.0 °C σ=495 µS cm^{-1}

It was possible to identify and count all three sizes of the fluorescent microplastic particles in the different samples, e.g. smooth and rough clay tiles, smooth and rough Plexiglas tiles, and biofilms. The particles were mainly attached to biofilm flocs and aggregations of sediments (Fig. 3.2.).

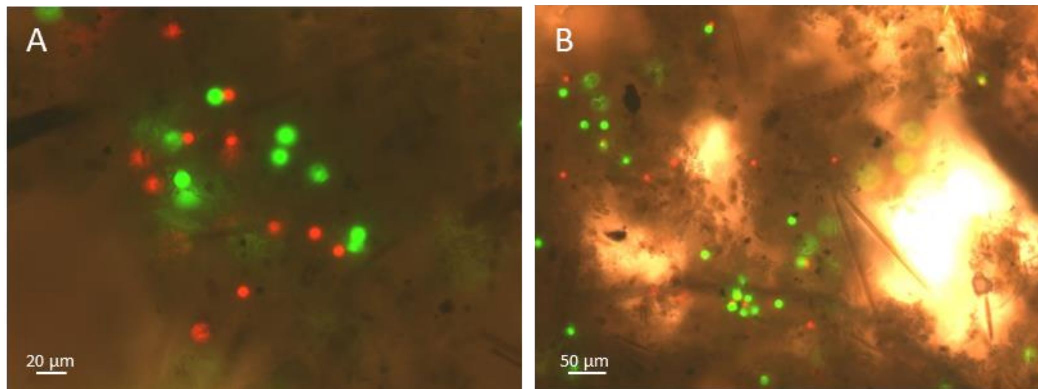

Fig. 3.2. Fluorescent microplastics of 6 µm (red) and 10 µm (yellow-green) in biofilm samples; scales = 20 µm (A), 50 µm (B).

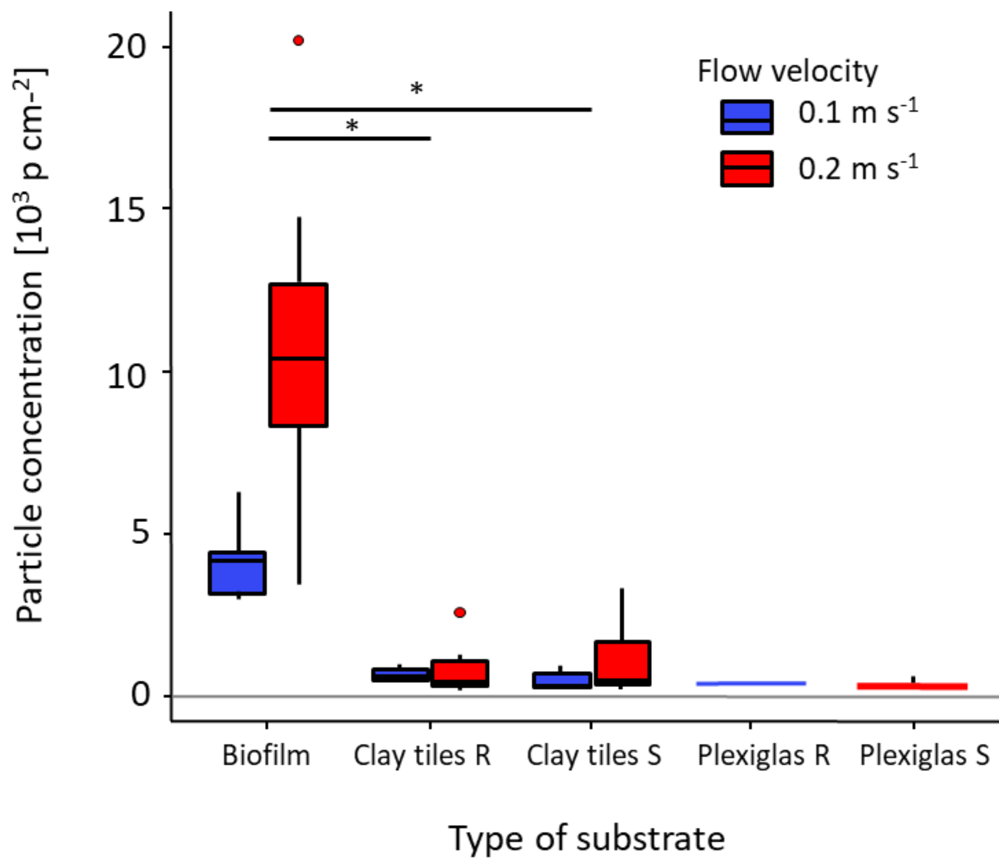

Fig. 3.3. Comparison of the microplastic particle retention (p cm^{-2}) of the 6 µm particle size by the substrates biofilm, clay tiles rough (R), clay tiles smooth (S), Plexiglas rough (R) and Plexiglas smooth (S) for two different flow velocities (0.1 m s^{-1} and 0.2 m s^{-1}). Kruskal-Wallis-Test (*$p < 0.05$), post-hoc Dunn-Test with method 'holm'.

The data of the first experiment, in which the retention of plastic particles by different substrates were compared to biofilms, showed no homogeneity and normality of the data of the comparison of particle retention by difference, thus a non-parametric Kruskal-Wallis-Test was chosen. The retention of MP particles by different substrates showed significant differences in the particle concentration in biofilms compared to the other substrates. The trapped amount of microplastic particles in biofilms were between 6 to 8 times for the velocity of 0.1 m s^{-1} and between 9 to 12 times for the velocity of 0.2 m s^{-1} significantly more than the rough and smooth clay tiles ($p < 0.05$; Fig. 3.3.). The highest numbers of particles retained by biofilms were at the 0.2 m s^{-1} flow velocity with a mean concentration of 10899 particles per cm². The Plexiglas tiles were only tested at a flow velocity of 0.1 m s^{-1} and had

only few particles retained with 370 particles per m² for rough tiles and 305 particles per cm² for the smooth ones. There were no significant differences of MP retention between the two flow velocities (0.1 m s^{-1}, 0.2 m s^{-1}) for each substrate.

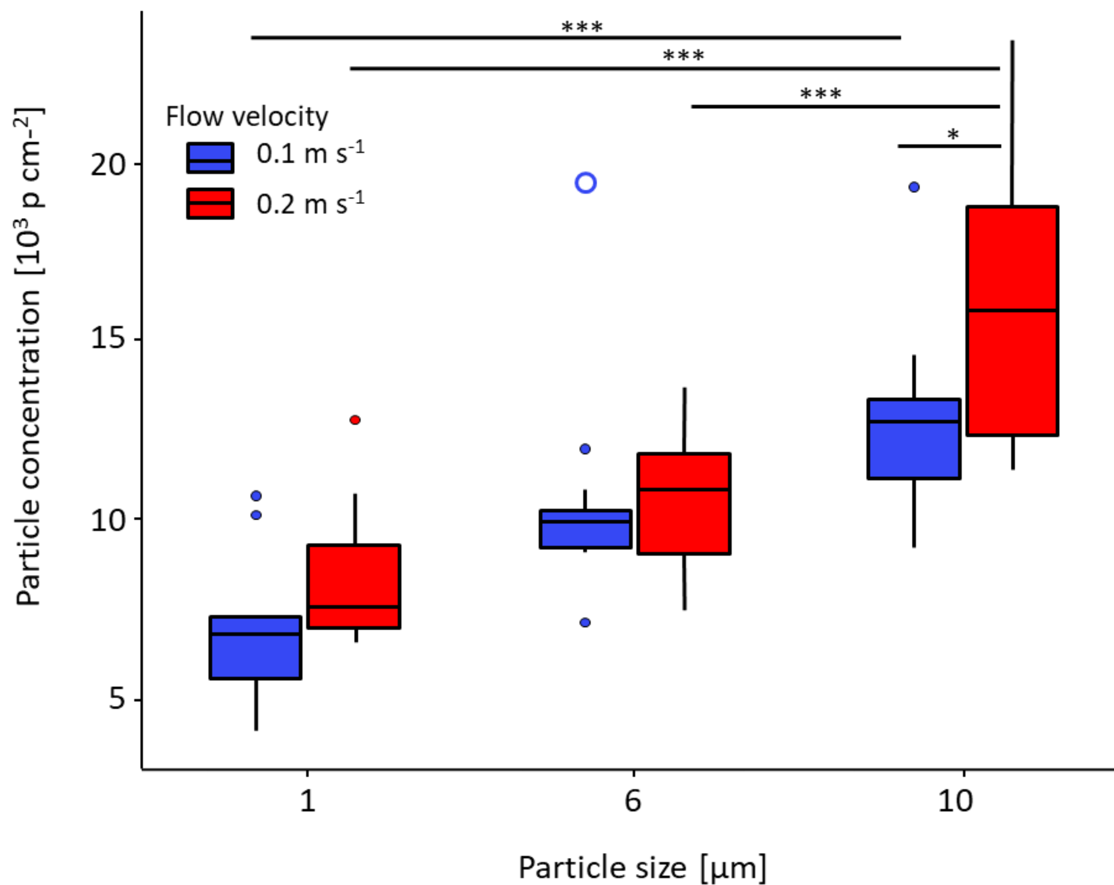

Fig. 3.4. Concentration of microplastics (particles cm^{-2}) in the biofilms compared between microplastic particles sizes (1 µm, 6 µm, 10 µm) for two flow velocities (0.1 m s^{-1}, 0.2 m s^{-1}). Outlier removed (grey circle), ANOVA $p < 0.05$, post-hoc TukeyHSD: * < 0.05, *** < 0.001.

The particle size significantly influences the amount of retained particles and decreases with decreasing particle size ($p < 0.001$; Fig. 3.4.). The concentration of the retained particles was at least twice as high in the 10 µm particles as in the 1 µm particles. The highest concentrations of particles were found for the 10 µm particle sizes and differed significantly between the flow velocities with 12,639 p cm^{-2} for 0.1 m s^{-1} and 16,164 p cm^{-2} for 0.2 m s^{-1} ($p < 0.05$, Fig. 3.4.). There were no significant differences between the flow velocities for the particles sizes 1 µm and 6 µm.

The particle recovery was calculated for experiment 2 by the sum of the particles in biofilms and bulk water divided by the number of particles at the beginning of the experiment (500 p ml^{-1}). On average for all particle sizes, 42.64 ± 15.4% (27.8 - 70%) were recovered (Fig. 3.5.). This is a little less than presented by Arnon et al. (2009), where the recovery of microplastics was 50 - 70%. In the channels with the higher flow velocity (0.2 m s^{-1}), more microplastic particles (regardless which size) are entrapped in the biofilm (Figs. 3.4., 3.5.).

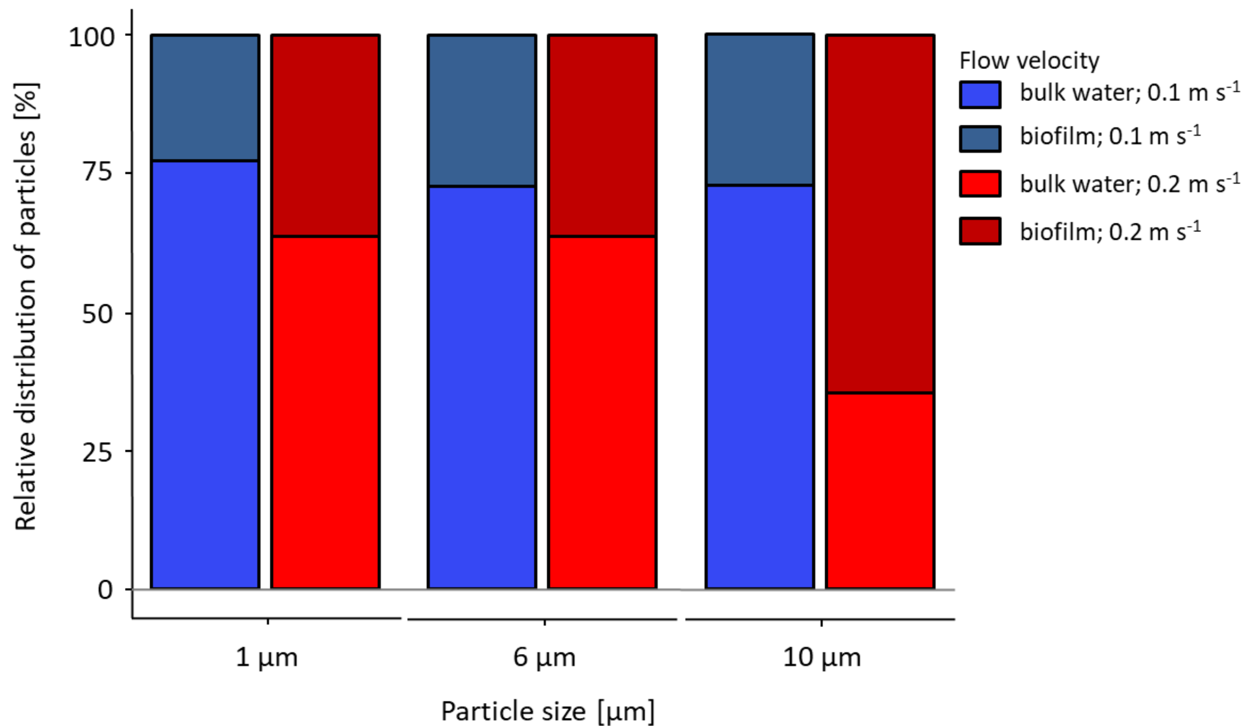

Fig. 3.5. Distribution (%) of microplastic particles (1 µm, 6 µm, and 10 µm) in biofilms (dark grey) and bulk water (light grey) in experiment 2 in channels 1 (0.1 m s^{-1}) and 3 (0.2 m s^{-1}) after 24 hours.

3.4.2 Microplastic uptake by *Stentor coeruleus* and trophic transfer

The highest number of particles was counted in the living cells with a mean of 11.21 p Ind^{-1} for the 6 µm particle size and 19.39 p Ind^{-1} for the 10 µm particle size (Fig. 3.6. A). The formalin fixation led to significant fewer particles within the cells with 2.6 p Ind^{-1} and 2.0 p Ind^{-1} respectively. The cells with the Lugol's solution did not contain any particles. We assume that most if not all plastic particles are egested by *Stentor coeruleus* during the

fixation process. A similar observation was also made for flagellates that released particles when killed with preservatives, which lead to a high underestimate of ingested particles (Pace & Bailiff, 1987). Therefore, it was chosen to count the concentration of microplastics in living cells at the end of the experiments.

The average time for particle egestion was 167 min for the 6 µm particles (n =4) and 185.57 min for the 10 µm particles (n = 7) (Fig. 3.6. B). Thus, the duration of the experiment was set to 60 minutes so the ciliates had enough time to ingest particles, but did not start to egest.

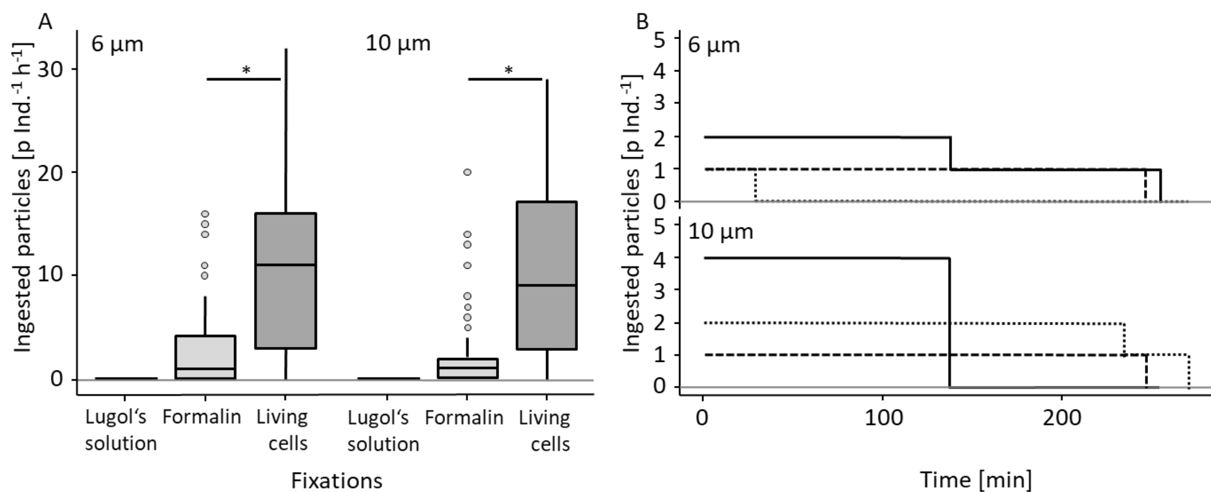

Fig. 3.6. Results of the pre-experiments. (A) Ingestion rate of particles (6 µm and 10 µm) remaining within the cells after fixation with Lugol's solution, formalin, and in living cells as control with an initial particle concentrationfor of 2500 p ml^{-1} for one hour. (B) Cyclose time of *Stentor coeruleus* (n = 3) for 6 µm and 10 µm particles. * < 0.05 (Kruskal-Wallis-Test)

It was possible to identify and count the plastic particles (6 µm and 10 µm) ingested by *S. coeruleus* after one-hour exposure at different microplastic particle concentrations (0 p ml^{-1}, 500 p ml^{-1}, 1,250 p ml^{-1}, 2,500 p ml^{-1}, 5,000 p ml^{-1}; Fig. 3.8.). At the initial concentration of 10,000 p ml^{-1}, it was too difficult to identify individual particles within the moving cells.

The ciliate *S. coeruleus* ingested microplastic particles of both sizes but more at higher particle concentrations in the given medium compared to lower concentrations (Figs. 3.7., 3.8.). For the 6 µm particles all given concentrations showed significantly different particles

abundances within the *S. coeruleus* individuals except for the concentrations of 2,500 p ml^{-1} to 5,000 p ml^{-1}. For ingestion of the 10 µm particles the concentration of 500 p ml^{-1} is significantly different to all other concentrations and the ingestion of particles at the concentrations of 1,250 p ml^{-1} and 5,000 p ml^{-1} are significantly different ($p < 0.05$; Fig. 3.8.). The control of both approaches showed no ingestion (0 p ml^{-1}) and thus was significantly different to all other concentrations.

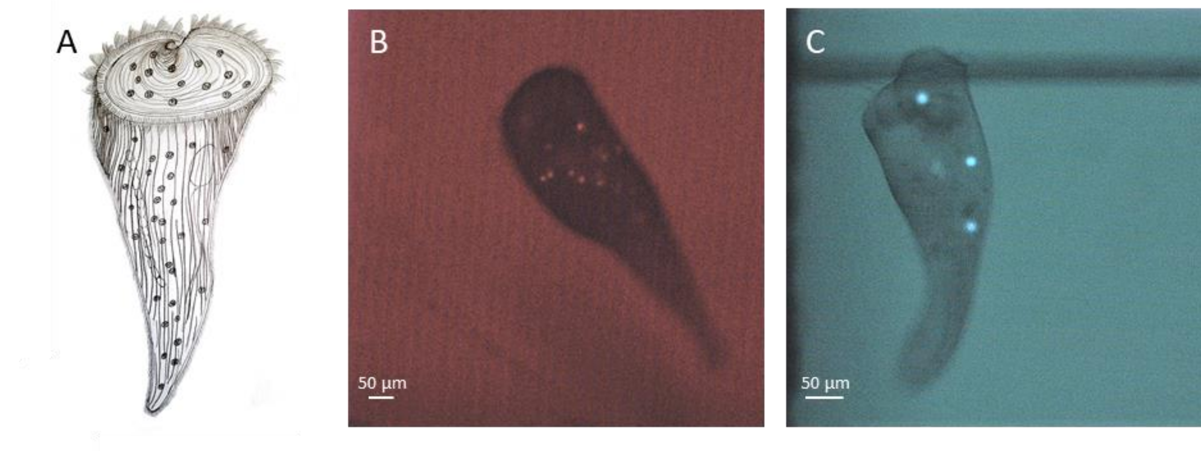

Fig. 3.7. (A) Schematic drawing of *Stentor* sp. (J. Werner). (B) *S. coeruleus* with 6 µm red microplastic particles (M. Thiel). (C) *S. coeruleus* with 10 µm yellow-green microplastic particles (M. Thiel); scales = 50 µm.

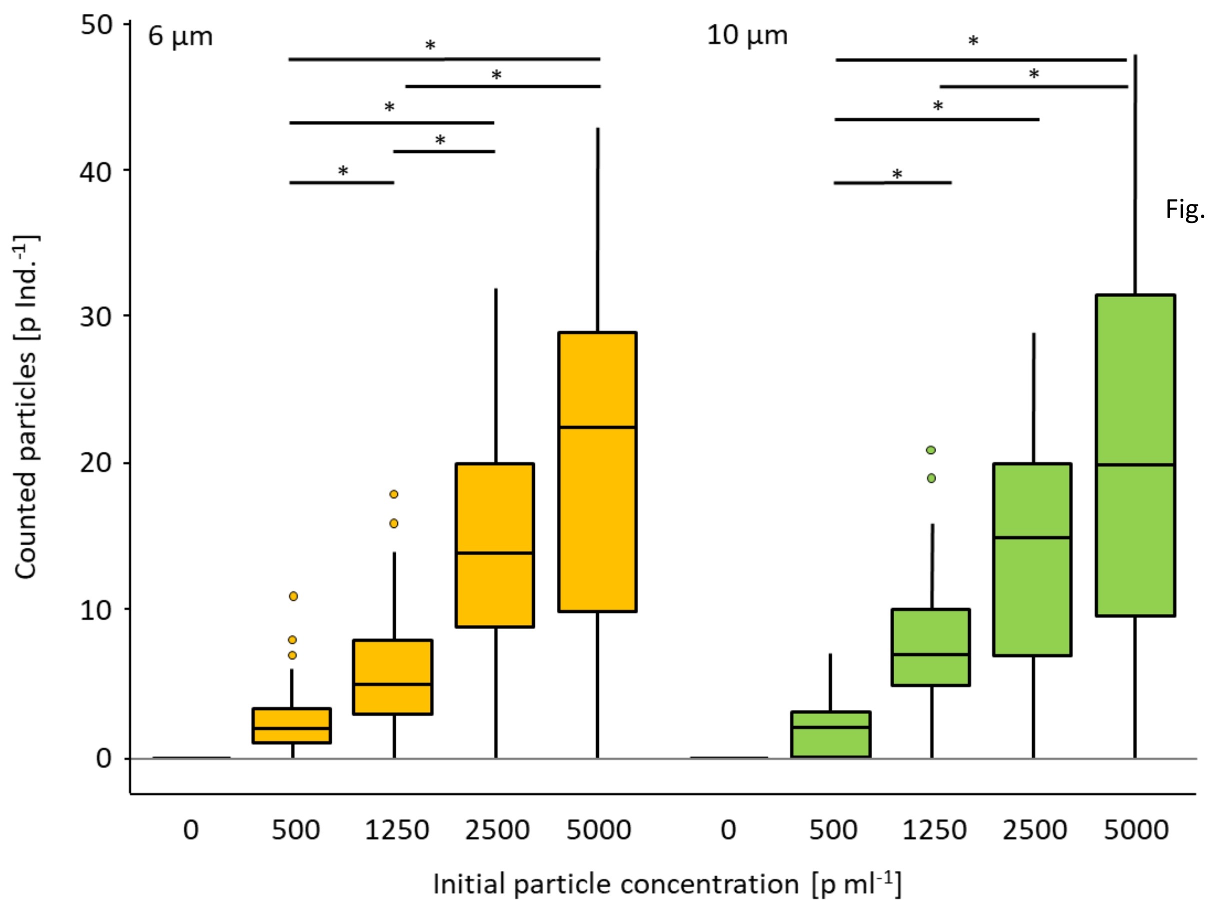

Fig. 3.8. Ingestion of MP (p Ind.$^{-1}$) for the particle sizes of 6 µm and 10 µm by *S. coeruleus* in presence of varying concentrations (p ml^{-1}). Kruskal- Wallis-Test, post-hoc Dunn-Test (* < 0.05). The control (concentration of 0 p ml^{-1}) was significantly different to all other groups (not indicated).

The video analysis showed that individuals of *S. coeruleus* were ingested by one of three *D. villosus* specimens. The path of *S. coeruleus* with incorporated microplastic particles was followed towards the mouthparts of the amphipod and the dissection of its intestinal tracts revealed plastic particles (Fig. 3.9.). There was no MP found in the medium, thus it was assumed, that the microplastic uptake only occurred via the protists.

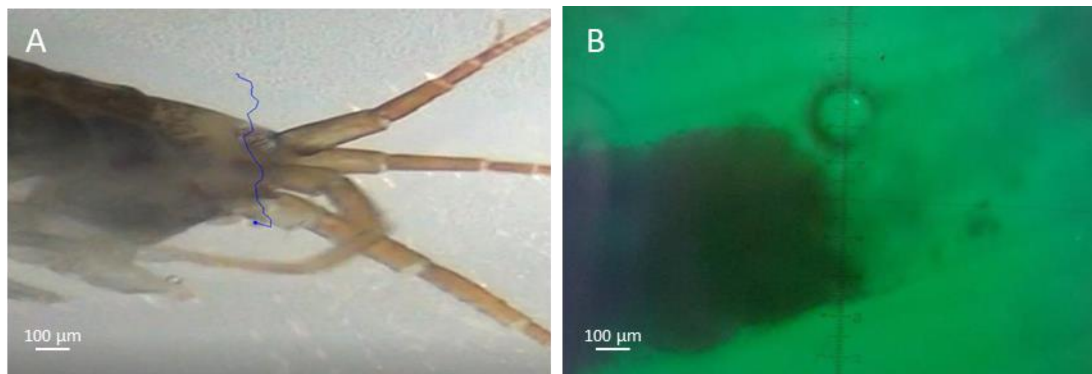

Fig. 3.9. (A) *Dikerogammarus villosus* feeding and ingesting *Stentor coeruleus*. The blue line shows the path of the moving cell (ImageJ); (B) Microplastic particle (10 µm) in the intestinal tract of *Dikerogammarus villosus* (Thiel, 2019); scales = 100 µm.

3.5 Discussion

There are three mechanisms of particle attachment and retention in biofilms: 1) passive interception by biofilm flocs, 2) sorption of particles to the basic biofilm, and 3) grazing activities of ciliates at the surface biofilm (Eisenmann et al., 2001). The first two concern the morphology and material of biofilms, the latter the microbial feeding activity. The share of particles ingested by ciliates is estimated as 10 % of the loaded suspension, leaving effects influenced by biofilm morphology as the major share (Roche et al., 2017).

With the focus on biofilm morphology, we consider the retention of particles in four individual steps: flow field and particle encounter, particle attachment, particle transport within biofilms, and particle detachment. We will discuss our results following these consecutive steps and take a closer look on the role of protists and the uptake of plastics particles in the later part of this discussion (Tab. 3.3.).

3.5.1 Retention by biofilms

Biofilms are complex microhabitats with a three-dimensional structure forming grooves and channels, which enable water flow and nutrient availability for inhabiting organisms (Costerton et al., 1987; Battin 2003; Vasudevan, 2014). They form intricate communities and are involved into the energy flux and the benthic-pelagic coupling of the river (Arndt et al., 2003; Weitere et al., 2018). The morphology and composition of biofilms depend not only on their age but also on the water parameters of the different seasons. The organisms of the pelagic and the benthos in rivers prefer different conditions, concerning ciliates peritrichs prefer colder while hetrotrichs prefer warmer periods (Ackermann et al., 2011). Our biofilms grew in December of 2018 and March of 2019 in the River Rhine and probably had different organismic compositions. Because the experiments were conducted in different seasons with different conditions, their results were not compared.

The general retention of microplastic particles (6 µm) by biofilms was significantly higher than the retention by different clay tiles ($p < 0.05$) and not comparable to different treated Plexiglas tiles because here nearly no microplastic particles were found (Fig. 3.3.).

Particles, microplastics and other organic and inorganic substances are transported to the biofilms by the currents of the river. The hydrodynamic conditions in our experiments resemble those in natural rivers. Here, the flow velocity of the river, roughness, height, age as well as the EPS of the biofilm, and the size and shape of the particles play important roles for the attachment of particles (De Beer et al., 1996; Arnon et al., 2009; Walker et al., 2013; Roche et al., 2017). In a second experiment with three sizes of microplastic particles (1 µm, 6 µm, 10 µm) and two different flow velocities (0.1 m s^{-1}, 0.2 m s^{-1}), the abundance of microplastic particles in the biofilm increases with increasing particle size and flow velocity (Arnon et al., 2009); similar results are given in the present study (Fig. 3.10.). An increased flow velocity influences the turbulent boundary layer over biofilms and pushes particles into the three-dimensional structure of the biofilms (De Beer et al., 1996). Here, larger sizes are more and longer entrapped in the biofilms grooves, voids and channels underlining the importance of the structure and roughness of the biofilm explaining our results of higher abundances in the biofilms of 10 µm particles (Fig. 3.10.). A slight increase in flow velocity

from 0.1 m s^{-1} to 0.2 m s^{-1} resulted in higher abundance of microplastic particles in the biofilm. A higher flow velocity resulted in water following the biofilm structure rather than flowing across it as it did at lower flow velocities (De Beer et al., 1996). The particles are pushed into the biofilms by the forces of the higher flow velocity and are trapped in the grooves and cavities of the biofilm structure (Reynolds and Carling, 1991; Risse-Buhl and Küsel, 2009). The relative abundance of 10 µm particles in the biofilm is around 25 % at the flow velocity of 0.1 m s^{-1} but twice as high at the flow velocity of 0.2 m s^{-1} (around 60 %; Fig. 3.5.). Smaller particles did not settle in the biofilm in the same amount and appear to be stronger influenced by viscous forces.

Further, the abundances of microplastic particles in the biofilms were increasing with increasing particle size (Fig. 3.4.). A similar result was shown by Arnon et al. (2009), though their experiments were conducted with even lower flow velocities of 0.9 cm s^{-1} and 5 cm s^{-1} (is equal to 0.009 m s^{-1} and 0.05 m s^{-1} respectively) the abundance of 5 µm particles in the biofilms are higher than the abundances of the 1 µm particles. In contrast, the biofilm in this study mainly consisted of algae with filamentous structures (up to several centimeters). The particles in such a loose surficial biofilm can be different than in a compact and only few millimeter thick biofilm as we used. In opposite to the results with natural biofilms, model calculations have shown that particles of an intermediate size of 5 µm have a lower retention than particles smaller or larger (1 µm and 10 µm). A similar pattern was shown with biofilms present, though biofilms did not act as a sink but as affecting the shape and density of particles by colonizing those (Besseling et al., 2017). The complex structure of the biofilms with pores and grooves used in this study have high surface enlargements and can explain the retention of at least 25 % of the microplastic particles of the three sizes and at both flow velocities (Drury et al., 1993; Roche et al., 2017).

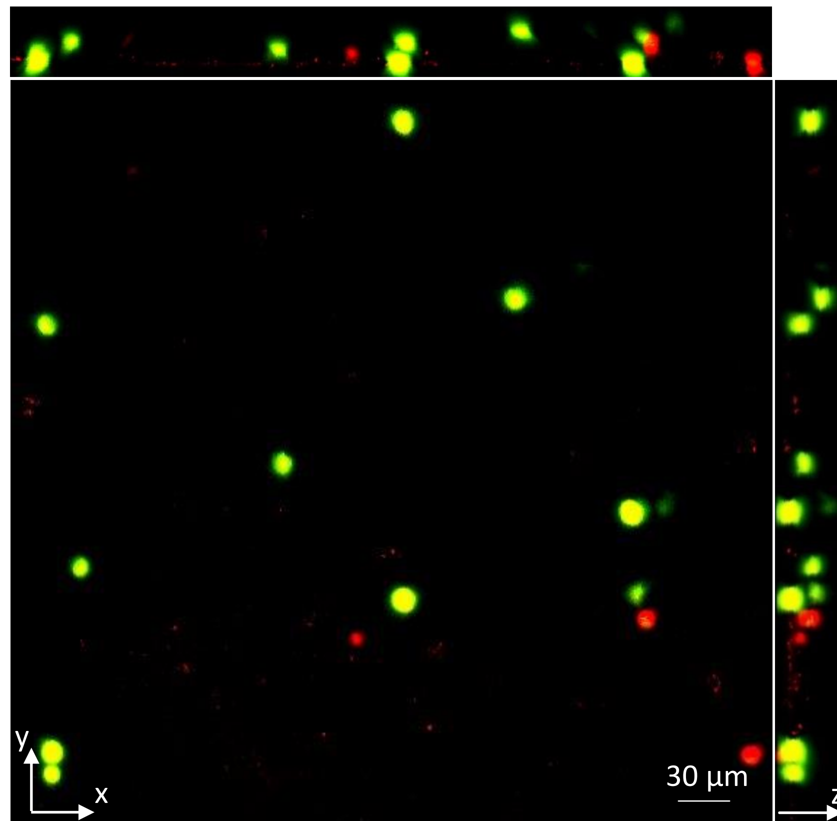

Fig. 3.10. Projection of x, y- and z-axis of microplastic particles (yellow-green: 10 µm, red: 6 µm) incorporated in the biofilm matrix on a glass slide visualized using LSM 510 (Zeiss, Germany) with a magnification of 20x using no transmitted light (F. Haase).

Extensive research has been done on particle retention by biofilms in reactors or bio-filters for wastewater treatment. Here, the biofilms form a dynamic and permeable membrane on support structures are used for microfiltration or ultrafiltration (Sprouse & Rittmann, 1990; Drury et al., 1993; Huang et al., 2019). This research helps to understand the general mechanisms of particle retention and transports, yet, the conditions are different from those in natural streams. Okabe et al. (1997) found that particles penetrated the biofilm within 23 minutes and spatial distribution reached equilibrium after 90 minutes with more particles near the surface than near the substratum. The fast penetration time leads to the conclusion that mechanisms other than diffusion play a role, which contrasted earlier studies (Perry and Chilton, 1973). Okabe et al. (1997) concluded that retention and transport of particles are accomplished through pores and water channels, which was confirmed by Stoodley et al. (1994). Cell abundance is higher near the substrate than near the surface

(Okabe, 1997), also mean pore size decreases from top to bottom (Zhang and Bishop 1994). These findings underline the potential for microplastic particles to attach to biofilms and be entrapped in its structure. In our experiment, we did not distinguish between the effects of biofilm structure and biofilm-associated organisms (for a potential influence of biofilm ciliates, see 3.5.2. and Figs. 3.7., 3.8.). Our experiments show that 6 to 8 times (at a flow velocity of 0.1 m s^{-1}) and 9 to 12 times (at a flow velocity of 0.2 m s^{-1}) more microplastic particles were found in biofilms than on clay tiles without biofilms (Fig. 3.3.). The three-dimensional structures of the biofilms form with biofilm growth, but complex structures are present after only a few days (Battin et al., 2003). Especially algae are responsible for the complex three-dimensional structure forming pits, grooves and caves as the biofilms grow and can cause the trapping of various particles (Roche et al., 2017). Further, the EPS layer can play an important role in attaching of microplastic particles to the biofilms, after which they get pushed into the layers of the biofilm by the forces of the flow velocity (Mikos & Peppas, 1990). Though most of the particles were still found in the bulk water, this can be explained by the small proportion of biofilm surface of 240 cm^2 compared to a large amount of surrounding water of 20 liters within the endless flow channels (Figs. 3.1., 3.5.). A study with bacterial biofilms showed that the number of attached plastic particles of 1 µm increased proportionally with the amount of biofilm present, which did not incorporate any protists. Additionally, particle concentration was also proportional to the standard deviation of biofilm thickness, thus indicating that biofilm age and morphology plays an important role in particle retention (Drury et al., 1993). Finally, the influence of biofilms in particle dynamics in streams was modelled based on experimental studies with biofilms (Roche et al., 2017). The authors found that particle retention correlated with biofilm height which can depend on the age of the biofilms, roughness and fraction substrate coverage. These studies show that there is a lack of studies about the effect of naturally grown biofilms on microplastic particles and underline the importance of the results shown in our study.

Our experiments were conducted for only 24 hours. Other studies show the fate of microplastic particles in biofilms over a more extended period. For more than 20 days, microplastic particles, here called microbeads, remained in biofilms and were transported into the deeper layers of the biofilms very fast via the pores and channels of the biofilms

(Stoodley et al., 1994; Okabe et al., 1997). Transport of plastic particles inside biofilms is accomplished by advection resulting from cell growth and re-adhesion of particles that detached from pore walls causing redistribution (Okabe et al., 1997). Reichert and Wanner (1997) reported that microbeads persisted longer in the biofilm than bacteria naturally occurring in the biofilm. The pores become significantly smaller towards the substrate, the biofilm becomes increasingly dense (Zhang and Bishop, 1994) and can quickly become clogged with microplastic particles. Particles trapped in the biofilm can thus be flushed out less efficiently by the forces of the flowing water and remain there, occupying the protective microhabitats for protozoa such as bacteria and flagellates. Therefore, not only biofilms have an impact on microplastic particles, but the particles can also change the nature of the biofilm and thus change the habitats and conditions for the organisms of the river and of the biofilm.

Mature biofilms often reach a thickness that cannot withstand the forces of river flow velocities. Parts of the biofilm can tear off and reattach to other substrates in the river, causing the biofilm and its organisms to disperse (Schönborn, 1992; Risse-Buhl and Küsel, 2009). Pits and voids of the biofilm can bind protozoa and organic particles, as well as microplastic particles as shown in our experiments. When parts of the biofilm are torn off, microplastic particles can also be dispersed within the environment (Drury et al., 1993). Detachment from biofilms can happen as separate particles, as part of biofilm particles or by biofilm sloughing (Wanner and Gujer 1986; Okabe et al., 1997). An average of 20 % of the entrapped particles in older, thicker biofilms were released to the bulk water after 20 days; thus detachment rates are low (Okabe et al., 1997). The release rate coefficient indicates that it is slower in mixed population biofilms than in young and mono-population biofilms, thus being dependent on biofilm properties (Okabe et al., 1997). In our study, we did not focus on the detachment of biofilm parts and the studies for too short to get any results if microplastic particles detach with the biofilm and show a potential do disperse in the river. But during our studies we were able to observe that flocks of biofilms from the River Rhine which were not treated with fluorescent microplastic particles did contain plastic particles, especially filamentous plastic particles (Fig. 3.11.).

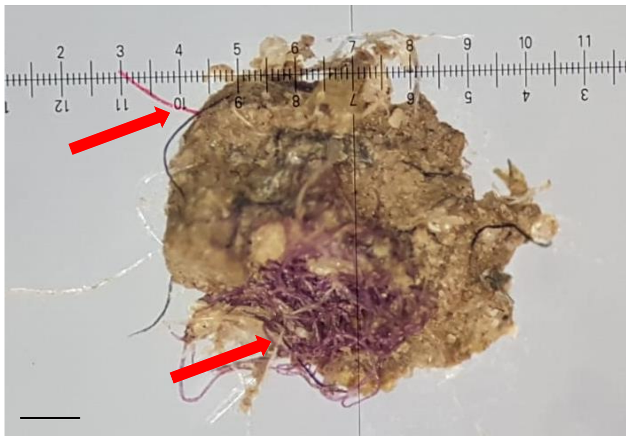

Fig. 3.11. Biofilm from the River Rhine with filamentous plastic particles (indicated by red arrow); scale = 2.5 mm

3.5.2 Ingestion of microplastics by protists

The ingestion of particles strongly depends on the feeding types of the protist (Fenchel, 1987). Even though benthivorous, many suspension-feeding and filter-feeding protists in biofilms have been shown to feed on suspended particles (Ackermann et al., 2011). Other feeding types, such as raptorial feeding amoeba, are more likely to feed on particles attached to the biofilms (Arndt et al., 2003; Böhme et al., 2009).

The fluorescent microplastic particles were selected according to the size classes of bacteria and algae preferred by the genus *Stentor* (Wenzel and Liebsch, 1975; Foissner et al., 1992). The results of the experiment show, that *Stentor* specimens were able to ingest microplastic particles of the sizes 6 µm and 10 µm and the number of particles ingested increases with rising initial particle concentration in the medium (Fig. 3.7.). Unfortunately, no maximum uptake was achieved and the experiment had to be terminated at a concentration of 10,000 p ml^{-1} since a quantitative evaluation was no longer possible at this concentration due to cluster formation of the microplastic particles. These results show that non-selective ciliates, such as *Stentor*, are capable of ingesting microplastic particles. They use a filter-feeding mechanism to ingest particles of any kind (Foissner et al., 1992), but it was also observed that *Stentor* individuals do not digest but eject the microplastic particles after a particular time (Fig. 3.6. B). *Stentor coeruleus* has a body size of 1 - 2 mm and prefers food particles of 7 - 22 µm (Foissner et al., 1992). The size range of the preferred food

particles underlines our result that *Stentor* individuals showed the highest abundances of ingested microplastic particles for the largest used size of 10 µm. This indicates that particle size is an important factor in particle retention by protists.

3.5.3 Trophic transfer

To find out if trophic transfer of microplastic particles is possible, we placed *Stentor* individuals with ingested particles into a medium in which *Dikerogammarus villosus* was present. In this experiment, we were able to show video graphically that *Dikerogammarus* ingests *Stentor* individuals and thus microplastic particles could enter the digestive tract of the gammarid (Fig. 3.9.). Other ingestion could be ruled out because the medium was free of microplastic particles and fluorescent microplastic particles were only present within the *Stentor* individuals.

In our experiments, the uptake of *S. coeruleus* by *D. villosus* and the transfer of microplastics from the ciliate cell to the intestinal tracts of the amphipod could only be shown for one feeding event. It is assumed that the density of *S. coeruleus* individuals was too low to lead to more feeding events. Additionally, *D. villosus* might have been too stressed by the experimental setup and thus show no normal feeding behavior. However, we think that these first findings are enough to assume that trophic transfer of microplastics from protists to grazers or predators take place. Initial experiments have also shown that microplastic particles can be passed on to fish larvae via the ingestion of ciliates. Microplastic transfer from a copepod to a mysid shrimp was already observed and supports the findings of the present study and underlines the needed sensitivity towards possible microplastic transfer into higher trophic levels (Setälä et al., 2014).

Furthermore, microplastic particles were observed to be embedded in the spongin of *Spongilla lacustris* and on the material the tubes of *Chelicorophium curvispinum* are made of. In indeterminate ciliates and amoboezoans a transport of microplastic particles could also be observed. Based on these observations, it can be assumed that the various organisms in rivers can ingest microplastic particles or that these particles can get attached to the

organisms. In order to make reliable statements about the different pathways of microplastics in rivers, further targeted studies are necessary (Fig. 3.12.).

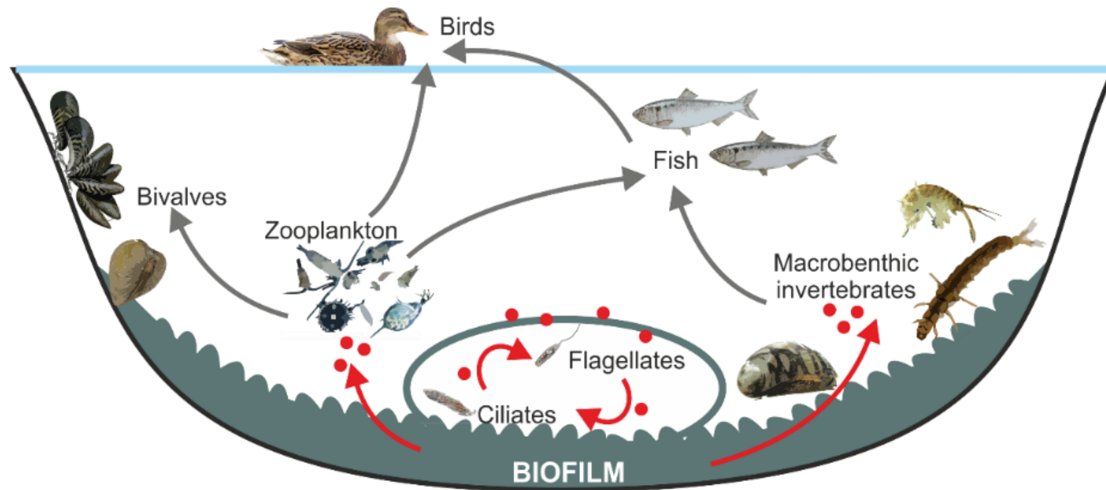

Fig. 3.12. Schematic drawing of possible pathways of microplastics (red) in the food web in rivers entering through biofilms.

3.5.4 Conclusion: ecological implications

We conclude that biofilms play an important role in the fate and effects of microplastic in freshwater streams. Especially due to the complex three-dimensional structure of biofilms, microplastic particles are bound and can remain in the voids and channels of the biofilm for a longer period of time. This reduces the abundance of microplastic particles in the surrounding water. However, due to sloughing, microplastics are released back into the water.

It is shown that the ciliate *Stentor* is able to ingest microplastics and concluded that other ciliates might be able to do so if particle size is similar to natural food sources and if particle concentration is high enough. Due to the role of ciliates within the food chain of biofilms but also higher trophic levels, it is likely that those particles are transferred to predators of rivers, such as gammarids, as shown in Figure 3.9.

It is important to have sufficient and reliable data to make reliable numerical models and execute risk assessments. First risk assessments show, that harmful effects are not expected until 2100. However, high concentration in local areas can lead to earlier effects (Everaert et al., 2018). More research is required to test the effects of microplastics with various polymer

types, shapes and sizes on single protists, microbial communities and general ecology in freshwater, similar as it has been done with metazoan. Additionally, more insight into the particle retention mechanisms of microplastics by biofilms might influence the application of biofilms in waste water treatments to retain plastic particle and reduce microplastic emissions (More et al., 2014; Li et al., 2018b).

3.5.5 Literature review

Exemplary studies on single protozoan species and the uptake of plastic particles were found for ciliates, flagellates and amoeba (Tab. 3.3.). The feeding types included filter-feeding and suspension-feeding in ciliates, but often the information on feeding types were missing. The tested plastic particles were mainly latex (polystyrene) particles and usually ranged in size from 0.09 µm to 10 µm; single experiments were conducted with larger particles. Because cell size was rarely given, a general relation of cell size to tested particle size could not be made. Many studies aimed to observe if and how particles are ingested; therefore the concentrations of particles ranged and the length of exposure varied widely. Further, concentrations were particularly high in certain studies to increase the chance of encounter. In studies in which particle uptake was given additionally to natural food sources, the particle concentration was only a fraction of e.g. bacterial concentration (McManus & Fuhrman, 1986; Pace & Bailiff, 1987). In general, particle concentration was usually set minor than the naturally occurring food particle concentration (Borsheim, 1984). As results, the number of particles within the cells varied depending on particle concentration and exposure time. Additionally, not all studies measured the ingested particles because the aim was instead the clearance rate, which is given in µl per individual (Borsheim, 1984; Jonsson, 1986; Pace and Bailiff, 1987).

Eisenmann et al. (2001) investigated the particle concentration in the ciliate genus *Epystilis* from biofilms. They recorded up to 1,000 particles per individual after 3.5 hours of the 24 h experiment. After this peak, the number of ciliates and the ingested particles decreased rapidly. At the end of the experiment, ciliates could be found in the biofilm but did not contain any particles. Particles were rather attached to the stalks of the ciliates,

which contribute to the structure of the biofilms (Eisenmann *et al.,* 2001). Also, other studies with longer exposure times show that the highest peak of ingested particles is rather at the beginning of the experiment than at the end (Batz and Wunderlich, 1976; Jonsson, 1986). As in the experiments of this study, the rate of uptake is high, then slows down and might even decrease because more particles are egested than ingested (McManus and Fuhrman, 1986; Pace and Bailiff, 1987). This could indicate adaptive behaviour. It was shown that particle discrimination by size in suspension-feeding protozoans could be explained due to the functionality of the mouth apparatus, but no discrimination for particle type (Fenchel, 1980). However, subsequent studies showed that particle uptake by ciliates correlated with particle concentration and particle size, the ladder also depending on the cell size of the protozoans (Jonsson, 1986).

Sherr et al. (1987) compared the uptake by ciliates and flagellates of fluorescently labelled bacteria and fluorescent latex particles, which showed that the uptake ratios were 10:1 for the ciliates and 6:1 for the flagellates, thus preferring the bacteria over the plastic particles, indicating the ability to distinguish between different particle types. A similar trend was found by Mc Manus and Fuhrmann (1986) and also for amoeba (Bowser and Olszweski, 1983; Winiecka-Krusnell et al., 2009). Additionally, in 1916 Metalnikov observed that food vacuoles of ciliates containing digestible material circulate much longer than those containing inert material (Bowser and Olszweski, 1983).

Oxyrrhis marina, a heterotrophic dinoflagellate, was found to ingest microplastic particles of 7.3 µm, but no larger particles in laboratory studies (Cole et al., 2013). The pelagic ciliate *Tintinnopsis lobiancoi* was tested for microplastic particle (10 µm) uptake with varying concentrations (1,000 p ml^{-1}, 2,000 p ml^{-1}, 10,000 p ml^{-1}). Between 55 up to 85 particles were found per cell and thus were higher than particle uptake by other zooplankton taxa, such as copepoda, cladocera, polychaeta, rotifera or mysida (Setälä et al., 2014). A similar uptake of microplastic particles was observed for the ciliate *Stentor coeruleus*, though the ingested particles were not countable with certainty at the initial particle concentration of 10,000 p ml^{-1} because they clustered in the ciliate’s food vacuoles (Fig. 3.8.).

Besides other factors like particle shape and polymer type (Botterell et al., 2020), particle size plays a crucial role in particle retention by protists. The different genera of flagellates, ciliates, and other protists prefer different size ranges of food particles; thus the range for the uptake of microplastic particles also differs for every organism. In our experiments, *Stentor coeruleus* individuals ingested 6 µm and 10 µm microplastic particles, which fit into the given food particle size range of 7 - 22 µm (Foissner et al., 1992). These sizes represent only a fraction of the body size of the ciliate genus *Stentor* (1 - 2 mm, Foissner et al., 1992), indicating that the food particle size ranges for smaller protest are even smaller (e.g. for flagellates, body size of 1 - 450 µm (Jeuck and Arndt, 2013)). The results of the Literature review showed that particle sizes from 0.1 to 10 µm have been used in experiments. When detecting microplastics in environments, the applied methods often excluded small-sized particles (< 10 µm; Kanhai et al. 2017). Thus, it is often neglected which influences these particle sizes might have on protists in natural habitats ingesting them.

The size range of present microplastics particles is not available for specific environmental compartments yet. Hence, it is currently impossible to make assumptions on how natural concentrations are similar to the ones in the experiments and have to be estimated. The results of this study show that particle concentration influenced particle uptake in *Stentor coeruleus*. The chosen initial microplastic particle concentrations of up to 10,000 particles per milliliter represent the abundance of heterotrophic flagellates and algae of rivers, but the abundance of planktonic bacteria even exceeds the initial microplastic particle concentration (see chapter 1 Figs. 2.5., 2.6.; Kathol et al., 2011). Thus, the absence of a saturation point in the feeding experiments with *Stentor coeruleus* can be explained because they mainly feed on algae and bacteria (Foissner et al., 1992). To study the possible ingestion of microplastic particles by different organisms, it is essential to adapt the offered concentration of microplastic particles to that of the preferred food particles of the examined organisms. Büks et al. (2020) compared different studies dealing with microplastics in soil and estimated that more than half of the studies did not use the appropriate plastic concentration. Further, shape and size are of importance for feeding experiments. In the present study, round spheres of small sizes were used, which fit to the size and shape of bacteria and are used for easy detection and availability, but do not

represent the microplastic particles present in the environment (Yokota et al., 2017). In different ecosystems, fibres are often present, but due to the limited availability of fibres for experiments, their effects are rarely investigated (Bükes et al., 2020).

The literature and the present experiments on *Stentor coerules* show, that many protists are able to ingest microplastics (Figs. 3.7., 3.8.). These findings give rise to the question: Which effects have microplastics on protists? One factor that is often neglected, including in this study, is the time of exposure to microplastic particles and fate of particles and organisms after ingestion. Cyclose time of *Stentor* individuals show the egestion of microplastic particles, but it was not examined if the individuals can survive constant ingestion of microplastic particles instead of sufficient amount of and appropriate food particles. Microplastic particles showed no effects on the movement and metabolism of the benthic foraminiferan *Haynesina germanica*; however, these experiments were also performed for short durations only and underline the need for long-term experiments (Langlet et al., 2020). In amoeba, the presence of monodisperse Stöber silica microspheres of 1 µm, 3 µm and 6 µm in diameter or 4 µm polystyrene spheres dramatically increased the rate of *Difflugia* growth and foreign microspheres became the dominant construction material (Bian et al., 2019). Further, due to the ingestion of microplastic particles and their stay in food vacuoles of the protists, the surface of egested particles might be altered and therefore be more attractive or disguised for protists (Dolan and Coats, 1991). The examination of these particles is challenging, but their alteration has to be considered. Yokota et al. (2017) observed dynamic interactions and effects between two cyanobacteria species and microplastic particles. The settlements of bacteria or algae on plastic particles can enhance the bioavailability of these particles, increase their ingestion and, therefore the abundance in higher trophic levels of food webs (Yokota et al., 2017).

Many studies investigated the effects of microplastic particles on only a single or few species, though the effect can be different in communities (Böhme et al., 2009). Aggregation and sinking, egestion and enhanced bioavailability of microplastic particles and the effect on particular but not all species can change the species composition of communities. The decrease of one species can increase the abundance of another competing species and affect preying species. None of the publications observed any ecological effects on single

protist species. Most particle uptakes resulted in a seemingly harmless expel of particles. However, none of the studies tried to investigate possible effects. Therefore, a variety of experiments is needed to understand the effects of microplastics on single species but also interacting communities. With a better understanding of these effects, a better solution to the problem of plastic particles in different ecosystems can be established.

Table 3.3.: Exemplary studies of the ingestion of plastic particles by biofilm communities, ciliates, flegaellates and amoebae.

Species	*Environment*	*Feeding type of protozoans*	*Size of particles [µm]*	*Polymer type*	*Exposure concentration [p ml^{-1}]*	*Length of exposure*	*Number of particles per cell*	*Source*
Biofilms								
Mono-population biofilms of *Pseudomonas aeruginsa*	Biofilm bacterium (gram-negative)	Chemo-organotroph	1	NA (Polyscience Inc., No. 15702)	na	90 - 145 hours	na	Drury et al., 1993
Mixed-population biofilms growing on basalt particles	BAS-reactor	Autotrophic and heterotrophic	0.87	Fluorescent latex beads (Polyscience Inc., Cat. no. 15702)	Added stepwise	Up to 1,500 hours	3.6 * 10^9 p m^{-2} biofilm, 4.6 * 10^{12} p m^3 bulk water	Tijhuis et al., 1994
Mixed-population biofilms growing on basalt particles	BAS-reactor	Heterotrophic, mixed and autotrophic biofilms	1	Fluorescent latex beads (Polyscience Inc., Cat. no. 15702)	Added stepwise	Up to 1,400	3.6 * 10^9 p m^{-2} heterotroph biofilms and 4.6 * 10^{12} p m^3 bulk water; 1.7 * 10^9 p m^{-2} mixed biofilms and 1.7 * 10^{12} p m^3 bulk water; 0.74 * 10^9 p m^{-2} autotroph biofilms and 1 * 10^{12} p m^3 bulk water	van Benthum et al., 1995
Mixed-population biofilms grown from waste water treatment plants	Rotating disc reactor; young and old biofilms	na	1	Fluorescent latex beads (Polysciences Inc., No. 15702)	5×10^{10} to 2×10^{11}	24 hours	Up to 3.5 * 10^7 p cm^{-2} in biofilms ; p ml^{-1} decreased from 3.2 to 1.2 * 10^7 in bulk water	Okabe et al., 1997

Species	*Environment*	*Feeding type of protozoans*	*Size of particles [µm]*	*Polymer type*	*Exposure concentration [p ml^{-1}]*	*Length of exposure*	*Number of particles per cell*	*Source*
Mixed-population biofilm grown on clay marbles; sessile ciliates *Epistylis* domianting	Waste water reactor (biofilter)	grazing	1	Fluorescent latex beads (Polysciences Inc., No. 17154) and fluorescing hydrophilic beads (car- boxylated polystyrene (Molecular Probes, No. 17154)	3.23 x 10^7	24 hours	10,700 – 37,000 p mm^{-2}; Up to 958 p Ind^{-1} and up to 1,209 p Ind^{-1} h^{-1}	Eisenmann et al., 2001
Primarily of green algae *Spirogyra* and *Mougeotia*	Biofilm and sand in laboratory flume	na	1, 5	carboxilated fluorescently-labeled microspheres (Polysciences)	500 for 1 µm 200 for 5 µm	80 – 100 hours	Up to 3x and40x more 1 µm and 5 µm beads in biofilm than in sand respectively	Arnon et al., 2009
na	Natural growth within test flume	na	~ 8.4	fluorescent particles (EcoTrace, ETS Worldwide Ltd.)	na	30 min	Significantly more particles in 42 days old biofilm than 18 days old biofilm	Roche et al., 2017
Ciliates								
Tetrahymna pyriformis	PPL medium	phagotrophic	2.02	Polyvinyl-toluene-latex beads	10^8	3 h	186 p Ind^{-1} after 45 min; 166 p Ind^{-1} egested after 145 min	Batz and Wunderlich, 1976
13 species of ciliates	Isolated from ponds, streams, soil	Suspension-feeding	0.09, 0.11, 0.23, 0.36, 1.09, 2.02, 5.7	Latex beads (Dow Chemical Company)	Different concentrations (no concentrations given)	5 min	Clearance rate up to 9*10^5 p Ind^{-1} (*Stylonychia*)	Fenchel, 1980
Epistylis rotans, *Strombidium* sp.	Isolated from lake, pelagial	Filter-feeding	0.57, 1.04	Monodisperse fluorescent latex beads (Fluoresbrites, Polysciences)	2.6 - 6.5 x 10^5	0.05, 2, 4 and 6 min	Clearance rate of 0.23-1.26 µl Ind^{-1} h^{-1} *Epistylis* and 0.26-0.9 µl Ind^{-1} h^{-1} *Strombidium*	Borsheim, 1984

Species	*Environment*	*Feeding type of protozoans*	*Size of particles [µm]*	*Polymer type*	*Exposure concentration [p ml^{-1}]*	*Length of exposure*	*Number of particles per cell*	*Source*
Strombidium reticulatum, *Strombidium vestitum*, *Lohmanniella spiralis*	Planktonic, isolated from surface waters; laboratory cultures	Filter-feeding	1.01, 2.11, 2.87, 5.7, 6.4, 9.7, 14.4, 19.1	Latex beads (Dow Chemical Company, Coulter Electronics Lt.)	na	2 - 20 min	Clearance rates (µl h^{-1}) depending on particle size: *L. spiralis* 0-26, *S. reticulatum* 0-1.7, *S. vetitum* 0-0.52	Jonsson, 1986
Mixed culture with oligotrichs, scuticociliates	Marine, isolated from Dublin River	phagotrophic	0.51	Fluorescent latex beads (Polysciences Inc.)	na	20 min	2.7 p Ind^{-1}	Sherr et al., 1987
Cyclidium sp.	culture	grazing	0.57	Fluorescent latex beads (Polysciences Inc.)	4-6 x 10^5 in field experiments	90 min	Around 14 p Ind^{-1} after 90 min; clearance rate of 1-2% of added particle concentration in field experiments	Pace and Bailiff, 1987
Predator: *Mesodinium pulex*, *Euplotes vannus*, *Euplotes woodruffi* Prey: *Metanophrys* sp., *Cyclidium* sp, *Pleuronema* sp.	Estuarine ciliates in laboratory experiments	raptorial, filter-feeding	1	Yellow-green fluoresbrite plain microspheres, Polysciences Inc.	1 * 10^2 - 10^9 p ml^{-1}	10 - 140 min	*M. pulex* 0 p Ind^{-1}, *Euplotes* low ingestion rates of free microspheres; *Metanophrys* sp., *Cyclidium* sp, *Pleuronema* sp. ingested microspheres at different concentrations	Dolan and Coats, 1991

Species	*Environment*	*Feeding type of protozoans*	*Size of Particles [µm]*	*Polymer type*	*Exposure concentration [p ml^{-1}]*	*Length of exposure*	*Number of particles per cell*	*Source*
Epistylis	As part of biofilm in waste water reactor (biofilter)	na	1	Fluorescent latex beads (Polysciences Inc., No. 17154), fluorescing hydrophilic beads (car- boxylated polystyrene (Molecular Probes, No. 17154)	3.23 x 10^7	24 h	Ca 1,000 p Ind^{-1} after 3.5 hours	Eisenmann et al., 2001
Tintinnopsis lobiancoi	Isolated from Finnland, Baltic Sea	Filter-feeder	10	Floureszent Polystyrene spheres (Polyscience Inc.)	1,000, 2,000, 10,000	3 h	55 - 85 p Ind^{-1} after 30 min; ingestion of microspheres by all tested taxa (copepod, cladocera, polychaeta, rotifer, mysida)	Setälä et al., 2014
Halteria grandinella Strombidium sp. Vorticella microstoma	Monomictic man-made lake	bacterivorous	0.57	carboxylated fluorescent microspheres (Fluoresbrite, Polysciences, Inc.)	5 - 9 * 10^5 p ml^{-1}	15 min to 1 hour	Clearance rates (nl h^{-1}): 126 *of H. grandinella*, 30 of *Strombidium* sp., 138 of *V. microstoma*	Sanders et al., 1989
Flagellates								
Attached to aggregates and unattached flagellates	Isolated from Long Island Sound	heterotrophic	2 - 4	Dyed trialdehyde resin (Radiant Color Co.)	1.8, 3 x 10^6	10 h	Up to 0.8 p Ind^{-1} in attached and up to 0.7 p Ind^{-1} in unattached flagellates	McManus and Fuhrman, 1986
Mixed culture	Isolated from estuarine water	phagotrophic	0.51	Fluorescent latex beads (Polysciences Inc.)	na	40 min	0.07-0.1 p Ind^{-1}	Sherr et al., 1987

Species	*Environment*	*Feeding type of protozoans*	*Size of particles [µm]*	*Polymer type*	*Exposure concentration [p ml^{-1}]*	*Length of exposure*	*Number of particles per cell*	*Source*
2 heterotrophic flagellates (bodonid and chrysomonad)	Isolated from sediments	heterotrophic	0.57	Fluorescent latex beads (Polysciences Inc.)	4-6 x 10^5 in field experiments	250 min	0.01 and 1.7 p Ind^{-1} after 250 min (depending on species)	Pace and Bailiff, 1987
Bodo spp., *Paraphysomonas* sp., *Pteridomonas danica*, *Diaphanoeca* sp., *Bodo parvulus*, unidentified heterotrophic flagellates	Isolated from Sørfjorden, Norway and Barents Sea, field experiments in Masfjorden	bactivorous	0.57	Monodisperse yellow-green latex beads (Polysciences Inc.)	na	48 hours	Clearance rate of beads by *Bodo* sp. 0.61-0.9 nl Ind^{-1} h^{-1}, 1.9 * 10^4 - 5 * 10^5 nl Ind^{-1} h^{-1} flagellates of field samples; all tested flagellates were able to ingest beads	Nygaard and Thingstad, 1988
Monas sp. 1 *Monas* sp. 2 Colonial heterotroph *Ochromonas*	Monomictic man-made lake	Heterotroph, mixotroph	0.57	carboxylated fluorescent microspheres (Fluoresbrite, Polysciences, Inc.)	5 - 9 * 10^5 p ml^{-1}	15 min to 1 hour	Clearance rates (nl h^{-1}): 0.8 *of Monas* sp. 1, 0.6 of *Monas* sp. 2, 3.9 of colonial heterotroph, 0.1 of *Ochromonas*	Sanders et al., 1989
Paraphysomonas imperforata	Isolated from marine snow	heterotrophic	0.5	Monodispersed, fluorescent polystyrene beads	4.1 * 10^6	64.5 hours	Due to ingestion and egestion by flagellates particles aggregate	Fukuda and Koike, 2000

Species	*Environment*	*Feeding type of protozoans*	*Size of particles [µm]*	*Polymer type*	*Exposure concentration [p ml^{-1}]*	*Length of exposure*	*Number of particles per cell*	*Source*
Paraphysomonas vestita, Pteridomonas danica	Marine cultures, sessile and motile	heterotrophic	0.2, 25	Latex beads	10^{10} of 0.2 µm		No uptake of 0.2 µm particles, attachment to large particles	Christensen-Dalsgaard and Fenchel, 2003
Amoeba								
Difflugia globulosa	Algae biofilm	phagocytotic	1, 3, 6 stöber 4 poly	Stöber silica microspheres , Polystyrene spheres	0.1 g in 50 ml deionized water	Several weeks	*D. globulosa* used particles for theca development	Bian et al., 2019
Acanthamoeba castellanii	Medium (as in Neff et al., 1964)	phagocytotic	2.02 vinyl 1.8 styrene	Polyvinyltoluene (Dow Chemical co. Indianapolis, IN) Polystyrene, fluorescent, carboxylated (Polyscience, Inc., Warrington, PA)	0.8 mg latex beads in medium with10^{6} amoebae	155-170 mins	Different amounts depending on treatment, uptake up to over 100 p Ind^{-1}	Bowser and Olszewski, 1983
Acanthamoeba sp.	Proteose-peptone-glucose medium (Weisman and Korn, 1966)	phagocytotic	0.126, 0.264, 0.557, 1.305 styrene 1.9, 2.68 vinyl	Polystyrene and Polyvinyltoluene latex beads (Dow Chemical Co.)	14 * 10^{6}, 70 * 10^{6}	10, 15, 20, 30 mins	Up to 14 p Ind^{-1}	Weisman and Korn, 1967

Species	*Environment*	*Feeding type of protozoans*	*Size of particles [µm]*	*Polymer type*	*Exposure concentration [p ml^{-1}]*	*Length of exposure*	*Number of particles per cell*	*Source*
Acanthamoeba castellanii	Proteose-peptone-glucose medium (Weisman and Korn, 1966)	phagocytotic	1.3 styrene 1.857 vinyl	Polystyrene and Polyvinyltoluene latex beads (Dow Chemical Co.)	$1.6 * 10^{10}$	10, 20 and 30 mins	Up to 40 p Ind^{-1}	Weisman and Moore, 1969
Acanthamoeba castellanii	PYG medium Different inhibition media	phagocytotic	10	Latex-coated polystyrene beads (Sigma, Cat. No. 55463, Macro beads)	na	16h	4.4+-2.7 p Ind^{-1} in solution with *Toxoplasma gondii* oocysts	Winiecka-Krusnell et al., 2009

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4. General conclusions and perspectives

Biofilms play an important role in rivers, forming complex communities which are influenced by water parameters and are feeding on planktonic organisms and matter, thus contributing to the river's energy flow (see Fig. 1.1; Costerton et al., 1987; Arndt et al., 2003; Battin et al., 2003; Weitere et al., 2003; Kathol et al., 2009; Ackermann et al., 2011; Kathol et al., 2011; Vasudevan, 2014; Weitere et al., 2018).

The focus of this study was on the composition of organisms of the biofilms in large rivers using the River Rhine as a model system and studying their effects on the pelagic organisms. There are a few studies that have examined the effects of biofilms under semi-natural or laboratory conditions (e.g. Wey et al., 2008; Norf et al., 2009; Kathol et al., 2011). The present study is the first one, which was carried out *in situ* in a large river and was carried out for a long-term period. This means that influences on the organisms of the river due to seasonal changes and varying water parameters could be considered.

The summer and autumn of 2018 were characterized by high water temperatures, and the lowest water level ever recorded during the last decades was reached at October 23rd of 2018 (Fig. 2.2, https://www.elwis.de, status as of 27.10.2021). Though, biofilm structure and biofilm organisms are affected by changing abiotic parameters, still their retention effects on pelagic bacteria was mostly significant ($p < 0.05$; Fig. 2.6.). Macrofauna such as bivalves can be affected to a greater extent by high water temperature and may stop their reduction of pelagic bacteria, so the effect of biofilms on river water quality is particularly important under these conditions (Matthews and McMahon, 1999; Belz et al., 2004; Norf et al., 2007, 2009; Willkomm et al., 2007). Unfortunately, the survey had to be interrupted in May 2019 for operational reasons, and no further data could be collected. Thus, a comparison to other years with less extreme conditions was not possible, and it is not known whether the determined retention would be lower or even higher under different conditions. However, retention effects on the pelagic of August 2001 to January 2002 showing similar results with high retention effects on pelagic bacteria was also positive throughout the whole experimental period (Rapp, 2002). Other studies dealing with the effects of biofilms on the pelagic were carried out under laboratory conditions, so these results are only partially comparable with those presented here (e.g. Wey et al., 2008; Norf et al., 2009; Kathol et al., 2011). Under stable conditions in laboratories, the organisms of the biofilms did not have to cope with changes or adapt their feeding behavior. Another noticeable occurrence in the

study period between April 2018 and May 2019 were the few flood events, which also showed relatively low water level rises (less than 600 cm). During floods and heavy rainfall, bacteria, e.g. from fields and sewers, are increasingly introduced into rivers (Jokinen et al., 2010; Tillburg et al., 2015). Here no conclusion can be made whether the retention increases or whether the biofilms and their organisms would also have been affected by water levels above 800 cm. Nevertheless, flood and drought events have increased due to anthropogenically induced climate change, thus changing the water parameters and influencing river communities (Belz et al., 2004; Huang et al., 2015; Wolff et al., 2021).

Furthermore, sampling during flood events is more dangerous than at normal water levels. The Ecological Rhine Station of the University of Cologne offers safer sampling procedures than sampling directly in the river's water on the bank or in the riprap. Another advantage of the Ecological Rhine Station during level fluctuations is that the experimental setup was always in the water of the Rhine, avoided dry periods to which the riprap was exposed and therefore represented the conditions at the river bottom. In order to quantify the influence of the biofilm on the abundance of bacteria, algae and heterotrophic flagellates of the pelagial, a close-meshed net was installed in the front part of the experimental design, excluding the macrofauna. Therefore, the biofilm could grow and function undisturbed by predators, but therefore the values of the biofilm retention were probably estimated higher than they are *in situ*, because the feeding pressure on the biofilms and their inhabitants did not exist.

The retention effect was mainly attributable to the ciliates of the biofilm. In our study, the heterotrich stentorid, peritrich zoothamniid and vorticellid ciliates dominated, as they did in other studies of the River Rhine (Wey et al., 2008; Kathol et al., 2009; Wey et al., 2009; Ackermann et al., 2011; Kathol et al., 2011). In the cited studies, open surfaces were used to grow biofilms and still they show a similar species composition compared to biofilms grown in our experimental setup. Biofilm communities dominated by heterotrich and peritrich ciliates as in the model system River Rhine cannot be generalized to all rivers. But the prevalence of bacterivorous organisms, especially of ciliates due to their high biovolume, will generally play a large role in reducing pelagic bacteria.

For the first time, a study was conducted *in situ*, and our results on the clearance rate of bacteria by the biofilm exceeded that of biofilms maintained under semi-natural conditions

(Fig. 2.7. A; Kathol et al., 2011), thus underlining the importance of *in-situ* studies. The channels of the experimental setup were covered with an opaque PVC plate to represent the bottom of the Rhine River as this is where most of the substrates for biofilm growth are present. A light-exposed experimental setup could have produced different results, but would only have represented the upper 20 cm of the River and could also have become clogged by the increased accumulation of algae, so that the biofilms would have had to be removed and the experiment restarted. By using glass spheres as substrates for biofilm growth, a large surface area could be created, but these were also very compact and the free spaces were very small. In rivers, increasing surfaces at the river bottom can enhance biofilm growth and promote the effects of biofilms on water quality. Experiments with stones as surfaces for biofilm growth showed similar retention effects and even more stable effects on the retention on heterotrophic flagellates (Rapp, 2002).

Biofilm ciliates may also contribute to the reduction of phytoplankton and heterotrophic flagellates from the pelagic; *Stentor* spp., for example, also feeds on algae, and raptorial genera such as *Lacrymaria* and *Litonotus* prey on heterotrophic flagellates (Foissner et al., 1992, 1995). However, especially the biofilm structure might have greatly influenced the phytoplankton and heterotrophic flagellates of the pelagic and thus their retention (Fig. 2.6. B, C; Risse-Buhl and Küsel, 2009; Früh et al., 2011). Bacteria are essential for the formation of biofilms through their excretion of EPS, and other organisms can settle on the biofilms (Costerton et al., 1987; Battin et al., 2003; Vasudevan, 2014). The high biovolume of algae suggested a quick attachment of algae, here mainly diatoms form the complex three-dimensional structure of the biofilms. The grooves and channels of the biofilm's structure protect smaller organisms such as bacteria and flagellates and its porosity also provides a constant flow of nutrients (Reynolds and Carling, 1991). Rivers represent a transport route not only for shipping, but also for substances dissolved in water, organic and inorganic substances, as well as for the certain organisms of rivers. Without the retention of these, everything can ultimately end up and accumulate in the sea. High concentrations of bacteria, pathogens and microplastics, as well as nutrients from fertilizers, can have an effect on marine organisms and algal blooms (Djakouré, et al., 2017; Wang et al., 2019).

In the second part of the study, it was hypothesized that the biofilms' three-dimensional structures might have an impact on the retention of microplastics from the surrounding

water as they have on the retention of pelagic bacteria. Further, the effect of the heterotrich ciliate *Stentor* sp., partly dominates the natural biofilms of the River Rhine, on microplastic particles was investigated and experiments to examining the trophic transfer of microplastic particles were conducted.

To study the hypothesis concerning the biofilms' structures, biofilms were grown for three months in the raft of the Ecological Rhine Station and then exposed to microplastic particles in an experimental setup on the Rhine Station (Fig. 3.1.). In order not to further pollute the environment with microplastic particles, the experiments were carried out in endless flow channels in which the conditions of the River Rhine could be simulated and even the water of the river could be used. After 24 hours, abundances between 5,000 to almost 20,000 microplastic particles per square meter of biofilm were found, with higher abundances at higher flow velocities for all size classes tested and the highest abundances for the largest particles (Fig. 3.4.). The attachment and settlement of the microplastic particles into the grooves and channels of the complex three-dimensional structure of the biofilms were possible because the biofilms had previously grown in the river for three months and were able to form complex structures. If it is assumed that the EPS alone can be responsible for the attachment of microplastics to surfaces, this has to be considered for the other tested surfaces, which were in the same basin with biofilms (Tab. 3.1.). Bacteria could have colonized them and thus the EPS on these surfaces could have played a role in the attachment and retention of microplastic particles (Fig. 3.3.).

It must be assumed that microplastic particles from the River Rhine water were already embedded in the biofilms naturally grown in the river and used for the experiments (Klein et al., 2015; Mani et al., 2016). These may have already influenced the structure of the biofilms and might have had an impact on the retention, since the tested microplastic particles might not been embedded as much as they would have been without the plastic particles of the river present. To exclude this effect, experiments have to be performed in which the biofilms can grow without microplastic particles present. In addition, a specific concentration of microplastic particles was assumed for the experiments, which might exceed the value in rivers and can vary during different seasons, because e.g. flood events can increase the plastic concentrations in rivers (Roebroek et al., 2021). Independent of these experiments, samples of biofilms grown for half a year and one year in the River Rhine were taken most

recently. Since the evaluation of microplastic particles from the environment is not as simple as that of the tested fluorescent particles, the field samples were sent to an external laboratory which, with the help of a sensitive Raman spectrometer, will provide information about the type and amount of microplastics in the River Rhine and Rhine-grown biofilms. Unfortunately, these results are not yet available at the point of completion of this study.

High concentrations of microplastic particles were also offered in the feeding experiments with the ciliate *Stentor*. The focus in these experiments was primarily on the question of whether *Stentor* individuals ingest the particles and on what amount (Fig. 3.8.). The particles were of the size of the ciliate's preferred food and showed that it is non-selective while ingesting. No saturation point was reached, suggesting that *Stentor* can ingest and excrete particles not depending on the type of particles. This excretion process of fluorescent microplastic particles was observed under the microscope. However, due to the short duration of the experiments, it is not clear whether the ciliate individuals would have survived permanent ingestion of microplastic particles that might have prevented the ingestion of digestible food particles.

The study results show estimates that indicate what is possible with the use of biofilms in rivers to clean them from bacteria and microplastics. The clearance rate shows that even a tiny amount of biofilm of 7,000 cm^2 per channel can clear thousands of liters of water per day from bacteria. In order to preserve water quality and thus the high importance of rivers for mankind, measures must be taken to reduce bacteria, pathogens and microplastics or plastic pollution. Here, the use of biofilms can be a possibility. Increasing the number of surface areas for biofilm growth, which were reduced by the canalization of rivers in the past, one can assume a lower bacterial load in the pelagic. The targeted use of biofilms as an additional treatment step in wastewater treatment plants could prevent a large number of bacteria pathogens and microplastics from entering rivers in the first place. Furthermore, the use of biofilms to retain microplastics could play an essential role in rivers. They could be grown and removed regularly so that the concentration of microplastics in rivers can be reduced. Biofilms are especially suitable for the retention of microplastics because they occur naturally in the river and, above all, quickly form a three-dimensional structure that promotes the attachment and settlement of microplastic particles.

Furthermore, special attention must be paid to the transfer of microplastic particles to higher trophic levels. First results show that this transfer and the accumulation of microplastics by higher trophic levels of the food chain are possible (Fig. 3.9.; Yokota et al., 2017). Thus, an uptake of microplastic particles by humans via consumption of fish should also be possible. However, since plastic pollution is not only a problem in aquatic but also in other ecosystems, the transfer in the food chain is also conceivable here and must be investigated (Van Cauwenberghe et al., 2015; Horton et al., 2017; Peeken et al., 2018; Rillig and Bonkowski, 2018).

5. References of introduction and general conclusion

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6. Supplementary material

Table 4.1. Water parameters. Water level (cm), flow velocity ($m^3 s^{-1}$), temperature (°C), conductivity ($\mu S cm^{-1}$), oxygen content ($mg L^{-1}$) and oxygen saturation (%) plus standard deviation during experiments of Chapter 1 from April 2018 to May 2019 of both chapters.

Month	Water level [cm]	Water discharge [$m^3 s^{-1}$]	Temperature [°C]
April '18	291.1 ± 1.45	1850 ± 4	16.3 ± 0.3
May '18	294.91 ± 23.49	1753.4 ± 135.69	18.43 ± 1.49
June '18	296.79 ± 59.71	1862.07 ± 349.74	22.49 ± 1.04
July '18	172.43 ± 25.28	1135.68 ± 184.99	25.03 ± 1.6
August '18	118.7 ± 7.05	769.94 ± 38.51	25.35 ± 1.79
September '18	127.08 ± 13.82	842.13 ± 75.19	21.09 ± 1.72
October '18	90.78 ± 16.61	676.81 ± 73.51	16.21 ± 1.94
November '18	90.91 ± 11.65	682.37 ± 51.24	10.61 ± 1.96
December '18	279.95 ± 111.23	1788.81 ± 780.45	8.6 ± 1.54
January '19	323.68 ± 76.42	2006.16 ± 521.52	5.72 ± 1.03
February '19	305.43 ± 63.17	1898.07 ± 462.69	6.06 ± 0.97
March '19	385.6 ± 134.23	2405.87 ± 961.64	8.89 ± 0.76
April '19	238.62 ± 24.64	1413.7 ± 119.24	13.35 ± 1.81
May '19	278.67 ± 53.65	1679.26 ± 327.4	14.79 ± 0.92
Mean	235.33 ± 92.83	1483.16 ± 542.27	15.21 ± 6.45

Month	Conductivity [$\mu S cm^{-1}$]	Oxygen content [$mg L^{-1}$]	Oxygen saturation [%]
April '18	486.5 ± 61.5	9.6 ± 0.12	102.51 ± 0
May '18	462.17 ± 14.7	9.28 ± 0.73	100.46 ± 2.17
June '18	425 ± 37.09	7.77 ± 0.4	94.53 ± 7.07
July '18	457.24 ± 25.55	8.05 ± 0.57	98.89 ± 5.97
August '18	478.23 ± 26.35	8.75 ± 1.22	105.88 ± 16.72
September '18	526.17 ± 24.62	8.03 ± 0.35	91.63 ± 1.78
October '18	559.19 ± 22.15	9.2 ± 0.48	97.23 ± 2.66
November '18	585.67 ± 17.4	10.36 ± 0.61	97.06 ± 0.95
December '18	515.16 ± 62.28	10.58 ± 0.92	87.85 ± 6.07
January '19	543.48 ± 61.61	11.74 ± 0.2	95.33 ± 0.6

February '19	588.71 ± 92.6	11.89 ± 0.17	98.39 ± 1.03
March '19	455.97 ± 47.27	10.98 ± 0.27	98.12 ± 1.16
April '19	447.17 ± 8.63	10.2 ± 0.37	100.12 ± 4.91
May '19	433.48 ± 20.45	9.43 ± 0.32	96.24 ± 3.47
Mean	497.44 ± 53.53	9.7 ± 1.27	97.45 ± 4.28

Table 4.2. Mean abundances (Ind. ml^{-1}) of biofilm organisms per sampling date from May 24th, 2018 to May 23rd, 2019 of chapter 1.

		May 24th	Jun 7th	Jun 14th	Jun 21st	Jul 5th	Jul 12th	Jul 19th
Algae	Centric Diatoms	111.00	339.66	117.66	259.74	7221.66	927.96	2994.78
	Chlorophytes	0.00	8.88	0.00	17.76	284.16	128.76	142.08
	Cryptophytes	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Pennate Diatoms	73.26	301.92	146.52	119.88	295.26	155.40	450.66
	Xanthophyceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Flagellates	Cercozoans	0.00	0.00	0.00	0.00	0.00	19.98	0.00
	Chaonoflagellats	0.00	0.00	0.00	0.00	0.00	0.00	8.88
	Chrysomonads	0.00	0.00	0.00	0.00	0.00	2.22	17.76
	Cryptomonads	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Euglenida	0.00	0.00	0.00	0.00	0.00	11.10	0.00
	Kathablepharids	0.00	0.00	0.00	0.00	0.00	0.00	2.22
	Kinetoplastida	0.00	66.60	31.08	35.52	597.18	330.78	228.66
Ciliates	Heterotrichia	0.00	8.88	93.64	0.00	128.76	37.74	6.66
	Litostomatea	0.00	0.00	167.57	0.00	31.08	53.28	0.00
	Oligohymenophorea	0.00	39.96	93.64	44.40	0.00	33.30	6.66
	Phyllpharingea	0.00	4.44	0.00	0.00	0.00	2.22	17.76
	Spirotrichia	0.00	4.44	0.00	0.00	0.00	0.00	0.00
Meiofauna	Amoebae	0.00	19.98	0.00	6.66	66.60	33.30	37.74
	Gastrotrichia	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Heliozoa	0.00	0.00	0.00	0.00	2.22	13.32	0.00
	Nematoda	0.00	2.22	2.22	0.00	2.22	2.22	0.00
	Oligochaeta	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Rotifera	0.00	2.22	0.00	2.22	0.00	2.22	0.00

		Aug 23rd	Sep 20th	Sep 26th	Oct 4th	Oct 18th	Oct 25th	Nov 8th
Algae	Centric Diatoms	1325.34	284.16	239.76	299.70	1644.53	266.68	1177.84
	Chlorophytes	843.60	11.10	17.76	8.88	288.90	0.00	0.00
	Cryptophytes	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Pennate Diatoms	59.94	126.54	115.44	259.74	2689.02	2889.03	4778.02
	Xanthophyceae	219.78	0.00	0.00	0.00	0.00	0.00	0.00
Flagellates	Cercozoans	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Chaonoflagellats	1081.14	397.38	594.96	0.00	911.16	1022.27	0.00
	Chrysomonads	8.88	8.88	22.20	8.88	44.45	44.45	22.22
	Cryptomonads	0.00	0.00	0.00	0.00	0.00	0.00	44.45
	Euglenida	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Kathablepharids	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Kinetoplastida	44.40	35.52	193.14	88.80	133.34	355.57	177.79
Ciliates	Heterotrichia	2.22	11.10	8.88	8.88	0.00	22.22	22.22
	Litostomatea	2.22	4.44	6.66	2.22	88.89	44.45	88.89
	Oligohymenophorea	6.66	135.42	102.12	102.12	1666.75	1444.52	1200.06
	Phyllpharingea	31.08	71.04	6.66	0.00	22.22	22.22	0.00

	Spirotrichia	0.00	0.00	0.00	0.00	22.22	66.67	0.00
Meiofauna	Amoebae	39.96	6.66	13.32	13.32	22.22	22.22	66.67
	Gastrotrichia	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Heliozoa	0.00	2.22	0.00	0.00	0.00	44.45	0.00
	Nematoda	0.00	0.00	0.00	6.66	22.22	22.22	44.45
	Oligochaeta	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Rotifera	2.22	2.22	0.00	2.22	0.00	0.00	0.00

		Jul 27th	Aug 3rd	Aug 10th	Aug 17th	Dec 12th	Dec 19th	Dec 27th
Algae	Centric Diatoms	1722.72	3221.22	3820.62	1198.80	1266.73	1088.94	555.58
	Chlorophytes	2322.12	1178.82	1669.44	532.80	288.90	88.89	177.79
	Cryptophytes	2.22	0.00	0.00	0.00	0.00	0.00	0.00
	Pennate Diatoms	355.20	37.74	175.38	48.84	5155.81	6333.65	6222.53
	Xanthophyceae	1733.82	2517.48	2994.78	182.04	44.45	0.00	0.00
Flagellates	Cercozoans	2.22	0.00	2.22	0.00	0.00	0.00	0.00
	Chaonoflagellats	55.50	0.00	26.64	86.58	0.00	0.00	0.00
	Chrysomonads	35.52	22.20	57.72	31.08	111.12	22.22	0.00
	Cryptomonads	0.00	0.00	0.00	0.00	0.00	311.13	266.68
	Euglenida	0.00	0.00	0.00	0.00	44.45	0.00	0.00
	Kathablepharids	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Kinetoplastida	28.86	133.20	468.42	64.38	1555.63	733.37	955.60
Ciliates	Heterotrichia	33.30	79.92	31.08	17.76	44.45	22.22	0.00
	Litostomatea	6.66	57.72	51.06	0.00	0.00	44.45	0.00
	Oligohymenophorea	4.44	6.66	0.00	0.00	533.36	1488.96	911.16
	Phyllpharingea	19.98	11.10	19.98	2.22	0.00	0.00	0.00
	Spirotrichia	0.00	0.00	0.00	0.00	66.67	66.67	0.00
Meiofauna	Amoebae	22.20	93.24	230.88	31.08	0.00	44.45	0.00
	Gastrotrichia	0.00	2.22	0.00	0.00	0.00	0.00	0.00
	Heliozoa	19.98	6.66	0.00	0.00	0.00	0.00	0.00
	Nematoda	0.00	4.44	0.00	2.22	22.22	44.45	44.45
	Oligochaeta	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Rotifera	6.66	4.44	6.66	2.22	0.00	44.45	0.00

		Nov 15th	Nov 22nd	Nov 29th	Dec 6th	Mar 21st	Apr 11th	Apr 18th
Algae	Centric Diatoms	600.03	1733.42	1266.73	2555.68	2244.56	1200.06	9156.01
	Chlorophytes	44.45	88.89	355.57	133.34	88.89	0.00	822.26
	Cryptophytes	0.00	0.00	0.00	0.00	0.00	0.00	311.13
	Pennate Diatoms	3577.96	4444.67	4044.65	3977.98	13756.24	10600.53	19423.19
	Xanthophyceae	0.00	66.67	0.00	0.00	0.00	0.00	777.82
Flagellates	Cercozoans	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Chaonoflagellats	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Chrysomonads	88.89	44.45	0.00	22.22	0.00	0.00	0.00
	Cryptomonads	0.00	22.22	22.22	200.01	0.00	66.67	311.13
	Euglenida	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Kathablepharids	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Kinetoplastida	444.47	288.90	688.92	1733.42	311.13	244.46	1311.18
Ciliates	Heterotrichia	0.00	0.00	22.22	22.22	0.00	22.22	155.56
	Litostomatea	0.00	0.00	0.00	0.00	0.00	0.00	44.45
	Oligohymenophorea	133.34	355.57	1111.17	1111.17	4955.80	6444.77	1822.31
	Phyllpharingea	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Spirotrichia	0.00	44.45	22.22	44.45	44.45	66.67	0.00
Meiofauna	Amoebae	22.22	44.45	0.00	0.00	0.00	0.00	22.22

	Gastrotrichia	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Heliozoa	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Nematoda	44.45	22.22	22.22	22.22	0.00	0.00	0.00
	Oligochaeta	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Rotifera	0.00	0.00	0.00	22.22	0.00	0.00	0.00

		Jan 10th	**Jan 17th**	**Jan 24th**	**Jan 31st**	**Feb 7th**	**Feb 14th**	**Feb 25th**
Algae	Centric Diatoms	688.92	1000.05	355.57	1577.86	888.93	244.46	1555.63
	Chlorophytes	88.89	266.68	177.79	88.89	200.01	88.89	288.90
	Cryptophytes	0.00	0.00	0.00	0.00	0.00	0.00	22.22
	Pennate Diatoms	4955.80	4111.32	3933.53	4378.00	4044.65	4422.44	5044.70
	Xanthophyceae	133.34	155.56	0.00	111.12	0.00	0.00	0.00
Flagellates	Cercozoans	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Chaonoflagellats	0.00	0.00	0.00	0.00	0.00	0.00	66.67
	Chrysomonads	88.89	22.22	111.12	133.34	0.00	22.22	0.00
	Cryptomonads	0.00	0.00	111.12	244.46	44.45	0.00	288.90
	Euglenida	133.34	244.46	0.00	0.00	0.00	0.00	0.00
	Kathablepharids	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Kinetoplastida	422.24	311.13	355.57	844.49	111.12	155.56	311.13
Ciliates	Heterotrichia	0.00	111.12	0.00	22.22	0.00	0.00	0.00
	Litostomatea	66.67	44.45	66.67	88.89	0.00	44.45	0.00
	Oligohymenophorea	5000.25	4155.76	8311.53	5733.62	5511.39	5178.04	1177.84
	Phyllpharingea	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Spirotrichia	66.67	0.00	44.45	0.00	66.67	0.00	66.67
Meiofauna	Amoebae	0.00	22.22	0.00	0.00	22.22	0.00	22.22
	Gastrotrichia	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Heliozoa	0.00	0.00	22.22	0.00	0.00	0.00	0.00
	Nematoda	0.00	0.00	22.22	44.45	22.22	0.00	0.00
	Oligochaeta	0.00	0.00	22.22	0.00	0.00	0.00	0.00
	Rotifera	266.68	22.22	0.00	22.22	22.22	0.00	0.00

		Mar 14th	**Apr 25th**	**May 2nd**	**May 9th**	**May 15th**	**May 23rd**
Algae	Centric Diatoms	2422.34	8867.11	8689.32	12022.82	11000.55	12889.53
	Chlorophytes	0.00	1466.74	1622.30	3177.94	4311.33	6289.20
	Cryptophytes	0.00	644.48	488.91	333.35	422.24	400.02
	Pennate Diatoms	10667.20	24823.46	21401.07	23978.98	23267.83	25201.26
	Xanthophyceae	88.89	1022.27	1444.52	1622.30	2533.46	3155.71
Flagellates	Cercozoans	0.00	0.00	0.00	0.00	0.00	0.00
	Chaonoflagellats	200.01	0.00	0.00	177.79	911.16	1066.72
	Chrysomonads	44.45	0.00	0.00	0.00	0.00	0.00
	Cryptomonads	88.89	22.22	44.45	155.56	244.46	266.68
	Euglenida	0.00	0.00	0.00	0.00	0.00	0.00
	Kathablepharids	0.00	0.00	0.00	0.00	0.00	0.00
	Kinetoplastida	488.91	1000.05	1044.50	1777.87	2311.23	2266.78
Ciliates	Heterotrichia	0.00	0.00	66.67	88.89	244.46	644.48
	Litostomatea	22.22	0.00	0.00	0.00	0.00	0.00
	Oligohymenophorea	3555.73	1600.08	2266.78	4000.20	4133.54	4111.32
	Phyllpharingea	0.00	0.00	0.00	0.00	0.00	0.00
	Spirotrichia	0.00	0.00	111.12	177.79	155.56	222.23
Meiofauna	Amoebae	66.67	133.34	88.89	177.79	200.01	355.57

Gastrotrichia	0.00	0.00	0.00	0.00	0.00	0.00
Heliozoa	0.00	311.13	577.81	200.01	0.00	66.67
Nematoda	44.45	111.12	244.46	488.91	0.00	0.00
Oligochaeta	0.00	0.00	0.00	0.00	0.00	0.00
Rotifera	0.00	0.00	22.22	44.45	111.12	244.46

Table 4.3. Mean abundance (Ind. ml^{-1}) of pelagic bacteria per sampling date from June 28th, 2018 to May 23rd, 2019 of sampling spot a (before the biofilm) and sampling spot b (behind the biofilm) of chapter 1.

	Jun 28th	**Jul 5th**	**Jul 12th**	**Jul 19th**	**Jul 26th**	**Aug 2nd**
A before BF	1249993.73	2527495.02	1523955.33	1219621.67	1931814	1430464.33
B after BF	430199.76	1485099.36	904177.67	551645	1355374.67	909554.333
	Aug 9th	**Aug 16th**	**Aug 23th**	**Aug 30th**	**Sep 7th**	**Sep 14th**
A before BF	1549251	1798257	1594709	2021115.33	3830604.04	1839557
B after BF	1163579.67	1240952	1111568.33	1118993.67	1957127.56	1189317.33
	Sep 19th	**Sep 26th**	**Oct 4th**	**Oct 12th**	**Oct 18th**	**Oct 25th**
A before BF	2765730.33	1962819.67	1916437.33	1894031	1791638.33	3060612.21
B after BF	2130599.33	1118517.67	964327	1013007.67	1121053.33	1397313.14
	Oct 31st	**Nov 8th**	**Nov 15th**	**Nov 22nd**	**Nov 29th**	**Dec 6th**
A before BF	2791116.57	2817692.97	2535546.84	1946185.34	2253093.3	3930596.07
B after BF	1463042.5	1151447.52	1176687.08	892335.979	1243608.6	2223847.35
	Dec 12th	**Dec 20th**	**Dec 27th**	**Jan 10th**	**Jan 17th**	**Jan 24th**
A before BF	3281193.24	2987510.34	3051128.92	3048748.95	3028953.59	2204377.55
B after BF	1461055.27	1773818.77	1580057.74	1483291.56	1249131.35	1136103.46
	Jan 31st	**Feb 7th**	**Feb 14th**	**Feb 26th**	**Mar 14th**	**Mar 21st**
A before BF	1888715.9	3010669.33	2876002.37	2868069.12	3174887.66	2736163.54
B after BF	1670743.65	1157126.36	1046158.01	1134007.32	1549029.89	1177086.41
	Apr 4th	**Apr 11th**	**Apr 18th**	**Apr 25th**	**May 2nd**	**May 9th**
A before BF	2816426.7	2743135.65	3541536.15	4039325.08	3625384.02	3649454.83
B after BF	1195061.67	1712650.78	1596667.21	2273235.15	1675687.73	1905153.09
	May 19th	**May 23rd**				
A before BF	3019990.91	3582260.18				
B after BF	1382783.16	1624459.83				

Table 4.4. Mean abundances (Ind. ml^{-1}) of pelagic organisms of sample spot A before the biofilms per sampling date from May 5th, 2018 to May 23rd, 2019 of chapter 1.

		May 5th	May 18th	May 24th	Jun 7th	Jun 14th	Jun 21st	Jun 28th
Algae	Centric diatoms	3591	2061.5	3178.7	7953.4	1502.9	3577.7	8857.8
	Chlorophytes	106.4	106.4	252.7	26.6	119.7	106.4	359.1
	Cryptophytes	0	0	0	0	0	0	0
	Pennate diatoms	239.4	186.2	159.6	385.7	226.1	385.7	305.9
	Xanthophyceans	0	0	0	0	0	0	0
	undetermined	0	0	13.3	66.5	0	0	0
Flagellates	Cercozoans	0	0	0	0	0	0	0
	Choanoflagellates	13.3	0	0	0	0	13.3	0
	Chrysomonads	13.3	0	0	0	0	0	0
	Cryptomonads	0	0	0	0	0	0	0
	Euglenids	0	0	0	0	0	0	0
	Kathablepharids	0	0	0	0	0	0	53.2
	Kinetoplastids	0	0	0	0	13.3	0	212.8
	undetermined	252.7	199.5	359.1	665	172.9	345.8	532
Others	Heterotrichs	0	0	0	0	0	0	0
	Oligohymenophoreans	0	0	0	0	39.9	0	0
	Spirotrichs	0	0	0	0	13.3	0	0
	Amoebae	0	0	0	0	0	0	39.9
	Heliozoans	0	0	0	0	0	0	0

		Jul 7th	Jul 12th	Jul 19th	Jul 26th	Aug 2nd	Aug 9th	Aug 16th
Algae	Centric diatoms	12674.9	10334.1	28462	15667.4	13419.7	9522.8	4548.6
	Chlorophytes	292.6	279.3	970.9	1463	1596	4162.9	3950.1
	Cryptophytes	0	0	0	0	0	0	0
	Pennate diatoms	372.4	2673.3	2234.4	425.6	372.4	505.4	119.7
	Xanthophyceans	0	0	0	16891	14949.2	29592.5	1955.1
	undetermined	0	13.3	0	13.3	53.2	0	0
Flagellates	Cercozoans	0	13.3	66.5	66.5	13.3	0	0
	Choanoflagellates	39.9	13.3	0	39.9	0	305.9	0
	Chrysomonads	0	0	478.8	159.6	412.3	532	13.3
	Cryptomonads	0	0	0	106.4	186.2	212.8	146.3
	Euglenids	0	0	0	0	0	0	0
	Kathablepharids	0	0	0	0	0	0	0
	Kinetoplastids	39.9	26.6	558.6	0	26.6	26.6	0
	undetermined	319.2	372.4	532	159.6	106.4	39.9	79.8

Others	Heterotrichs	0	0	0	0	0	0	0
	Oligohymenophoreans	0	13.3	0	0	0	0	0
	Spirotrichs	0	0	0	0	0	0	0
	Amoebae	0	0	26.6	0	39.9	39.9	13.3
	Heliozoans	0	0	0	0	0	0	0

		Aug 23rd	Sep 19th	Sep 26th	Oct 4th	Oct 12th	Oct 18th	Oct 25th
Algae	Centric diatoms	8698.2	279.3	984.2	1223.6	824.6	133	172.9
	Chlorophytes	4056.5	252.7	305.9	39.9	53.2	93.1	106.4
	Cryptophytes	0	106.4	13.3	133	66.5	239.4	13.3
	Pennate diatoms	66.5	26.6	26.6	93.1	2380.7	359.1	585.2
	Xanthophyceans	3537.8	0	26.6	0	13.3	0	0
	undetermined	0	0	0	0	0	0	0
Flagellates	Cercozoans	0	0	0	0	0	0	0
	Choanoflagellates	226.1	0	0	0	0	0	0
	Chrysomonads	438.9	26.6	66.5	39.9	66.5	0	0
	Cryptomonads	212.8	0	26.6	93.1	0	0	0
	Euglenids	0	0	0	0	0	0	0
	Kathablepharids	0	0	0	0	0	0	0
	Kinetoplastids	13.3	13.3	0	13.3	0	13.3	0
	undetermined	39.9	13.3	0	0	53.2	0	0
Others	Heterotrichs	0	0	0	0	53.2	0	13.3
	Oligohymenophoreans	0	0	0	0	0	0	0
	Spirotrichs	0	0	0	0	13.3	0	0
	Amoebae	0	0	0	26.6	0	13.3	0
	Heliozoans	0	0	0	0	0	0	0

		Nov 8th	Nov 15th	Nov 22nd	Nov 29th	Dec 6th	Dec 12th	Dec 19th
Algae	Centric diatoms	79.8	146.3	126.35	126.35	512.05	152.95	46.55
	Chlorophytes	93.1	239.4	19.95	139.65	192.85	66.5	66.5
	Cryptophytes	13.3	119.7	113.05	6.65	0	0	13.3
	Pennate diatoms	412.3	239.4	365.75	212.8	505.4	452.2	505.4
	Xanthophyceans	0	0	0	0	19.95	53.2	0
	undetermined	13.3	0	0	0	0	0	0
Flagellates	Cercozoans	0	0	0	0	0	0	0
	Choanoflagellates	0	0	0	19.95	0	53.2	0
	Chrysomonads	26.6	53.2	66.5	59.85	53.2	73.15	19.95
	Cryptomonads	0	0	0	0	0	0	0
	Euglenids	0	0	0	0	0	6.65	0
	Kathablepharids	0	0	0	0	0	0	0
	Kinetoplastids	26.6	26.6	139.65	239.4	239.4	345.8	379.05
	undetermined	0	0	0	0	0	0	0
Others	Heterotrichs	0	0	0	0	0	0	0
	Oligohymenophoreans	0	0	0	0	0	0	0
	Spirotrichs	0	0	6.65	0	0	0	0

	Amoebae	0	0	0	13.3	0	19.95	6.65
	Heliozoans	0	0	0	0	0	0	0

		Dec 27th	Jan 10th	Jan 17th	Jan 24th	Jan 31st	Feb 7th	Feb 14th
Algae	Centric diatoms	399	638.4	844.55	46.55	139.65	179.55	146.3
	Chlorophytes	0	53.2	119.7	113.05	525.35	638.4	13.3
	Cryptophytes	0	6.65	0	0	0	0	0
	Pennate diatoms	864.5	518.7	864.5	126.35	292.6	345.8	498.75
	Xanthophyceans	0	0	46.55	26.6	0	19.95	0
	undetermined	0	0	0	0	0	0	0
Flagellates	Cercozoans	0	0	0	0	0	0	0
	Choanoflagellates	0	0	0	0	0	6.65	0
	Chrysomonads	106.4	19.95	106.4	13.3	19.95	0	0
	Cryptomonads	0	0	0	0	0	0	0
	Euglenids	19.95	0	66.5	6.65	0	0	0
	Kathablepharids	0	0	0	0	0	0	0
	Kinetoplastids	518.7	186.2	232.75	73.15	352.45	226.1	59.85
	undetermined	0	0	0	0	0	0	0
Others	Heterotrichs	0	0	0	0	0	0	0
	Oligohymenophoreans	0	0	0	0	0	0	13.3
	Spirotrichs	0	0	0	0	0	0	13.3
	Amoebae	0	6.65	6.65	6.65	6.65	0	0
	Heliozoans	0	0	0	0	0	0	0

		Feb 25th	Mar 14th	Mar 21st	Apr 11th	Apr 18th	Apr 25th	May 2nd
Algae	Centric diatoms	518.7	718.2	1137.15	744.8	2852.85	6849.5	2640.05
	Chlorophytes	305.9	33.25	0	53.2	86.45	252.7	186.2
	Cryptophytes	26.6	106.4	46.55	119.7	279.3	332.5	219.45
	Pennate diatoms	877.8	1044.05	2108.05	1064	1842.05	7295.05	3876.95
	Xanthophyceans	0	0	0	0	0	199.5	139.65
	undetermined	0	0	0	0	0	26.6	0
Flagellates	Cercozoans	0	0	0	0	0	0	0
	Choanoflagellates	0	0	73.15	0	1177.05	99.75	106.4
	Chrysomonads	33.25	13.3	26.6	0	418.95	0	0
	Cryptomonads	0	0	19.95	0	0	0	0
	Euglenids	0	0	0	0	0	0	0
	Kathablepharids	0	13.3	0	0	0	0	0
	Kinetoplastids	79.8	79.8	465.5	172.9	884.45	970.9	1449.7
	undetermined	0	0	0	0	0	0	0
Others	Heterotrichs	0	0	0	0	0	0	0
	Oligohymenophoreans	0	0	0	0	0	0	6.65

Spirotrichs	0	0	0	0	0	0	0
Amoebae	0	0	6.65	6.65	0	39.9	6.65
Heliozoans	0	0	0	0	0	0	0

		May 9th	May 15th	May 23rd
Algae	Centric diatoms	12648.3	12495.35	12654.95
	Chlorophytes	1615.95	1037.4	1270.15
	Cryptophytes	525.35	372.4	325.85
	Pennate diatoms	20169.45	18886	20641.6
	Xanthophyceans	438.9	1044.05	1349.95
	undetermined	0	0	0
Flagellates	Cercozoans	0	0	0
	Choanoflagellates	505.4	432.25	505.4
	Chrysomonads	139.65	139.65	186.2
	Cryptomonads	139.65	26.6	53.2
	Euglenids	0	0	0
	Kathablepharids	0	0	0
	Kinetoplastids	1210.3	645.05	618.45
	undetermined	0	0	0
Others	Heterotrichs	0	0	0
	Oligohymenophoreans	0	0	0
	Spirotrichs	0	0	0
	Amoebae	33.25	0	119.7
	Heliozoans	0	0	0

Table 4.5. Mean abundances (Ind. ml^{-1}) of pelagic organisms of sample spot B after the biofilms per sampling date from May 5th, 2018 to May 23rd, 2019 of chapter 1.

Abundance (in 1ml)		May 5th	May 18th	May 24th	Jun 7th	Jun 14th	Jun 21st	Jun 28th
Algae	Centric diatoms	4655	2128	3444.7	7341.6	864.5	2154.6	8884.4
	Chlorophytes	0	106.4	226.1	53.2	106.4	172.9	332.5
	Cryptophytes	0	0	0	0	0	0	0
	Pennate diatoms	106.4	93.1	133	159.6	106.4	133	199.5
	Xanthophyceans	0	0	0	0	0	0	0
	undetermined	39.9	26.6	0	146.3	0	53.2	0
Flagellates	Cercozoans	0	0	0	0	0	0	0
	Choanoflagellates	79.8	0	0	0	13.3	0	0
	Chrysomonads	13.3	0	0	0	0	0	0
	Cryptomonads	0	0	0	0	0	0	0
	Euglenids	0	0	0	0	0	0	0
	Kathablepharids	0	0	0	0	0	13.3	0
	Kinetoplastids	0	0	0	0	53.2	0	53.2
	undetermined	266	305.9	212.8	305.9	226.1	438.9	332.5
Others	Heterotrichs	0	0	0	0	0	0	0
	Oligohymenophoreans	0	0	0	0	0	0	0
	Spirotrichs	0	0	0	0	0	0	0
	Amoebozoans	0	0	0	0	0	0	0
	Heliozoans	0	13.3	0	0	0	0	0
		Jul 7th	**Jul 12th**	**Jul 19th**	**Jul 26th**	**Aug 2nd**	**Aug 9th**	**Aug 16th**
Algae	Centric diatoms	9828.7	3045.7	26240.9	14736.4	9908.5	6636.7	3724
	Chlorophytes	159.6	252.7	2061.5	518.7	1236.9	2527	3404.8
	Cryptophytes	0	0	0	0	0	0	0
	Pennate diatoms	186.2	332.5	266	226.1	425.6	119.7	66.5
	Xanthophyceans	0	0	0	11797.1	11305	14989.1	2048.2
	undetermined	13.3	0	0	0	13.3	0	0
Flagellates	Cercozoans	0	13.3	0	26.6	39.9	0	0
	Choanoflagellates	0	0	0	0	13.3	93.1	159.6
	Chrysomonads	0	0	359.1	186.2	332.5	372.4	13.3
	Cryptomonads	0	0	0	79.8	226.1	79.8	159.6
	Euglenids	0	0	0	0	0	0	0
	Kathablepharids	0	0	13.3	0	0	0	0
	Kinetoplastids	39.9	0	79.8	0	13.3	0	0
	undetermined	146.3	199.5	319.2	93.1	106.4	106.4	26.6

Others	Heterotrichs	0	0	0	0	0	0	0
	Oligohymenophoreans	0	0	0	0	0	0	0
	Spirotrichs	0	0	0	0	0	0	0
	Amoebozoans	0	0	0	0	0	13.3	0
	Heliozoans	0	0	0	0	0	0	0

		Aug 23rd	Sep 19th	Sep 26th	Oct 4th	Oct 12th	Oct 18th	Oct 25th
Algae	Centric diatoms	7541.1	372.4	1010.8	2048.2	585.2	66.5	146.3
	Chlorophytes	4415.6	332.5	305.9	26.6	159.6	79.8	133
	Cryptophytes	0	119.7	119.7	159.6	239.4	226.1	0
	Pennate diatoms	159.6	13.3	53.2	93.1	119.7	199.5	305.9
	Xanthophyceans	4269.3	0	0	26.6	119.7	13.3	0
	undetermined	13.3	0	0	0	0	0	0
Flagellates	Cercozoans	0	0	0	0	0	0	0
	Choanoflagellates	26.6	0	0	0	0	665	0
	Chrysomonads	412.3	13.3	212.8	26.6	0	146.3	26.6
	Cryptomonads	133	0	53.2	13.3	0	0	0
	Euglenids	0	0	0	0	0	0	0
	Kathablepharids	0	0	0	0	0	0	0
	Kinetoplastids	0	0	0	0	0	0	13.3
	undetermined	53.2	13.3	0	0	0	0	0
Others	Heterotrichs	0	0	0	0	0	0	0
	Oligohymenophoreans	13.3	0	0	0	0	0	0
	Spirotrichs	0	0	0	0	0	0	0
	Amoebozoans	0	0	0	13.3	0	0	0
	Heliozoans	0	0	0	0	0	0	0

		Nov 8th	Nov 15th	Nov 22nd	Nov 29th	Dec 6th	Dec 12th	Dec 19th
Algae	Centric diatoms	106.4	53.2	166.25	152.95	399	239.4	152.95
	Chlorophytes	53.2	79.8	59.85	113.05	0	73.15	59.85
	Cryptophytes	39.9	119.7	199.5	13.3	6.65	0	13.3
	Pennate diatoms	212.8	212.8	139.65	126.35	259.35	498.75	299.25
	Xanthophyceans	0	0	0	0	0	0	0
	undetermined	0	0	0	0	0	239.4	0
Flagellates	Cercozoans	13.3	0	0	0	0	0	0
	Choanoflagellates	0	0	0	0	0	0	0
	Chrysomonads	26.6	79.8	106.4	59.85	106.4	99.75	39.9
	Cryptomonads	0	0	0	0	0	0	0
	Euglenids	0	0	0	0	0	13.3	0
	Kathablepharids	0	0	0	0	0	0	0
	Kinetoplastids	53.2	39.9	119.7	126.35	212.8	631.75	299.25
	undetermined	0	0	0	0	0	0	0
Others	Heterotrichs	0	0	0	0	0	0	0
	Oligohymenophoreans	0	0	0	0	0	0	0
	Spirotrichs	0	0	0	0	0	0	0

Amoebozoans	26.6	26.6	13.3	0	0	39.9	0
Heliozoans	0	0	0	0	0	0	0

		Dec 27th	Jan 10th	Jan 17th	Jan 24th	Jan 31st	Feb 7th	Feb 14th
Algae	Centric diatoms	285.95	186.2	871.15	66.5	99.75	19.95	26.6
	Chlorophytes	0	73.15	66.5	405.65	1236.9	379.05	13.3
	Cryptophytes	0	0	0	26.6	6.65	0	0
	Pennate diatoms	512.05	305.9	232.75	33.25	53.2	146.3	152.95
	Xanthophyceans	0	0	6.65	0	0	0	0
	undetermined	0	0	0	0	0	0	0
Flagellates	Cercozoans	0	0	0	0	0	0	0
	Choanoflagellates	0	0	0	0	46.55	0	0
	Chrysomonads	186.2	26.6	106.4	6.65	0	0	6.65
	Cryptomonads	13.3	0	6.65	0	0	0	0
	Euglenids	6.65	0	13.3	0	0	0	0
	Kathablepharids	0	6.65	0	0	0	0	0
	Kinetoplastids	465.5	518.7	259.35	133	46.55	66.5	13.3
	undetermined	0	0	0	0	0	0	0
Others	Heterotrichs	0	0	0	0	0	0	0
	Oligohymenophoreans	0	0	0	0	73.15	0	0
	Spirotrichs	0	0	0	0	0	0	0
	Amoebozoans	0	0	19.95	0	0	26.6	0
	Heliozoans	0	0	0	0	0	0	0

		Feb 25th	Mar 14th	Mar 21st	Apr 11th	Apr 18th	Apr 25th	May 2nd
Algae	Centric diatoms	159.6	266	272.65	425.6	325.85	3211.95	1330
	Chlorophytes	126.35	13.3	0	6.65	392.35	0	93.1
	Cryptophytes	39.9	19.95	6.65	66.5	199.5	279.3	159.6
	Pennate diatoms	199.5	432.25	385.7	159.6	458.85	1050.7	658.35
	Xanthophyceans	0	0	0	0	0	0	146.3
	undetermined	0	0	0	0	0	19.95	0
Flagellates	Cercozoans	0	0	0	0	0	0	0
	Choanoflagellates	0	0	0	0	0	0	0
	Chrysomonads	0	6.65	0	0	0	79.8	19.95
	Cryptomonads	0	0	0	0	0	0	13.3
	Euglenids	0	0	0	0	0	0	0
	Kathablepharids	0	0	0	0	0	0	0
	Kinetoplastids	33.25	106.4	99.75	0	113.05	285.95	405.65
	undetermined	0	0	0	0	0	0	0
Others	Heterotrichs	0	0	0	0	0	0	0
	Oligohymenophoreans	0	0	0	0	0	0	0

Spirotrichs	0	0	0	0	0	13.3	0
Amoebozoans	13.3	13.3	0	0	0	26.6	6.65
Heliozoans	0	0	0	0	0	0	0

		May 9th	May 15th	May 23rd
Algae	Centric diatoms	1589.35	2021.6	1562.75
	Chlorophytes	219.45	59.85	119.7
	Cryptophytes	126.35	126.35	119.7
	Pennate diatoms	6783	9715.65	8239.35
	Xanthophyceans	46.55	0	0
	undetermined	0	0	0
Flagellates	Cercozoans	0	0	0
	Choanoflagellates	0	0	0
	Chrysomonads	26.6	0	0
	Cryptomonads	6.65	0	0
	Euglenids	0	0	0
	Kathablepharids	0	0	0
	Kinetoplastids	578.55	226.1	179.55
	undetermined	0	0	0
Others	Heterotrichs	0	0	0
	Oligohymenophoreans	0	0	0
	Spirotrichs	0	0	0
	Amoebozoans	0	0	0
	Heliozoans	0	0	0

Table 4.6. Abundances of 6 µm microplastic particles on different substrates (particle cm^{-2}) at two different flow velocities (0.1 m s^{-1}, 0.2 m s^{-1}) of experiment 1 (December 2018) of chapter 2.

Type of substrate	Flow velocity [m s^{-1}]	6 µm particle abundance [p cm^{-2}]
Biofilm	0.1	4071.22
Biofilm	0.1	3113.29
Biofilm	0.1	2993.54
Biofilm	0.1	3519.37
Biofilm	0.1	2998.75
Biofilm	0.1	4859.09
Biofilm	0.1	4498.13
Biofilm	0.1	4425.24
Biofilm	0.1	4390.53
Biofilm	0.1	6316.81
Biofilm	0.2	20234.62
Biofilm	0.2	9024.02
Biofilm	0.2	14785.51
Biofilm	0.2	11037.07
Biofilm	0.2	7542.00
Biofilm	0.2	3401.36
Biofilm	0.2	8093.85
Biofilm	0.2	9745.94
Biofilm	0.2	12272.66
Biofilm	0.2	12859.23
Clay tiles smooth	0.1	277.66
Clay tiles smooth	0.1	610.86
Clay tiles smooth	0.1	249.90
Clay tiles smooth	0.1	888.52
Clay tiles smooth	0.2	416.49
Clay tiles smooth	0.2	2859.92
Clay tiles smooth	0.2	555.32
Clay tiles smooth	0.2	27.77
Clay tiles smooth	0.2	3331.95
Clay tiles smooth	0.2	360.96
Clay tiles smooth	0.2	416.49
Clay tiles smooth	0.2	277.66
Clay tiles rough	0.1	749.69
Clay tiles rough	0.1	499.79
Clay tiles rough	0.1	472.03
Clay tiles rough	0.1	888.52
Clay tiles rough	0.2	2582.26
Clay tiles rough	0.2	1305.01
Clay tiles rough	0.2	888.52
Clay tiles rough	0.2	194.36

Clay tiles rough	0.2	472.03
Clay tiles rough	0.2	333.19
Clay tiles rough	0.2	388.73
Plexiglas tiles rough	0.1	305.43
Plexiglas tiles rough	0.1	444.26
Plexiglas tiles rough	0.1	360.96
Plexiglas tiles smooth	0.1	499.79
Plexiglas tiles smooth	0.1	166.60
Plexiglas tiles smooth	0.1	249.90

Table 4.7. Abundances of 1 µm, 6 µm and 10 µm microplastic particles on biofilm (particle cm^{-2}) at two different flow velocities (0.1 m s^{-1}, 0.2 m s^{-1}) of experiment 2 (March 2018) of chapter 2.

Type of substrate	Flow velocity [m s^{-1}]	1 µm particle abundance [p cm^{-2}]	6 µm particle abundance [p cm^{-2}]	10 µm particle abundance [p cm^{-2}]
Biofilm	0.1	10106.90	19491.88	19311.40
Biofilm	0.1	10603.92	11906.15	13394.42
Biofilm	0.1	5289.46	10755.24	10931.56
Biofilm	0.1	5853.12	9818.13	13027.91
Biofilm	0.1	7219.21	9024.02	11550.74
Biofilm	0.1	4692.49	9384.98	9204.50
Biofilm	0.1	3950.44	10158.27	12415.66
Biofilm	0.1	7095.65	9149.66	10083.30
Biofilm	0.1	6908.93	9709.84	14564.76
Biofilm	0.1	6883.24	7069.28	11906.15
Biofilm	0.2	7684.30	13681.80	23427.74
Biofilm	0.2	6559.77	11807.58	18929.61
Biofilm	0.2	10643.48	10083.30	18112.59
Biofilm	0.2	6883.24	8743.58	11348.05
Biofilm	0.2	8621.41	8246.56	12557.27
Biofilm	0.2	12789.81	12789.81	16989.45
Biofilm	0.2	6462.59	7385.81	12186.59
Biofilm	0.2	7253.92	11453.56	14698.74
Biofilm	0.2	6872.14	9544.63	12217.13
Biofilm	0.2	9371.10	11620.16	21178.68

Table 4.8. Cyclose time (in min) of different *Stentor coeruleus* individuals (n=3) with ingested microplastic particles (in p Ind^{-1}; 6 µm, 10 µm) of chapter 2. Showing ingestion at 0 min, egestion of particles and end of experiment. Initial particle concentration in medium was 2500 p ml^{-1}.

Particle size [µm]	Ingested particles [p Ind^{-1}]	Cyclose duration [min]
6	2	0
6	2	126
6	1	137
6	0	255
6	1	12
6	0	29
6	0	270
6	1	0

6	0	247
10	4	0
10	4	126
10	0	137
10	2	0
10	2	224
10	1	234
10	0	270
10	1	0
10	0	247

Table 4.9. Abundance of mean ingested particles (p Ind^{-1}) of counted *Stentor coeruleus* individuals for different microplastic particles (6 µm, 10 µm) at different fixations (Formalin, Lugol's solution) and in living cells als control of chapter 2. Initial particle concentration in medium was 2500 p ml^{-1}.

Type of fixation	Particle size [µm]	Mean of ingested particles [p Ind^{-1}]	Number of counted individuals
Formalin	6	2.57	113
Living control	6	7.30	43
Formalin	10	2.01	113
Living control	10	6.26	43
Lugol	6	0.00	20
Living control	6	14.79	50
Lugol	10	0.00	20
Living control	10	13.74	50

Table 4.10. Abundance of mean ingested particles (p Ind^{-1}) of counted *Stentor coeruleus* individuals for different microplastic particles (6 µm, 10 µm) at different initial particle (0 p ml^{-1}, 500 p ml^{-1}, 1,250 p ml^{-1}, 2,500 p ml^{-1}, 5,000 p ml^{-1}) of chapter 2.

Initial particle concentration [p ml^{-1}]	Particle size [µm]	Mean of ingested particles [p Ind^{-1}]	Number of counted individuals
500	6	2.44	115
0 (Control)	6	0.00	20
500	10	1.81	115
0 (Control)	10	0.00	20
1,250	6	5.83	93
0 (Control)	6	0.00	20
1,250	10	7.44	93
0 (Control)	10	0.00	20
2,500	6	14.79	50
0 (Control)	6	0.00	17
2,500	10	13.74	50
0 (Control)	10	0.00	17
5,000	6	20.63	52
0 (Control)	6	0.00	20
5,000	10	21.42	52

0 (Control)	10	0.00	20

7. Eidesstaatliche Erklärung

Erklärung gemäß § 4 Absatz 9 der Promotionsordung

"Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit einschließlich Tabellen, Karten und Abbildungen, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie abgesehen von unten angegebenen Teilpublikationen noch nicht veröffentlicht worden ist, sowie, dass ich eine solche Veröffentlichung vor 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."

Köln, den 12.11.2021
Ort, Datum

Unterschrift

8. Curriculum Vitae

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Education

03/2018 – today **PhD Student** at the University of Cologne at the department of general ecology with focus on limnology and protozoology

10/2013 – 11/2015 **Master of Science in "Organismic Biology, Evolutionary Biology and Palaeobiology" (OEP Biology) at the University Bonn**
Final mark: 1.7
Master Thesis: "Tidying up and Description of Eight New Species – A Taxonomic Revision of the Genus *Oodera* Westwood, 1874 (Hymenoptera: Pteromalidae: Cleonyminae)"

10/2010 – 09/2013 **Bachelor of Science in Biology at the University of Cologne**
Final mark: 2.5
Bachelor Thesis: "The influence of pressure on deep-sea isolates of heterotrophic protists"

08/2001 – 06/2010 **Abitur at Gymnasium Köln - Pesch, Cologne**
Final mark: 2.5
Focus subjects: Biology, English

Professional Experience

08/2020 – today **Research associate** at the TH Köln – University of Applied Sciences at the institute of Technische Gebäudeausrüstung of the faculty of Anlagen, Energie- und Maschinensysteme

02/2017 – 02/2018 **Technical assistant** at the Zoological Research Museum Alexander Koenig, Bonn at the department of Diptera

11/2015 – 12/2016 **Research associate** at the Zoological Research Museum Alexander Koenig, Bonn at the department of Hymenoptera

08/2015 – 10/2015 **Student associate** at the Zoological Research Museum Alexander Koenig, Bonn at the department of Diptera

10/2014 – 12/2014 **Student Internship** (7 weeks) **at the Zoological Research Museum Alexander Koenig**, Bonn at the department of Diptera

04/2014 – 05/2014 & 07/2013 – 08/2013 **Student Internship** (6 weeks) **at the Zoological Research Museum Alexander** & **Koenig**, Bonn at the department of Herpetology
Topic: "Different Developments of Sexual Dimorphism of *Lacerta* from Different Geographical Regions: An Analysis of Geometric Morphometrics"

Publications

Živaljić S., Schoenle A., Nitsche F., Hohlfeld M., Piechocki J., Reif F., Shuma M., Weiss A., Werner J., Witt M., Voss J. & Arndt H. (2017): Survival of marine heterotrophic flagellates isolated from the surface and the deep sea at high hydrostatic pressure: Literature review and own experiments. *Deep Sea Research Part II*. 148: 251 – 259.

Schoenle A., Nitsche F., Werner J. & Arndt H. (2017): Deep-sea ciliates: Recorded diversity and experimental studies on pressure tolerance. *Deep Sea Research Part I*. 128: 55 – 66.

Werner J. & Peters R. (2018): Taxonomic revision of the genus *Oodera* Westwood, 1874 (Hymenoptera, Chalcidoidea, Pteromalidae), with description of ten new species. *Journal of Hymenoptera Research.* 63: 73 – 123.

Conferences and Workshops

2018/02/27-03/02 **37th Meeting of the German Society for Protozoology (DGP)**, Köln, Germany

2018/09/10-13 **Annual meeting of the German Society for Limnology (DGL)**, Kamp-Lintfort, Germany. Oral presentation: "Retentionseffekte von Biofilmen im Rhein"

2019/02/20-22 **38th Meeting of the German Society for Protozoology (DGP)**, Vienna, Austria. Poster presentation: “Retention Effects of Biofilms of the River Rhine”

2019/02/19 Workshop on parasite microscopy, Vienna, Austria

2019/09/23-27 **Annual meeting of the German Society for Limnology (DGL)**, Münster, Germany. Poster presentation: “Retention Effects in the River Rhine (Cologne) – One Year Full of Sampling”

2020/03/03-06 **39th Meeting of the German Society for Protozoology (DGP)**, Kaiserslautern, Germany. Oral presentation: “Annual course of retention effects through protistan dominated biofilms regarding planktonic bacteria in the River Rhine.”

2021/02/23-25 **40th Meeting of the German Society for Protozoology (DGP)**, Duisburg-Essen, Germany, online. Poster presentation: “Retention of Microplastics by Biofilms and its Ingestion by Protists”

2021/02/22 Workshop on digital microscopy, BIIGLE, online

2021/09/27-10/01 **Annual meeting of the German Society for Limnology (DGL)**, Leipzig, Germany, online. Oral presentation: “Quantitative Abschätzung der Plankton-Elimination durch den Biofilm im Rhein“

Reviewer for scientific journals

Knowlegde and Management of Aquatic Ecosystems

ZooKeys

Awards

02/2021 2nd Poster award at the 40th meeting of the German Society for Protozoology, Retention of microplastics by biofilms and its ingestion by protists

09/2019 Poster award at the Jahrestagung der Deutschen Gesellschaft für Limnologie, Retention effects in the River Rhine (Cologne) – One year full of sampling –

06/2015 Scholarship by Alexander-Koenig-Gesellschaft e.V. for the scientific visit of the Musée royal de l'Afrique centrale and stay in Tervuren, Belgium

08/2014 Scholarship of the European Union for ERASMUS Intensive Program: “Origin, Evolution and Future of the Biosphere” in Banyuls, France

Voluntary Activities

10/2020 – today	KRAKE e. V., development of a garbage trap for the River Rhine
06/2005 – 05/2011	Handball-Coach for children (3 – 9 years old) at the sport clubs Longericher SC and Turnerkreis Nippes, Cologne Guided the children in sporty activities, taught the rules and strategy of Handball, managed applications

Skills

Languages	German	Native
	English	Fluent
	Latin	Basic
IT	MS Office	Good
	R-Statistic	Basic

References

Hartmut Arndt, Prof., Dr.	Head of the working group Arndt at the Department of General Ecology & Limnology, Zoological Institute, University of Cologne Hartmut.Arndt@uni-koeln.de
Nina Kloster, Prof., Dr.	Head of GreenING Lab at the TH Köln – University of Applied Sciences at the institute of Technische Gebäudeausrüstung of the faculty of Anlagen, Energie- und Maschinensysteme
Ximo Mengual, Dr.	Curator of the Section Diptera at the Research Museum Alexander Koenig, Bonn x.mengual@zfmk.de
Ralph Peters, Dr.	Curator of the Section Hymenoptera at the Research Museum Alexander Koenig, Bonn r.peters@zfmk.de

Köln, den 12.11.2021

Ort, Datum

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