

Aus dem Institut für Bodenkunde und Bodenerhaltung

Professur für Bodenressourcen und Bodenschutz

der Justus-Liebig-Universität Gießen

# **Effects of Polyamide Microplastic Particles on Aquatic Outdoor Ecosystems - A Mesocosm Study**

Dissertation

vorgelegt von

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# **Effects of Polyamide Microplastic Particles on Aquatic Outdoor Ecosystems - A Mesocosm Study**

INAUGURAL DISSERTATION

Zur Erlangung des Doktorgrades (Dr. rer. nat.)

Im Fachbereich Agrarwissenschaften, Ökotoxikologie und  
Umweltmanagement der Justus-Liebig-Universität Gießen

vorgelegt von

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Gießen, 2026



Mit Genehmigung des Fachbereichs Agrarwissenschaften,  
Ökotoxikologie und Umweltmanagement der  
Justus-Liebig-Universität Gießen

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Tag der Disputation: 05.03.2026



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## I. Glossary & List of abbreviations

Term	Explanation
Abundance	The abundance is a number of a respective taxon in a sample
Alternative hypothesis	The alternative hypothesis is the hypothesis that is accepted if the null hypothesis is rejected
Bioassay	A bioassay is an experimental procedure for the quantitative or qualitative determination of the effect of a substance on living organisms in a closed system
Biocenosis	Biocenosis refers to the totality of all living organisms (plants, animals, microorganisms) that occur in a specific habitat and interact with each other
Closure Principle Computational Approach Test (CPCAT)	The CPCAT is a statistical test, which is used for reproduction data
CMR	The CMR criteria refer to the classification of substances as carcinogenic (C), mutagenic (M) or toxic to reproduction (R) in accordance with European and international chemical regulations
Ecosystem	An ecosystem is a functional unit consisting of a biocoenosis (community of all organisms) and a biotope (inanimate environmental factors), which are connected to each other through a variety of interactions
Effect Concentration (EC)	The EC is the calculated concentration of a substance on which any percentage (often 50%) of the organisms show effects
European Food Safety Authority (EFSA)	The EFSA is an independent agency of the European Union that provides scientific opinions and risk assessments on food and feed
Emergence mean Time (EmT)	The EmT is the point in time at which a specific proportion of individuals of a taxon emerged
Emerging insects	Emerging insects are adult, winged invertebrates with aquatic larval stages
Enclosure	An enclosure is a stainless-steel ring that serves to separate individual test vessels in a larger pond
Food chain	A food chain consists of primary producers, consumers of various orders and decomposers
Higher tier	Higher tier studies are studies with a more realistic approach, such as mesocosm or field studies
Lowest Observed Effect Concentration (LOEC)	The LOEC is the lowest used concentration in an experiment that has significant differences compared to the control
Macroinvertebrate Artificial Substrate Sampler (MASS)	MASS is a method to enrich macroinvertebrates in an experiment
Macroinvertebrates	Macroinvertebrates are invertebrates recognizable with the eyes
Mesocosm	Mesocosms are independent but comparable ecosystems that are used for chemical testing, among other things
Microplastic	Microplastic are polymer particles that do not exceed a length of 5 mm in any of their dimensions

<b>Term</b>	<b>Explanation</b>
Microplastic continuum	The microplastic continuum is the concept that plastic particles are constantly changing in size, shape, chemical composition and bioavailability
Minimum Detectable Difference (MDD)	The MDD indicates the percentage value by which a treatment must differ from the control to determine statistical significance in a given test design
No Observed Effect Concentration (NOEC)	The NOEC is the highest used concentration in an experiment that has no significant differences compared to the control
Null hypothesis	The null hypothesis is the hypothesis of a statistical test that can either be rejected or accepted
Organisation for Economic Co-operation and Development (OECD)	The OECD is an international organization that analyses and coordinates economic, social and environmental policies to promote sustainable growth and prosperity.
PBT	The PBT criteria refer to the assessment of substances regarding their persistence, bioaccumulation and toxicity that may have an impact on the environment and human health
Predicted environmental concentration (PEC)	The PEC is the predicted environmental concentration of a substance in a specific environmental compartment (e.g., water, soil, air). It is based on model calculations, laboratory and field studies
Predicted no effect concentration (PNEC)	The PNEC is the predicted concentration of a substance in the environment at which no harmful effects on organisms are to be expected. It is calculated from ecotoxicity studies (e.g., on algae, fish, daphnia) with a safety factor.
Phytoplankton	Phytoplankton is a term for aquatic algae
Principle Response Curve (PRC)	A PRC is a multivariate statistical method for representing time-dependent changes in ecological communities, especially under experimental or environmental disturbances
Redundancy analysis (RDA)	RDA combines linear regression with principal component analysis (PCA) to investigate how a set of dependent variables (e.g., species composition) is explained by a set of independent variables (e.g., environmental factors)
Sexratio	The sexratio indicates which proportion of a population is male and female. Sexratio was calculated as $n_{\text{males}}/n_{\text{total}}$ with not distinguishable organisms disregarded
Significance level	The significance level $\alpha$ is the measure of a statistical test of the probability of error for first-order errors (often $\alpha = 0.05$ )
Zooplankton	Zooplankton is an umbrella term for small aquatic animals that do not or only hardly move independently

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## V. Zusammenfassung

Der Schutz und Erhalt der Ökosysteme sowie ihrer Biodiversität und der inhärenten Ökosystemleistungen sollte ein zentrales Ziel der aktuellen wissenschaftlichen Bemühungen sein. Nicht nur die steigende chemische Belastung, sondern auch das vermehrte Vorkommen von Plastik üben stetig Stress auf die Ökosysteme und deren unterschiedlichen Ebenen, vom Ökosystem bis zum Organismus aus. Zur Erfassung des potenziellen Risikos, das chemische Substanzen auf die Umwelt haben können, gibt es viele Regularien und standardisierte Vorgehen. Für Partikel, wie z.B. Mikroplastik, hat der Stand des Wissens noch nicht zu weitreichenden regulatorischen Maßnahmen geführt. Zwar wurden bereits die Aufnahme und Weitergabe von Mikroplastikpartikeln entlang der Nahrungskette von Mikroplastikpartikeln nachgewiesen, aber aufgrund der Vielzahl an kombinatorischen Möglichkeiten aus Basissubstanzen, Größen, Formen, chemischen Additiven und physikalisch-chemischen Eigenschaften ist es schwer, einen strukturierten Überblick über das ökotoxikologische Risiko zu erlangen. Stehende Gewässer, wie Seen oder Staustufen, sind aufgrund des fehlenden hydrodynamischen Drucks eine potenzielle Senke für solche Partikel. Dadurch kann es zu starken Anreicherungen gegenüber den Zuflüssen kommen. Die Plastikpartikel, die zum Teil von Biofilmen besiedelt sind, können von den Organismen mit Nahrung verwechselt oder unselektiv aufgenommen werden. In natürlichen Systemen kann es dabei zu einer Biomagnifikation kommen.

Da diese Zusammenhänge in klassischen Laborstudien nur unzureichend abgebildet werden können, wurde in dieser Arbeit eine Studie in einem aquatischen Freiland-Modellökosystem durchgeführt, um Einflüsse von Polyamid Partikeln auf die aquatische Biozönose unter realistischen Bedingungen zu untersuchen. Es wurden 5-50 µm große Partikel ohne Additive insgesamt viermal über einen Zeitraum von zehn Tagen in drei Konzentrationen in die Modellökosysteme appliziert, um einen kontinuierlichen Zustrom von Partikeln zu simulieren. Zusätzlich wurde eine Partikelkontrolle im Versuch eingesetzt, um potenzielle Effekte durch natürliche Partikel denen des Polyamids gegenüberzustellen. Über einen Zeitraum von über 100 Tagen wurden abiotische Parameter und Vertreter aller trophischen Ebenen regelmäßig beprobt und ausgewertet. Dabei wurden die Primärproduzenten durch die Chlorophyll a Messung von freischwebenden Algen und die Konsumenten und Destruenten abgebildet. Die in den etwa 1000 L fassenden Systemen eingesetzten Konzentrationen waren 1.5 mg L<sup>-1</sup>, 15 mg L<sup>-1</sup> und 150 mg L<sup>-1</sup>. Neben üblichen Abundanzzählungen der tierischen Organismen wurden zusätzlich das Biovolumen von adulten und juvenilen Makroinvertebraten erfasst und der Schlupfzeitpunkt und Schlupfrate der emergierenden Insekten als weiterer Endpunkt für Higher-Tier Studien etabliert.

Bei der Auswertung der Proben konnten auf große Teile der Biozönose keine Effekte festgestellt werden. Auf die Abundanz von Muschelkrebse (Ostracoden) jedoch gab es deutliche Effekte. Alle Polyamidbehandlungen zeigten signifikante Reduzierungen der Abundanz von Ostracoden im Vergleich zu der Kontrolle, wobei die Effekte in der höchsten Konzentration am längsten anhielten. In der Partikelkontrolle wurden keine Effekte beobachtet. Bei den emergierten Insekten einer Unterfamilie der Züchmücken (Orthocladinae) wurde ein signifikant verfrühter Schlupfzeitpunkt beobachtet. Die vorliegende Studie stellt mit einer Dauer von 114 Tagen, sowie der langen Exposition, der Partikelkontrolle und den realitätsnahen Konzentrationen von Polyamid-Partikeln ein Novum dar und trägt einen wichtigen Bestandteil zur realistischen Risikoabschätzung von Mikroplastik bei.

## VI. Abstract

The protection and preservation of ecosystems, their biodiversity and the inherent ecosystem services should be a central goal of current scientific efforts. Not only the increasing chemical pollution, but also the increased occurrence of plastic is putting constant stress on ecosystems and individuals. There are many regulations and standardized procedures to assess the potential risk that chemical substances can have on the environment. This is not the case for particles such as microplastic. Although the uptake and transfer of microplastic particles along the food chain has already been proven, it is difficult to obtain a comparative overview of the ecotoxicological risk due to the large number of different parent substances, sizes, shapes and chemical additives. Standing water bodies such as lakes or reservoirs are a potential sink for such particles due to the lack of hydrodynamic pressure. This can lead to strong accumulations compared to the lotic inflows. The plastic particles, some of which are colonized by biofilms, can be mistaken for food or ingested unselectively by the organisms. This leads potentially to biomagnification in natural systems.

Since this can only be inadequately reproduced in classical laboratory studies, an aquatic field model ecosystem study was carried out in this project to investigate the influence of polyamide particles on aquatic biocoenosis. Particles with a size range of 5-50  $\mu\text{m}$  were applied to the model ecosystems in three concentrations four times over a period of ten days in order to simulate a continuous influx of particles. In addition, a particulate control was used in the experiment to compare the potential effects of pure particles with those of the polyamide microplastic. Abiotic parameters and representatives of all trophic levels were regularly sampled and evaluated over a period of more than 100 days. The primary producers were represented by the chlorophyll a content in free-floating algae and the consumers and decomposers by animal plankton and larger invertebrates. The concentrations used in the approximately 1000 L systems were  $1.5 \text{ mg L}^{-1}$ ,  $15 \text{ mg L}^{-1}$  and  $150 \text{ mg L}^{-1}$ . In addition to pure abundance counts, the biovolume of adult and larval macroinvertebrates was also recorded and emergence time was established as a new endpoint for higher-tier studies.

The analysis of the samples revealed effects on the abundance of seed shrimps (Ostracoda). All polyamide treatments showed significant reductions compared to the control, with the effects at the highest concentration being the most long-term. No effects were observed in the particle control. A significantly earlier Emergence mean Time was observed in the emerged individuals of a subfamily of non-biting midges (Orthoclaadiinae). With a duration of 114 days this study is one of few to include long exposure, a particulate treated control and an environmentally realistic concentration of polyamide particles and gives important information for the risk assessment of microplastic under near-natural conditions.



## 1. Introduction

*“We do not inherit the earth from our ancestors, we borrow it from our children.”*

*old native American proverb*

This guiding principle demonstrates that it is vital to leave future generations an environment that is as unpolluted and unaltered as possible. However, anthropogenic activity is threatening the environment more acutely than ever before. The global forest area has shrunk by around 68% compared to pre-industrial times, 75% of the land area and 66% of the oceans are anthropogenically influenced and the negative consequences of climate change on ecosystems can be clearly seen in the rapid disappearance of unique habitats such as coral reefs (IPBES, 2019). There are various reasons for this, which often influence each other. On the one hand, there are direct anthropogenic interventions such as greenhouse gas emissions, changes in land and soil use and the increasing pollution of ecosystems. On the other hand, there are also indirect effects, such as the generation of electricity, technological progress and rapid population growth. Nevertheless, it is important to do justice to the advancing technologization and the growing world population. To ensure a constant supply of food, it is essential to use crop protection (Oerke and Dehne 2004, Ennis et al., 1975).

This use is regulated by various mechanisms, such as consumer advice and ecotoxicological risk assessment. However, the use of materials such as plastic is constantly increasing and is monitored by only a few national and international laws. In 1994, Denmark put levy on plastic bags and Bangladesh was the first country to put a ban on thin plastic bags eight years later (Syberg et al., 2021). Only nearly 20 years later the European Union banned certain disposable items and aims to reduce plastic raw materials and packaging by 20% (Syberg et al., 2021). As various polymers are used in almost all everyday processes, there is a high risk of plastic entering the environment, not just through improper disposal. An estimated amount of 19 to 23 million tons of plastic is released into the aquatic environment yearly (e.g., Kaandorp et al., 2023, Lebreton et al., 2018, Eriksen et al., 2014). So far, however, there are few data on the ecotoxicological risk of macro- and microplastic, especially in non-marine ecosystems. To answer the question whether plastic particles in environmentally relevant concentrations potentially endanger populations, the protection goal of ecotoxicology is not trivial.

Experiments have already demonstrated toxic effects on various organisms (Scherer et al., 2018), but some of these were in artificially high concentrations and under laboratory conditions. Contrary to the classical ecotoxicological risk assessment approach, which starts by performing lower tier experiments, beginning with a higher tier experiment can be helpful for non-standard stressors. In this study unaltered polyamide was chosen as test item. To gain an insight which species, life stages or trophic levels are potentially endangered, aquatic outdoor model ecosystems (so called mesocosms) may be useful to detect the potentially affected endpoints (Caquet, 2002). These mesocosms form a bridge between laboratory standardization capability and the realism of field experiments.



## 2. Current State of Research

### 2.1 Aquatic ecosystems

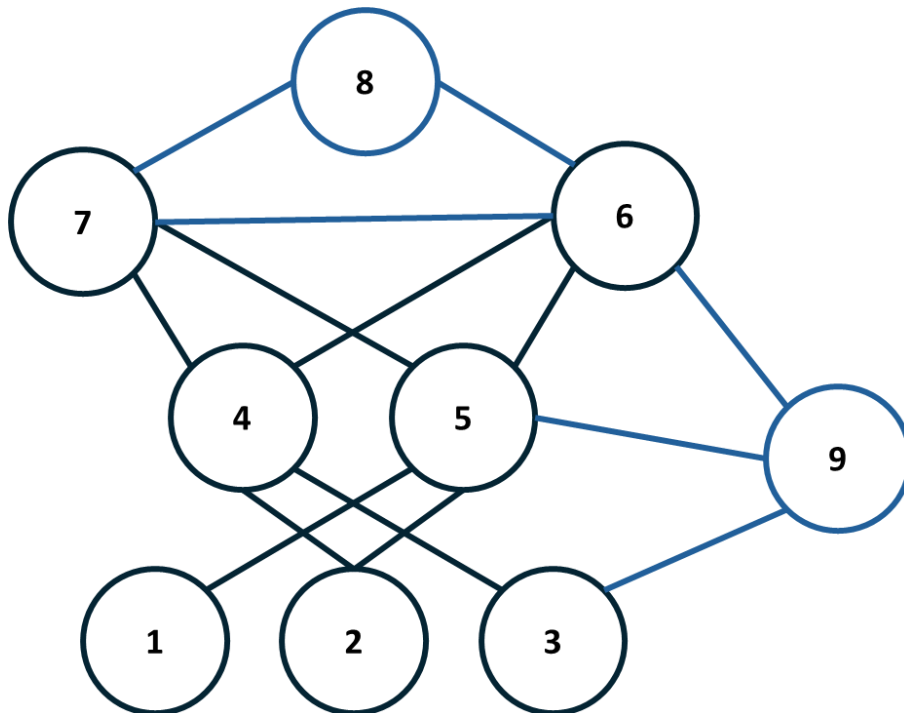
Two-thirds of the world's surface is covered with water. Less than 3% of that is freshwater. Although two-thirds of this is frozen in glaciers and ice on the polar ice caps, and almost the entire other third is groundwater, a disproportionately high number of animal species are present in surface inland waters, which make up 0.3% of the total fresh water (Baumgartner and Liebscher 1996). The very heterogeneous conditions of these waters offer around 126,000 species, i.e., around 10% of all species, many ecological niches in which they can spread and establish themselves (Dudgeon et al., 2006, Balian et al., 2008). These bodies of water are an important source of freshwater and food for humans as well. In Europe, small lentic ponds especially (low depth, area of up to 10 hectares) are not only very important as supplier of drinking water, source of socio-economic activities, but also a carbon dioxide sink and biodiversity hotspot (Millenium Ecosystem Assessment, 2005, Downing et al., 2008; Oertli et al., 2009; Bolpagni et al., 2019).

Lotic and lentic systems provide different habitats and challenges for the organisms, like insect larvae, gastropods or fishes. While rivers and streams are characterized by a more or less strong current, there are seasonal stratifications in lakes. This stratification depends on the temperature and develops in the lake in summer and winter. In summer, a distinction is made between the surface layer (epilimnion), thermocline (metalimnion) and deep layer (hypolimnion). This leads to an uneven distribution of dissolved oxygen in the summer. The oxygen content decreases with increasing depth up to the thermocline. In the deep layer, the oxygen content increases again with increasing depth. In spring and fall, this stratification in the lake is lost due to full circulation. Organisms must be able to cope with this seasonal change.

The physical and chemical characteristics of aquatic systems, such as temperature stratification and oxygen distribution, not only shape habitat conditions but also influence trophic interactions within the ecosystem. Therefore, analyzing the structure of the food web is essential for understanding the ecological dynamics of lakes and rivers. The food chain plays an important part in every ecosystem. It describes the feeding relationships between various organisms. A distinction is made between producers, consumers of various orders and decomposers. The first links in aquatic ecosystem are often composed of plankton. Plankton is an umbrella term for microorganisms in the water of seas, lakes and rivers. Phytoplankton consists of photoautotrophic algae of various algae classes and forms, together with macrophytes, the first link in the aquatic food chain. They produce organic materials such as leaves or roots from inorganic substances and sunlight. They are mainly found on the lake surface and shallow areas, where they have sufficient sunlight available. They contribute to the oxygen content in the lakes through photosynthetic processes. Animal plankton, also known as zooplankton, feeds on other organisms, like algae, and serves as food for many higher animals such as insect larvae and fish. Plankton is therefore an important part of the ecosystem in lakes and rivers and helps to maintain biodiversity. It is also an important

indicator of water quality as it is sensitive to changes in the ecosystem. If pollution increases, this can lead to a decline in plankton, which in turn has an impact on the entire food chain.

Consumers feed on other organisms and are further subdivided according to their consumption priorities (primary, secondary, tertiary consumers, etc.). Examples of consumers in the lake are waterbeetles, some insect larvae (e.g., Chaoborids) or fish, like trouts. Decomposers break down dead plant or animal matter and organic waste into inorganic material. Examples of decomposers in the lake are bacteria, fungi or water lice. The food chain in the lake is a complex system that is influenced by many factors. Changes in one part of the food chain can affect other parts and impact the entire ecosystem. It is therefore important to understand and protect it in order to maintain biodiversity and the ecosystem functions. Figure 1 shows a simplified example of an aquatic foodweb modified after Townsend (Townsend, 2009). Different species of primary producers, like macrophytes or phytoplankton are symbolized as numbers one to three. Grazers and filter feeders (numbers 4 and 5) feed on the above-mentioned. Predators (numbers 6 and 7) feed on the above-mentioned. 8 symbolizes a terrestrial predator and 9 the destructors. The lines indicate the different interactions between the different trophic levels (e.g., predator-prey relationships).



**Figure 1** Schematic representation of a simplified food web (modified (blue lines) after Townsend, 2009). Competition between different plant species (1, 2 and 3), grazers (4 and 5) and predators (6 and 7) is shown. 8 symbolizes a terrestrial predator and 9 the destructors. The lines indicate the different interactions between the different trophic levels (e.g., predator-prey relationships).

In this example, predators are represented by predatory insect larvae such as *Chaoborus obscuripes* (number 6) and small fry such as trout (number 7). Terrestrial individuals such as birds, for example, feed on these fish and the emerged insects. The decomposers (number 9) make the nutrients from animal remains, such as exuviae and plant remains, available again for the lower levels of the food web. With more species, more and more connections are created in the network. Top predators such as catfish or pike even feed partly on terrestrial individuals such as ducklings.

## 2.2 Usage of plastics

In 2023, around 414 million tons of plastic were produced worldwide (Statista, 2024). The amount of plastic produced worldwide has risen sharply over the last 70 years. The drivers of this increase over the past decades have been Asian countries, especially China. In Europe, development has been less rapid. Over the last ten years, the amount of plastics produced per year in the European plastics industry has even stagnated (Statista, 2024). Plastics are used in many different areas, including packaging, construction, electronics, textiles, agriculture and medicine (e.g., Castellano et al., 2008, Teymourian et al., 2021, Sacha et al., 2010). The largest portion of plastics is used for packaging, especially disposable packaging (Jepsen et al., 2020, OECD 2022). Until 2015, an estimated cumulated amount of 8.3 billion tons (Geyer et al., 2017) of plastics were produced. Around 600 million tons of that were recycled between 1950 and 2015. While 800 million tons were reused for energy and 4.9 billion tons were deposited or improperly dumped in the environment. The fact that plastic can be found almost everywhere, even in non-anthropogenic influenced deep-sea regions (Van Cauwenberghe et al., 2013) is a result of this mismanagement and longevity. Microplastic are found in all habitats of the marine environment (Barboza and Gimenez, 2015, Browne et al., 2015, Cózar et al., 2014). While at the beginning of industrial plastic production in the early 1950`s, 1.5 million tons were produced annually, by 2020 this figure had risen to 368 million tons (PlasticsEurope 2020).

The sharp rise in production volumes and the constantly growing demand for plastics can be explained by the advantageous product properties and the wide range of applications. For example, the production and use of single-use plastic products increased up to tenfold during the COVID - 19 pandemic (Patricio Silva et al., 2021). Single use plastics in medicine provide a fast, affordable and hygienic solution. Products made from plastic polymers are characterized by their high moldability, durability and low manufacturing costs (Waldschläger, 2019). In Europe, plastics are mainly used as packaging material (39.6% total share of the end consumer market), in the construction industry (20.4%), in the automotive industry (9.6%), for electrical goods (4.1%), in the household and leisure sector (3.7%) and in agriculture (3.4%) (PlasticsEurope, 2020). The wide range of applications shows that plastic products are of great benefit to society. For example, plastic products contribute to the safe storage of food and plastic constructions play an important role in saving energy, for example by producing lightweight components for vehicles (Andrady and Neal, 2009). Due to their diverse properties, various plastics are therefore used as simple packaging for products through to complex components in various branches of industry. Polyethylene (PE), polypropylene (PP) and polyvinylchloride (PVC) are the most widely produced polymers. However, microplastic particles made of polyamide (PA) and PE are among the most frequently detected in lakes and streams (Rehse et al., 2018). Despite its comparatively low production volume (~ 3%), PA is therefore of particular environmental relevance. Its use in fishing gear such as lines and nets may further contribute to its hazard for aquatic systems (e.g., Alijagic et al., 2024, Khosrovyan and Kahru 2021, Hasan et al., 2023, Choi et al., 2023, Zhang et al., 2023).

### **2.3 Microplastic definition**

In general, microplastic are particles that do not exceed a length of 5 mm in any of their dimensions. There are different ways to further distinguish microplastic (Wagner and Lambert 2020). On the basis of their:

#### ***Creation***

Primary microplastic are produced as such. They are used in cosmetics (e.g., peeling products), carrier material for pesticides or in paints and varnishes. Secondary microplastic, on the other hand, are produced by physical or chemical degradation of larger particles via different processes (Figure 2). The bigger plastic debris can be shredded biologically, chemically e.g., via oxidation or physically via mechanical or thermal processes. Microplastic fibers are produced, for example, due to abrasion when clothes made of synthetic fibers are washed.

#### ***Material and surface texture***

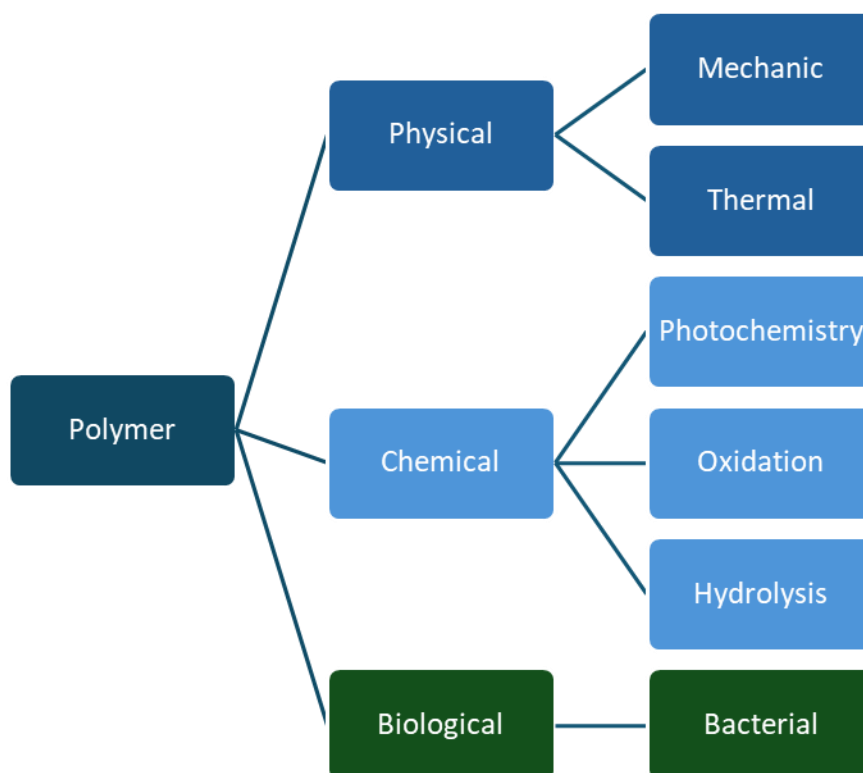
The presence of secondary microplastic particles from all existing plastics is conceivable. Various plastics such as PE, PP, Polystyrene (PS), Polyethylene Terephthalate (PET), PVC and PA are used. Due to weathering processes the surface texture of the microplastic particles can vary and change over time. The surface can roughen or edges can be smoothed. To serve different purposes, like reduced flammability or improvement of impact resistance, a wide range of additives find application (Lambert and Wagner 2018).

#### ***Shape and color***

The particles can also be differentiated by their color and shape. In addition to fibers, there are spherical particles with and without edges and irregularly shaped fragments. There are also sheets, films and foam particles (Wu et al., 2018). Due to the various possible applications of plastic particles different colors are used, e.g., red fibers for clothing or green plastics come as abrasion from cutting boards. The particles can inherit their colors from the original product or change it due to weathering processes. Fibers seem to be more present in populated areas and the different dominance of the shapes differs in different regions (Wu et al., 2018).

#### ***Size***

Microplastic can be further differentiated by size. In general, it can be said that the term microplastic refers to particles that do not exceed a length of 5 mm in any of their dimensions. Nevertheless, the particles can be further differentiated as suggested by Lambert et al. (2014) all particles over 5 mm are referred to as macroplastic, all between 5 mm and 1 mm as mesoplastic and between 1 mm and 0.1  $\mu\text{m}$  as microplastic. Even smaller particles can be categorized as nanoplastic.

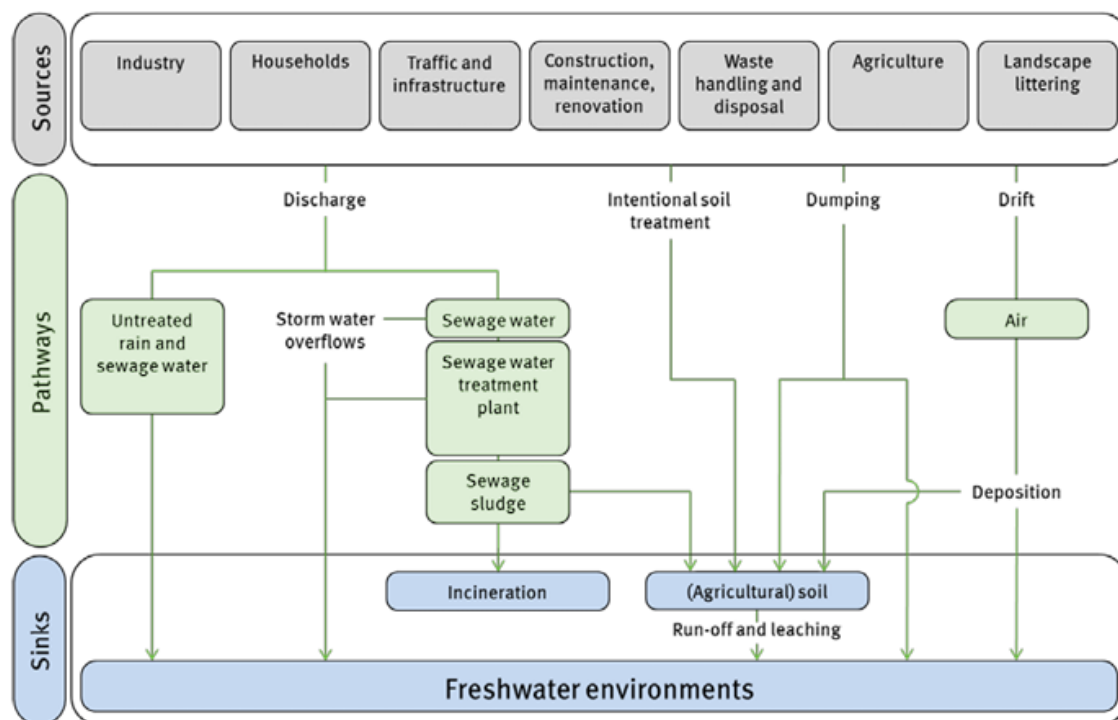


**Figure 2** Schematic representation of the physical, chemical and biological processes that play an important role in the in the formation of secondary microplastic (after Fath, 2019)

Environmental research with microplastic poses a number of special challenges. This begins, especially in (semi-)field research, with difficult detection and quantification. Due to the different materials, sizes and shapes of microplastic, it is difficult to ensure reliable analysis (Jin et al., 2022, Connors et al., 2017, Koelmans et al., 2019), especially in environmental samples. In laboratory studies, it can be assumed that nominal concentration prevails for the duration of the study due to a fixed weighed quantity. However, it should be noted that the materials remain in the water phase for different lengths of time or even float on the surface due to the different densities (Stride et al., 2024). This requires different methods, which makes standardization and comparability more difficult. The unclear long-term movement patterns and the extent to which it accumulates in biota are problematic too. This leads to not yet sufficiently researched effects of microplastic on biota and ecosystems. The risks of microplastic on human health are also still unclear. Microplastic have already been detected in the lungs, blood and placenta (Amato-Lourenco et al., 2021, Leonard et al., 2024, Ragusa et al., 2021). The health consequences, even on a longer-term scale, are uncertain. Unlike with pharmaceuticals or chemicals, there is also no uniform regulation or limitations of usage. Economic interests and the ubiquitous use of plastic products may hinder the reduction of general plastic use. Although there are no general databases on the publication of plastic-related articles 10 000 newly uploaded articles in 2021 in the web of science database show that microplastic are becoming increasingly relevant societally and scientifically.

## 2.4 (Micro-)Plastic in the environment

Plastic pollution is a global problem that affects both the environment and human health. Millions of tons of plastic are floating in the sea and polluting our oceans (Schwarz et al., 2023). The plastic that reaches the environment on land ultimately ends up in the sea via receiving waters, rivers and lakes. Nearly 75% of marine litter consists of plastic. Much of this waste is generated by pollution on land, where plastic bags, PET bottles or cigarette butts are carelessly thrown into nature. As soon as these anthropogenic pollutants are in the environment, they can be mistaken for food (e.g., Santos et al., 2021, Andrades 2019) by aquatic animals and thus end up in organisms like small invertebrates such as water fleas, chironomids or even in vertebrates like fish. The plastic then may bioaccumulate through the food chain up to organisms used by humans as food. The plasticizers, flame retardants and other contaminants that are potentially adsorbed or added to the plastic particles can harm both animals and humans. Up to 135 000 marine mammals and one million seabirds, turtles, fish and other species entangle every year due to ocean litter (Salomon and Markus, 2019). The preservation of these aquatic ecosystems is one of the most important goals of the 21st century, alongside the supply of clean water and the preservation of drinking water quality (Millenium Ecosystem Assessment 2005, Griggs et al 2013).



**Figure 3** Illustration of the possible entry paths of microplastic into rivers and lakes (according to Bänisch-Baltruschat et al., 2017)

Every year, around five to twelve million tons of plastic waste end up in the oceans worldwide (Jambeck et al., 2015), a lot of that via lakes and rivers (Figure 3). Microplastic enter the environment from a wide variety of anthropogenic sources (e.g., littering, roads and agriculture) (according to Bänisch-Baltruschat et al., 2017). Disposable plastic products can

release trillions of nanoparticles even when used properly (Zangmeister et al., 2022). One other important pathway is untreated wastewater and rainwater, which carries the microplastic load directly into bodies of water. Even wastewater treatment plants do not completely remove the microplastic. The use of sewage sludge as a fertilizer can also lead to microplastic being spread on agricultural land (e.g., Mason et al., 2016; Mahon et al., 2017). The particles are also transported and dispersed into aquatic and terrestrial ecosystems by wind drift, which may be far away from the actual source (Bänsch-Baltruschat et al., 2017). The last important transport pathway is from the soil to the aquatic environment. Particles that have accumulated in the soil can enter the aquatic environment with runoff and leaching into the aquatic environment.

Once the particles entered the aquatic environment, sedimentation is to be expected over time, so that the largest share of particles will accumulate on top of and in the sediment. Sedimentation rate is influenced depending on the density, shape, size and mass (Daily and Hoffman, 2020). Especially after aggregation of the particles biofouling processes and metal absorption influence the sinking behaviour (Reiser et al., 2020). Kowalski et al. (2016) have analyzed the sedimentation rates of PS, PA, PET and PVC, which are between  $-0.015 \text{ m s}^{-1}$  and  $0.05 \text{ m s}^{-1}$ . The negative sign signals that the particles are denser than water and therefore sink (Daily and Hoffman, 2020). Polymers such as PE, on the other hand, float on the water surface with a buoyancy velocity of  $0.0065 \text{ m s}^{-1}$  (Waldschläger and Schüttrumpf, 2019). Nevertheless, irregular movements in the water such as wave movements, currents, turbulences or the spring and fall circulation can change the sedimentation behavior. Furthermore, Mao et al. (2021) provide information on the penetration depth of microplastic particles into the sediment of Wuliangshai Lake in northern China. Half of the particles sank to a maximum depth of six to twelve centimeters within 100 years. These freshwaters especially are sinks for microplastic (Hurley et al., 2018). In the Three Gorges dam in China for example up to  $1.3 \cdot 10^7$  particles per  $\text{km}^2$  were found (Naqash et al., 2020).

It is estimated that between 4.8 and 12.7 million tons of plastic are disposed of in the oceans worldwide every year (Jambeck et al., 2015). However, the amount of plastic waste that ends up in the environment is much higher, as a large proportion of plastic waste ends up in landfills or is incinerated in the environment. The main pathway for the entry of plastic into the oceans is rivers (Meijer et al., 2021, Weiss et al., 2021). It is important to reduce the use of plastic and ensure that plastic waste is disposed properly to protect the environment. The following methods are generally used (by humans or nature) to decrease the burden of a chemical on the environment (Pandey et al., 2023):

### ***Adsorption***

Adsorption is a process in which pollutants stick to surfaces. There are various materials that absorb lipophilic chemicals, such as activated carbon, clay minerals and zeolites. Even microplastic particles potentially absorb other contaminants.

**Coagulation**

Pollutants can be coagulated by the addition of chemicals. This creates larger particles that can be more easily removed from the water. Particle crosslinking due to bridging in polymers can contribute to flake formation as well.

**Photocatalysis**

When pollutants are broken down by the effect of light it is referred to photocatalysis. A catalyst, like some plant enzymes, can accelerate the reaction. Energy transfer also allows reactive oxygen species to break down chemicals. Some plant extracts also contain natural reactants that can convert metal ions into reactive forms.

**Microbial degradation**

Microorganisms such as bacteria and fungi are used to break down particles. This can occur through enzymatic cleavage, catabolism, redox reactions or through cooperative degradation in biofilms

However, these methods are only partially applicable to plastic. There are also other approaches such as landfills, incineration or behavioral changes through reduction, reuse and recycling that can help limit the amount of plastic waste entering the environment (Pandey et al., 2023). It is important to note that these techniques and approaches are not perfect, and their effectiveness depends on various factors, such as the type, form and size of plastic and the environmental conditions. As plastic degrades very slowly in the environment, it can be assumed that it will continue to accumulate there. The risk of accumulation is especially high in dams and reservoirs, since there is a stoppage in flow and dense particles can sediment over time. Many of these lakes and reservoirs are used for (hobby) fishing, which increases the risk of secondary polyamide microplastic from the lines and nets (Koziol et al., 2022).

**2.5 The plastisphere and interactions with other environmental factors**

The environment has always been divided into different spheres (Martin and Johnson 2012). The atmosphere, which is the Earth's air envelope and contains various gases, is responsible for the weather and climate. All water vapor in the atmosphere and all water reservoirs, such as oceans, lakes and groundwater, are grouped together in the hydrosphere (Dobinski 2006). All frozen water areas can be divided into the cryosphere. The entirety of all habitats and living organisms is referred to as the biosphere. The solid, outer rock layer of the earth, which comprises the earth's crust and the uppermost part of the upper mantle, is the lithosphere, the uppermost layer of which, in which soil formation takes place, is called the pedosphere. Since mankind has played a major role in the transformation of the world, further spheres were created during the anthropocene. The technosphere, which comprises the entirety of all man-made and modified structures, technologies and material flows on earth (Zalasiewicz et al., 2017), is, like the plastisphere, a relatively new addition to these concepts and shows the extent to which human influence is changing the environment. The plastisphere

refers to biotic communities that have formed specifically on plastic waste, especially in bodies of water (Amaral-Zettler et al., 2020). In addition to the colonization of the surface of the (micro)plastic by algae, fungi and other microorganisms, invasive species or pathogenic germs can also adhere (Zhang et al., 2024). These biofilms can also release previously adsorbed chemical pollutants such as heavy metals or pesticides. An impact on the food chain is also conceivable. As the polymers that make up the plastic remain in the environment for a very long time, the plastisphere also remains for a long period. Plastic can serve as a substrate for microbes for decades and thus permanently changes the composition of marine and terrestrial microbiomes (Barros and Seena 2021, Rillig et al., 2024).

The behavior and risk potential of microplastic particles in the environment are strongly influenced by interactions between the particles, their polymer type and a variety of chemical substances and physical environmental factors. One of the most important interactions of microplastic particles is with pollutants such as heavy metals, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and pesticides (Tang et al., 2021). Due to their hydrophobic and often uneven surface, microplastic particles can serve as a carrier system for pollutants, adsorbing them and possibly transporting them over long distances. The pH value of the surrounding medium is a factor that significantly influences such interactions. A low pH value, such as in acidic soils or waters, can contribute to the release of certain pollutants from the microplastic, while other chemical reactions become relevant in alkaline conditions (Godoy et al., 2020). Microplastic can therefore act as a secondary source of pollutants in the environment if they have previously adsorbed pollutants (Hu et al., 2022, Hildebrandt et al., 2021).

Temperature and weather influences such as UV radiation and mechanical erosion caused by wind or waves are also significant for the properties of microplastic. Junck et. al (2024) found a decreased hydrophobicity and an increased surface negativity in aged microplastic particles. UV radiation promotes the degradation and structural change of the plastic, which can result in plastic particles becoming fragile and fragmenting further (Du et al., 2023). This not only results in a larger surface area of the particles and thus their adsorption capacity for pollutants but can also lead to changes in the chemical composition of the plastic material. Additives that contain plasticizers, flame retardants or stabilizers, for example, can then be released from the plastic, which can also have toxic effects on organisms living in the environment (Bolivar-Subirats et al., 2021, Barrick et al., 2021). Mechanical influences caused by currents, sediment abrasion or biogenic erosion can also produce reactive forms of microplastic particles in the environment, which play a role in chemical processes and therefore in the environment (Martinez and Barbosa 2024). Microplastic can also influence global warming, by aiding cloud formation or inducing greenhouse gas emissions from soil (Chia et al., 2023, Parvez et al., 2024). Salinity and the organic substances that occur in the environment are further environmental factors that influence the behavior of microplastic particles. In marine systems, for example, a high salt content causes microplastic particles to be distributed differently in the water and ionic interactions of pollutants can change (Zhang et al., 2024). Organic substances such as humic acids from dead

plant material can coat the surface of microplastic particles and thus change their chemical properties and their interaction with pollutants or organisms. These processes influence how long microplastic remain in the environment, how heavily they are loaded with pollutants and how they are consumed by living organisms (Wu et al., 2021, Chen et al., 2023).

In addition, microplastic interact with microbial communities that can colonize its surface and form biofilms. These biofilms can further modify the physico-chemical properties of microplastic and possibly influence the mobility and availability of pollutants and even the attractiveness as food source (Vroom et al., 2017). Microorganisms can also degrade or modify certain plastic components, which can potentially lead to the formation of even smaller secondary micro- or nanoplastic particles. (Zeenat et al., 2021). Valentine et al. (2022) have shown that caddisfly larvae are also a driver or biological breakdown of plastic litter, which likely increases bioavailability. Such complex interactions between microplastic, environmental factors and chemical pollutants complicate full assessment of the long-term impact of these particles on ecosystems and organisms.

## **2.6 Potential mode of actions**

Microplastic can affect organisms in a broad spectrum directly and indirectly. The different polymers may leach additives that were added to serve different purposes. Some plastics have added flame retardants to reduce flammability or additives that link together polymer chains. Also pigments for colorization, plasticizers, sensitizers, emulsifiers or more may be added to the polymers (Lambert and Wagner 2018). Additionally, to the chemicals that are intentionally mixed into the polymers, microplastic, with their big surface area, can act as a carrier for harmful substances. This allows pollutants such as pesticides to adsorb to the surface and be released later, for example in the body of an organism. During some recycling processes new additives can be added to the particles as well. Since the distribution of substances tends to follow equilibria, it is conceivable that the particles in a heavily polluted body of water absorb pollutants and release them where the environment is not saturated compared to the concentration in the particle.

Another potentially harmful effect of plastic can be blockage of the gastrointestinal tract. For example, plastic bags have already been shown to clog the stomach of sea turtles (Fukuoka et al., 2016). Something similar is also feasible for smaller particles in smaller organisms, such as daphnids for which it has already been shown that ingested particles can accumulate in the intestine (Polhill et al., 2022, Scherer et al., 2017). In addition to fragments with sharp edges that can cause internal damage, fibers can also cause physical damage. Fibers have the potential to entangle small invertebrates and limit their locomotion ability (Ma et al., 2024). This can result in reduced food intake or make the affected animals easy prey. Furthermore, it is also possible that, particularly in the case of accidents and large amounts of small particles with low density, these prevent sufficient sunlight from reaching the deeper water layers and thus prevent the primary producers from being sufficiently photosynthetically active. This would result in a drop in the oxygen content and cause problems for some organisms. Particles can also enter the lungs by inhalation or penetrate

other organs. Particularly small particles can even diffuse into cells or enter cells via transcytosis or endocytosis and cause inflammatory reactions or cell stress (Aloisi and Poma 2025).

### **2.7 Effects of microplastic particles on aquatic ecosystems**

It is important to understand the impact of microplastic on aquatic invertebrates and primary producers as these organisms play an important role in the ecosystem. They serve as a food source for other animals, help maintain biodiversity and to fulfil ecosystem services. Microplastic can have a significant adverse impact on aquatic invertebrates. The small plastic particles can be ingested by the animals and accumulate in the body, which can lead to impaired health and well-being. The effects of microplastic on aquatic invertebrates can vary depending on the type and size of the particles and the type of organism. Some studies have shown that microplastic can affect the reproduction, growth and survival rate of aquatic invertebrates. For example, ingestion of microplastic by mussels can lead to a change in the digestive enzyme activity, which can lead to decreased extraction of energy from food (Trestrail et al., 2021). Similarly, ingestion of microplastic by water fleas can lead to impaired reproduction and growth (Vieira et al., 2024). There is also an impact on fish and other marine animals. Small plastic particles can be ingested by animals and accumulate in the body, which can lead to impaired health and well-being (Miller et al., 2020). The effects of microplastic on fish can vary, depending on the type and size of the particles and the species of fish. It has been already shown that microplastic can affect the reproduction, growth and survival rate of fish (e.g., Bhat et al., 2024, Naidoo and Glassom, 2019).

Likewise, microplastic can have a significant impact on aquatic invertebrates. The small plastic particles can be ingested by the animals and accumulate in their bodies (Scherer et al., 2017). The effects of microplastic on aquatic invertebrates can vary depending on the type and size of the particles and the feeding type and size of the organism. In some studies, no toxic effects of microplastic were observed (e.g., Bruck and Ford 2018, Weber et al., 2018, Reichert et al., 2018, Hämer et al., 2014). Welden and Cowie (2016) found a reduced growth in a lobster species and Wang et al. (2019) have demonstrated damages in the digestive tract of *Artemia parthenogenetica* caused by ingested PS particles. The representative species *Daphnia magna* showed reduced feeding (Ogonowski et al 2016), increased mortality (Jemec et al., 2016), immobilization (Rehse et al., 2016) or less reproductive success (Besseling et al., 2014).

### **2.8 Regulation of environmental stressors**

Given the demonstrated biological effects of microplastic on aquatic organisms, particularly invertebrates, it becomes increasingly relevant to consider how such stressors are addressed within current regulatory frameworks. This necessitates a closer look at how environmental risks are assessed and managed at the legislative level, especially in the context of chemical substances that may pose similar ecological threats. Regulatory approaches of chemicals are based on ecotoxicological tests. The predicted environmental concentration (PEC) divided by the effect data obtained from these tests (PNEC) are used to calculate a

potential risk to the environment. The first acute effect data are performed with the aquatic triad (algae, daphnia, fish) to determine which group is the most sensitive. Chemicals are divided into four main categories for regulating purposes in the European Union. The four categories are industrial chemicals, veterinary and human pharmaceuticals, plant protection products and biocides. Some substances are categorized further. These chemicals meet the CMR criteria if they are either cancerogenic, mutagenic or toxic to reproduction. To meet the PBT criteria substances have to be persistent, accumulate in biota and be toxic. Another important criterion for risk analysis is the amount of this substance that is produced yearly. In general, the aim of the various laws and regulations is to identify a potential risk before any damage occurs, i.e., a prospective risk assessment.

### ***Plant protection products***

The risk assessment of plant protection products in the EU follows a strict, science-based procedure coordinated by the European Food Safety Authority (EFSA) and the European Chemicals Agency (ECHA). The procedure is based on Regulation (EC) No 1107/2009. This regulation determines rules for the authorization, placing on the market, use and control of plant protection products. The aim is to ensure a high level of protection of both human health and the environment and to improve the internal market and agricultural production by harmonizing these rules. The environmental risk assessment of plant protection products in the EU is carried out according to a tiered procedure that enables a gradual refinement of the risk assessment. The aim is to identify risks efficiently, economically and only carry out more complex investigations if necessary.

The first tier involves an initial assessment (worst-case assessment). This is the simplest and most conservative stage. It involves standard models and safety factors are used to calculate the PEC for various environmental compartments (soil, water, air). After a comparison with the PNEC, the risk is either acceptable and the assessment ends or a more detailed tier 2 assessment is required. This second tier contains a refined assessment with more realistic data. This includes more detailed exposure modeling, the consideration of site specifics (soil types, climate, water types), the use of more realistic application scenarios and a refined toxicity assessment through the use of specific laboratory and semi-laboratory studies. If there is still a risk ( $PEC > PNEC$ ), tier 3 must be performed. If the risk is manageable, risk reduction measures can be proposed (e.g., buffer zones, application requirements). The third tier has the highest level of detail with field studies (higher-tier studies). This involves the observation of real effects on ecosystems through complex ecotoxicological models. If no significant effects occur, the plant protection product can be approved. If there are still risks, the product is not authorized or is banned. In 2022, the European Commission presented a proposal for a Regulation of the European Parliament and of the Council on the sustainable use of plant protection products. This regulation is intended to supplement and update existing rules and, in particular, promote the reduction of the use of chemical plant protection products and the promotion of alternatives such as biological plant protection products and integrated pest management.

### ***Human and veterinary pharmaceuticals***

As medicinal products are biologically active and can enter the environment directly (e.g., via excretions, wastewater, manure), they must be assessed with regard to potential risks to ecosystems. Medicinal products for human use are regulated by Regulation (EU) 2019/6 and Directive 2001/83/EC. An evaluation is carried out by the European Medicines Agency (EMA). Veterinary medicinal products are governed by Regulation (EU) 2019/6. There are special requirements for medicinal products used in livestock farming (e.g., antibiotics). In stage one of the tiered procedure, it is assumed that 100% of medicinal products for human use are excreted and that veterinary medicinal products are released into the environment via manure. There is a special case for medicinal products for human use, as no further assessment is necessary if the PEC in surface water is  $< 0.01 \mu\text{g/L}$ . According to Regulation (EU) 2019/6 and Directive 2001/83/EC, medicinal products for human use may not be rejected solely on the basis of an environmental risk. If there are significant environmental risks, conditions may be imposed, such as disposal instructions in the package leaflet or restrictions on certain uses.

### ***Biocides***

The environmental risk assessment of biocides in the EU is similar to that of plant protection products, but in accordance with the requirements of the Biocidal Products Regulation (EU) No. 528/2012. The risk to various environmental compartments (water, soil, air) and non-target organisms (fish, birds, insects) is examined. Nevertheless, the two groups of substances differ in terms of the routes of exposure, which in the case of biocides occur via sewage systems, buildings and drinking water, and the places of application, which do not dominate in agriculture and forestry as in plant protection, but in the case of biocides in households, industry, medicine and public areas.

### ***Industrial chemicals***

The environmental risk assessment of industrial chemicals under REACH (Regulation (EC) No. 1907/2006) also follows a tiered procedure. The aim is to assess the risk to the environment (water, soil, air) and organisms from the manufacture, use and disposal of chemicals and to take measures if necessary. All chemicals manufactured or imported in quantities above 1 ton/year must be registered. The data requirements increase depending on the quantity. The PBT/vPvB assessment is carried out as part of the risk assessment of industrial chemicals under REACH and is required for all substances manufactured or imported in quantities over 10 tons per year. If a substance is classified as PBT or vPvB, it is included in the candidate list for substances of very high concern (SVHC). It may then be subject to authorization (Annex XIV REACH) or regulated by restrictions (Annex XVII REACH). Substances are considered persistent if the half-life in fresh water is more than 40 days and very persistent, if the half-life is more than 60 days. This is determined, for example, using simulation tests (OECD 308, OECD 309). If the bioconcentration factor in fish is over 2000, a substance is to be classified as bioaccumulative and as very bioaccumulative if the

bioconcentration factor is over 5000. Bioconcentration tests in fish (OECD 305) and modeling with QSAR (Quantitative Structure Activity Relationship) data are used for this purpose. A substance is considered toxic, if it is either chronically toxic to aquatic organisms ( $\text{NOEC} \leq 0.01$  mg/L), meets the CMR criteria or is endocrine disruptive. This can be assessed with long-term ecotoxicity studies (OECD 210, 211) or reproductive toxicity studies (OECD 416, 443).

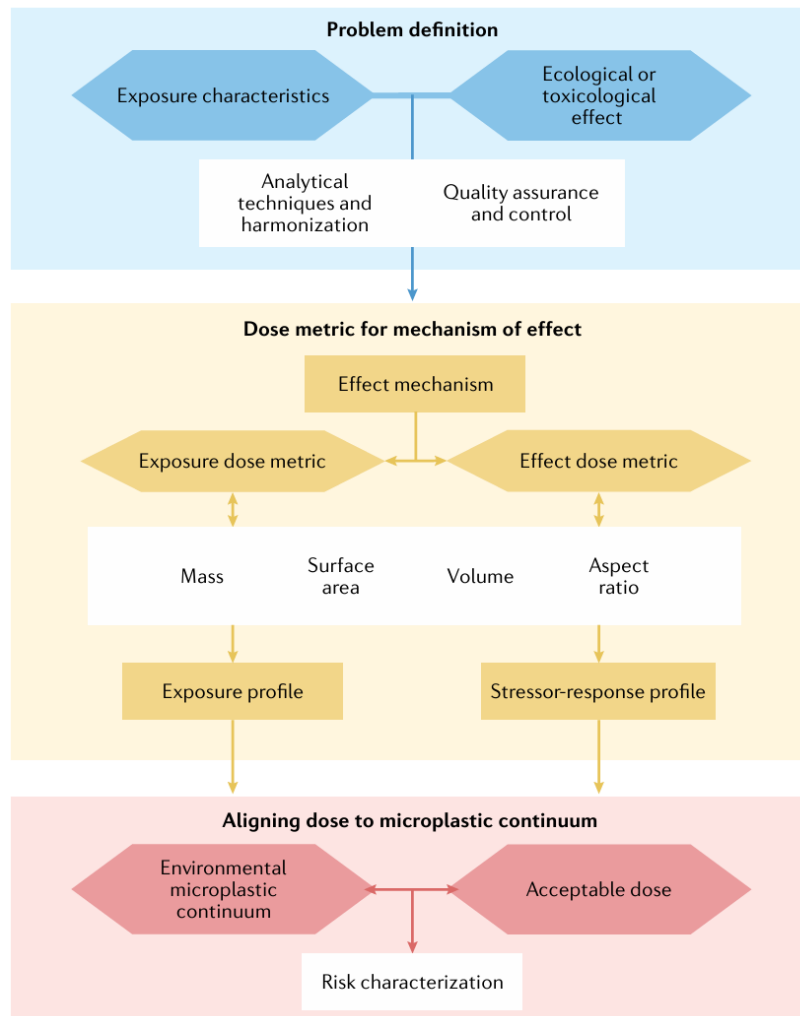
### ***Particles and plastic***

The regulation of particles, such as microplastic and other nanomaterials, is subject to complex legislation and can vary depending on the application and source. Nanomaterials are particles with a size of 1 to 100 nm and are used in various areas such as cosmetics, pharmaceuticals, electronics and food. Due to their special properties (e.g., high surface area), they require special regulation. According to the REACH Regulation (EC) No. 1907/2006, nanomaterials contained in chemicals or products must be subject to special monitoring. They must be explicitly mentioned as nanomaterials, on the safety data sheet for chemicals. For nanomaterials covered by REACH, manufacturers must provide toxicological data and an environmental risk assessment, and nanomaterials in food must be specifically approved and labeled on the food packaging in accordance with the Food Regulation (EC) No. 2015/2283 and Regulation (EU) No. 1169/2011.

In the EU, there are various legal frameworks that deal with the regulation of particles, particularly in the context of environmental protection, environmental health and safety. Regulation (EC) No 1107/2009 determines rules for the authorization, placing on the market, use and control of plastic materials and articles used in safe contact with food (Regulation (EU) No. 10/2011). The aim is to ensure a high level of protection of both human health and the environment and to improve the internal market and agricultural production by harmonizing these rules. Regulation (EC) No. 1223/2009 on cosmetic products regulates the use of microplastic in cosmetics. Since 2018, the use of microplastic in cosmetic products used to scrub or exfoliate skin or teeth, e.g., in scrubs and toothpastes, has been prohibited in the EU. The use of microplastic in industrial products (e.g., as fillers or conditioning agents) is not always directly regulated. In some areas, however, there are requirements to minimize the release of microplastic, such as in Regulation (EC) No. 1907/2006 (REACH) for chemicals. In coatings and paints, the REACH process is used to ensure that such products are safe for the environment and humans. Microplastic can enter the environment in agriculture through the use of plastic films, mulch materials or pesticides. The REACH regulation is important to control the voluntary disclosure of microplastic in products by manufacturers and to minimize potential risks. Microplastic are also used in medicine, e.g., as carriers in medicines or medical devices. These are also regulated by Regulation (EU) 2017/745 on medical devices and Regulation (EU) 2017/746 on in vitro diagnostic medical devices.

Another important instrument for regulating plastic is Directive 2019/904 on the reduction of the impact of certain plastic products on the environment. This directive sets out measures to reduce the environmental impact of certain single-use plastic products, including plastic products such as cutlery, plates, straws and cotton buds. Member States are required

to draw up national action plans to achieve these targets. The regulation of microplastic varies in different areas depending on the product and use. Koelmans et al. (2022) provide an approach that includes the multidimensionality of microplastic particles (Figure 4).



**Figure 4** Risk assessment scheme addressing the multidimensionality of microplastic (Koelmans et al., 2022)

The approach is divided into three steps: In the first step a definition of the problem is given on the basis of a protective objective, e.g., the protection of a population. This step includes a quality-controlled assessment and the harmonization of methods and the selection of suitable input data. In the second step relevant dose metrics are defined, based on selected effect mechanisms. Effect thresholds concentrations and exposure profiles are assessed after, leading to a stressor-response profile and an exposure profile. In the last step for each of the profiles of step 2 the bioavailable fraction of the microplastic continuum is applied. This is done by using probability density functions. The microplastic continuum describes the concept that plastic particles are constantly changing in size, shape, chemical composition and bioavailability. Lastly, actual exposure and effects thresholds are compared in a risk characterization. This allows to calculate a risk of each effect mechanism (Koelmans et al., 2022).

## 2.9 Ecotoxicological experiments and MDD concept

The transferability of laboratory experiments to the real world depends on various factors, such as the type of experiment and environment, in which it is conducted. External validity is a measure of how well the results of an experiment can be transferred to the real world. In laboratory experiments, various standardized single species tests are used to determine different effects on organisms at different trophic levels. In aquatic test systems, for example, at the level of the destruents the effect of a chemical on the bioluminescence of *Aliivibrio fischeri* is investigated, while a growth inhibition test for the green alga *Scenedesmus subspicatus* is carried out for primary producers (Fent, 2013). Among primary consumers, acute and chronic toxicity plays an important role for the two crustaceans *Daphnia magna* and *Daphnia pulex*. The same test parameters are important indicators for the primary, secondary and tertiary consumers, which are represented by different fish species such as the zebrafish (*Danio rerio*) or trout (*Oncorhynchus mykiss*) (Fent, 2013). However, in order to carry out an efficient risk assessment of potential pollutants, it is important to gather as much detailed information as possible about the behavior of a pollutant under realistic environmental conditions. The complex ecological aspects such as the impact on different populations of a biocoenosis, must be included into the assessment (Chapman, 2002). Especially substances that occur in different environmental compartments can have a negative impact on ecosystems (Pepper et al., 2014). There are multiple methods to increase the external validity of experiments, such as using samples that are representative of the target group or conducting experiments in more realistic environments, like outdoor mesocosms.

### ***Aquatic mesocosms***

Mesocosms are artificial ecosystems that are used to study the effects of environmental changes on biodiversity and ecosystem functions. These semi-natural systems are a compromise between the more unrealistic laboratory tests and the uncontrollable field studies. Aquatic mesocosm experiments in artificial pond systems were conducted for the first time conducted at the end of the 1960`s in order to answer fundamental ecological questions and thus gain new insights into the functions and structures of ecosystems (Hall et al., 1970; James and Boone, 2005). From this approach they developed into a holistic ecotoxicological research method that attempts to bridge the knowledge gap between laboratory experiments and real environmental conditions (Odum, 1984). As artificially created model ecosystems that can be used to assess the fate and effects of a substance on this test system mesocosms are used to assess appropriate endpoints for the different biological levels (Caquet et al., 2000). The different approaches of an aquatic model ecosystem differ primarily in the size of the respective experimental set-up. However, the aim, assessing realistic effects of chemicals on the environment, of the different models is identical. The results of laboratory experiments that were carried out in the first stage as part of a risk assessment are to be validated under realistic exposure scenarios and environmental conditions in combination with an ecosystem model that is as natural as possible (van Wijngaarden et al., 2005). Aquatic mesocosms are well suited to investigate the fate of chemicals in the environment (Van den Brink et al., 2005).

Due to the combination of the advantageous properties of the mesocosm as a model aquatic ecosystem, the stability of the system over a longer period and the ecological and environmental chemistry aspects, it can be concluded that mesocosm experiments can be used to draw more precise conclusions about the potential environmental risk of a test substance. In addition to this ecologically far more complex and realistic approach, mesocosms differ in terms of their water volume ( $>15 \text{ m}^3$ ) from microcosms ( $<15 \text{ m}^3$ ) (Hoffman, 2003). This experimental design is suitable for modeling the ecosystems of different types of water bodies in a near-natural way over longer periods of time. For example, standing water bodies can be modeled in large containers, while the ecosystem of flowing waters can be simulated with the help of river channels (e.g., Pérez et al., 2007; Beuter et al., 2019). Mesocosm systems offer a compromise between reproducibility and ecological aspects (Landner et al., 1989). They might also play a role in the approval of substances by serving as a model for the environment. Mesocosm experiments can help to study and assess the effects of substances on the environment. The results of these experiments can then be used by regulatory authorities such as the EFSA or the US Environmental Protection Agency (EPA) in decision finding processes on the authorization of substances. Mesocosm experiments can also help to study and assess the impact of environmental changes such as climate change or pollution on biodiversity and ecosystem functions (Sharma et al., 2021).

The transferability of laboratory experiments to the real world depends on various factors, such as the type of experiment and the environment in which it is conducted. There are various methods to increase the external validity of experiments, such as using samples that are representative of the target group or conducting experiments in realistic environments. External validity is a measure of how well the results of an experiment can be transferred to the real world. Since running waters are not expected to be a sink for plastic particles due to the current, this experiment used static mesocosms, which model dams and reservoirs where the particles can accumulate.

### ***Minimum detectable difference***

Unlike laboratory experiments, with certain criteria of validity and guidelines providing a framework for the experiments, mesocosm experiments are not bound by fixed criteria. Nevertheless, for regulatory acceptance, these higher tier studies must follow the concept of the Minimum Detectable Difference (MDD). The MDD gives an insight into the statistical power of a set experimental setup (Brock et al., 2015). The higher the MDD the higher the effects must be to show significant differences compared to the control. Each mesocosm experiment should have at least eight taxa that are potentially harmed by the stressor, in such high abundances that the MDD is below 100% (EFSA, 2006). This guarantees that no false negative decisions are made regarding the environmental risk. The MDD depends on sample size, number of replicates and variance. The variance again results from the natural variability of the replicates and the variability in sampling. The MDD is therefore a method for estimating statistical power retrospectively. These MDD values are also important for effect classification according to EFSA (EFSA, 2006). For some of the classes it is important to show recovery. It

does not matter whether the recovery is intrinsic, i.e., coming from the system (e.g., in daphnids or rotifers) or extrinsic, i.e., coming from other systems such as the control (e.g., in chironomids or baetids). Recovery can only be shown if the MDD criteria are met, i.e., the proportional MDD is below 100%. Recovery in this context means a disappearance of statistical significance between treatment and control. However, it is always important to consider whether the treatment values are approaching the control or whether the control is approaching the low treatment level due to ecological processes, such as a coordinated emergence event. Although there is also class 4B, which covers the case of decreasing control abundance and excessive MDD in the recovery period, all other classes (3A, 3B, 4A, 5A and 5B), where there are clear effects, require a low MDD after the effect period (Table 4).

## 2.10 Aim and research questions

The aim of this study is to investigate the risk posed by untreated spherical polyamide microplastic particles to aquatic biocenosis. For this purpose, three different concentrations and additionally a particle control with silicate particles were applied in a semi-field mesocosm study lasting over 114 days. Additional to the endpoints and evaluation methods that are conventionally used in mesocosm experiments, new approaches were used to investigate sublethal effects. The potential effects of the particles on individual taxa were analyzed as well as on the community and different trophic levels and developmental stages. The thesis describes the overall experimental set-up and evaluation methodology. The results are divided into four main chapters intended to answer the following research questions and hypotheses. In order to be able to assess the effects of the particles on the model ecosystems and to carry out a risk analysis, all compartments must be examined. The relevance of the topic described above gives rise to the following questions.

- A) *Does the presence of the added particles have an influence on the physicochemical composition of the model ecosystems and thus on the living conditions of the organisms?*
- B) *Is there a group in the aquatic invertebrate food web whose abundance is particularly influenced positively or negatively by the particles, i.e. which are the species of risk?*
- C) *Does a refined evaluation in higher tier studies, especially for stressors that are not typically tested in mesocosms, provide the opportunity to better assess effects and expand a risk analysis?*

Analysing the material properties and the biocenosis in the model ecosystems lead to three main hypotheses.

- 1) *As the particles have a higher density than water and are relatively small, they will sediment slowly. They can limit the light intensity available to the algae and higher aquatic plants for a short period of time.*
- 2) *Zooplankton organisms, especially filter feeders, will ingest the particles unselectively as they are similar in size to their preferred food. Once the particles have been ingested, they can have an effect on the small animal organisms in various ways.*
- 3) *Larger aquatic invertebrates, so-called macroinvertebrates, can ingest plastic particles directly as well as through their food and in some cases pass them on ontogenetically. This can cause effects on the organisms.*

As the characteristics of the habitat are equally relevant for all groups of organisms and have an effect on all of them, research question 1 is addressed in the results section before the 4 chapters. The four chapters of this dissertation address

***a. The influence of the particles on the primary producers***

By measuring the content of chlorophyll-a from different phytoplanktonic color classes and visually mapping the ground coverage of macrophytes, the effects of the particles on the primary producers were examined. Occasionally, the phytoplankton samples were sieved to measure the fraction with a similar size range as the particles. Dominance ratios of the color classes and a principal response curve were calculated. This chapter aims to answer research question B and hypothesis 1.

***b. The influence of the particles on the zooplankton***

Approximately 2% of the water volume were sieved and sampled in every mesocosm per sampling. The sampled organisms were taxonomically determined and the abundances per liter were statistically evaluated. In addition to the dominance ratios and diversity indices, a principal response curve was also calculated. This chapter aims to answer research question B and hypothesis 2.

***c. The influence of the particles on the larger invertebrates***

The macroinvertebrates were sampled using three different techniques, differentiated taxonomically and counted alive. The organisms were fixed after counting on three occasions to measure biovolume. In addition to the biovolume, dominance ratios, diversity indices and principal response curve was calculated. This chapter aims to answer research question B and C and hypothesis 3.

***d. The influence of the particles on adult, emerging insects***

The emerging insects were sampled and evaluated weekly. The organisms were taxonomically determined. Two genera were separated by their sexes additionally. In addition to the abundance data and the sex ratios dominance ratios, diversity indices and principal response curve was calculated. Furthermore, the emergence time was calculated for certain proportions of the total emergence. This chapter aims to answer research question B and C and hypothesis 1.

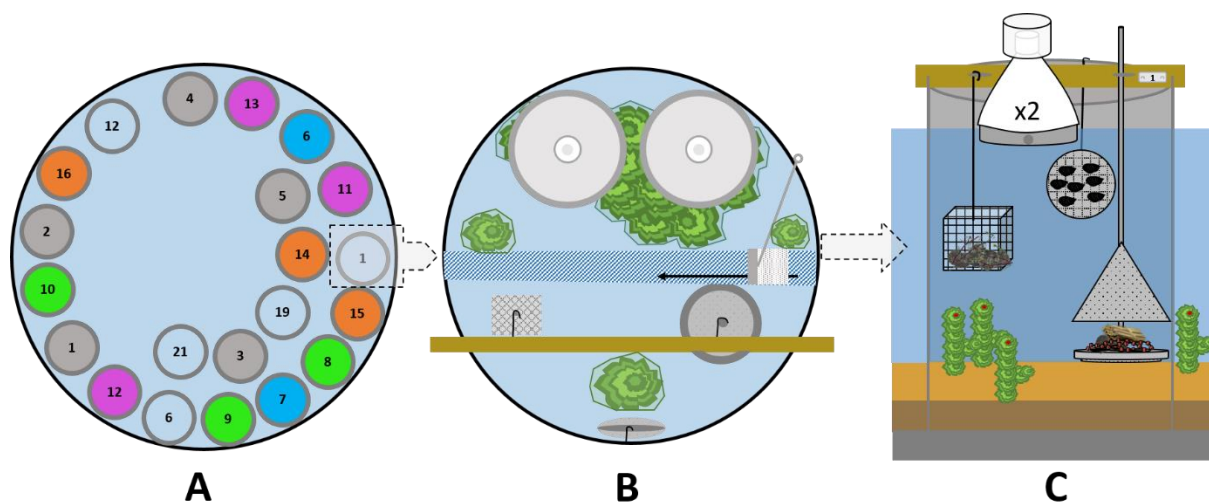
### 3. Experimental Methods

#### 3.1 Pond setup

An outdoor enclosure mesocosm study was conducted in an artificial pond at the test facility Neu-Ulrichstein (FNU; 50°45′06.1″N 9°02′02.8″E; Germany) starting in May 2020. The big outer pond (diameter 7.68 m) was freshly set up with sediment and water from a nearby natural pond in October 2018 (Appendix I). A 10 cm thick natural sediment layer was filled on an equally thick compacted layer of clay. Approximately 45.000 L natural pond water was filled in the big outer pond up to a water level of 1.20 m. Then, sand was uniformly added to the pond to lower the organic content of the sediment. Prior to the application of this study, a representative aliquot of pond water and a well-mixed subsample of sediment were sampled and analyzed for heavy metals (Mn, Pb, Cd, Cr, Ni, Hg, Zn), PCBs (6 components), pesticides (27 components) and selected chemical parameters (Na, K, S). Parameters were analyzed by Dr. Graner & Partner GmbH, Lochhausener Str. 205, D-81249 München, Germany (Appendix H). No microplastic could be identified after filtering 1000 l of pond water and sample preparation at UFZ Magdeburg in 2019 (Korinna Altmann, BAM, personal communication).

The climatic conditions at the outdoor facility site in Homberg (Ohm) correspond to Middle and Northern European conditions. During the in-life assessment phase, weather data (at least air temperature, solar radiation and precipitation) from the German Weather Service (DWD) weather station Neu-Ulrichstein (ID 10537) were monitored. The weather station is located about 800 m west of the test facility.

The macrophytes in the outer pond were harvested prior to the start of the experiment. Harvesting mobilizes the macrophyte-associated organisms and allows a more homogeneous distribution in the individual mesocosms. The macrophytes were planted specifically in the individual mesocosms before the start of pre-monitoring. Six weeks (April 2020) before the first application a series of 21 (of which 16 were actively used) of stainless-steel enclosures (diameter 1.10 m; approx. 1050 l) were installed by pressing them into the clay layer of the big pond. After pressing the enclosures into the clay layer 21 comparable, but unconnected mesocosm systems were formed. After an acclimatization phase of three days the macrophytes were harmonized and sampling equipment was placed in all mesocosms (Figure 5). The allocation of the mesocosms to the different concentration levels was done randomly, respecting abundances and physicochemical parameters, in the big outer pond, after a pre-sampling phase. This was done to avoid accidental effects that could be caused by solar radiation, for example.



**Figure 5** Top view of the big outer pond with 21 separate mesocosms (A). Mesocosms 1 - 5: control (grey); mesocosms 6 + 7: Silica (darkblue); mesocosms 8 – 10: LowPA (green); mesocosms 11 – 13: MedPA (violet); mesocosms 14 - 16: HighPA (red). Unused mesocosms: ID 1,6,12,18 and 21 filled colorless. Top view of a single mesocosm with traps and netting zone (B). Cross section of a mesocosm with trap techniques (C).

### 3.2 Test substances

#### 3.2.1 Polyamide

The polyamide particles (Goodfellow GmbH, Hamburg, Germany, item no. AM306010) were classified as fine white powder. The spherical particles had an average size of 15-20  $\mu\text{m}$ . The maximum particle size is 50  $\mu\text{m}$ , while the smallest particles in the batch have a size of 5  $\mu\text{m}$ . The density of the polymer is 1.13  $\text{g cm}^{-3}$ . Further physical, electrical, thermal, mechanical and chemical properties of the substance according to the manufacturer are listed in the appendix (Appendix A). The concentrations used in the study were 1.5  $\text{mg L}^{-1}$ , 15  $\text{mg L}^{-1}$  and 150  $\text{mg L}^{-1}$  with three replicates each.

#### 3.2.2 Silica

The silica particles (S3 Chemicals, Bad Oeynhausen, Germany, item no. S100252) used in the study is an amorphous oxide of silicon with the chemical formula  $\text{SiO}_2$ , which was also available in powder form. The powder is white and is odorless. All particles have a diameter of less than 45  $\mu\text{m}$ , with an average particle size between 11.5 and 15.5  $\mu\text{m}$ . The density of the  $\text{SiO}_2$  is 1.9  $\text{g cm}^{-3}$ . The melting point of the non-flammable silicon dioxide is 1700  $^\circ\text{C}$  and the boiling point is reached at 2300  $^\circ\text{C}$ . According to the manufacturer, the pH value of the product is 6. Further characteristics of the substance are listed in the appendix (Appendix A). The concentration used in the study was 150  $\text{mg L}^{-1}$ , comparable to the highest polyamide concentration. Two replicates were used in the study.

### 3.3 Application procedure

A range of concentrations of the test item (polyamide microparticles) and the particle control (silica microparticles) were applied to the mesocosms (Table 1). The pre-weighted test item was transferred in a suspension (63  $\mu\text{m}$  mesh sieved pondwater) for application. The test

item was dosed using the prepared suspensions and uniformly distributed below the water surface using a specially adapted separating funnel (the glass tip of the separating funnel is extended to 30 cm). The suspension was introduced while moving the glass tip of the opened funnel in a circular pattern approximately 15 to 25 cm below the water surface. After application of an individual mesocosm, the application equipment was rinsed three times with 500 ml sieved pond water each, which was added to the mesocosm as well. Each application process took three to five minutes (Appendix B).

**Table 1** Concentrations and applied amount per application in all mesocosms. The applied amount is based on a mean water volume in all mesocosms of 1050 L

Mesocosm	Treatment code	Amount applied per application [g]	Nominal concentration after all applications
Mesocosms 1-5	Control	0	0 mg L <sup>-1</sup>
Mesocosms 6+7	Silica	39.375	150 mg L <sup>-1</sup> Silica
Mesocosms 8-10	LowPA	0.394	1.5 mg L <sup>-1</sup> Polyamide
Mesocosms 11-13	MedPA	3.937	15 mg L <sup>-1</sup> Polyamide
Mesocosms 14-16	HighPA	39.375	150 mg L <sup>-1</sup> Polyamide

The application was repeated four times over a span of 10 days, with each quarter of the final particle burden.

### 3.4 Sampling methods and procedures

The day of the first application was designated as „day 0“ (start of experimental phase). For twelve weeks after the first application, samplings of different endpoints were performed regularly. The exact sampling scheme is shown in Table 2). An additional sampling of macroinvertebrates and physicochemical parameters was carried out 16 weeks post application. Up to two weeks after the application, the water level was  $\pm 10\%$  of the nominal depth on the first application day. Thereafter, the water level was  $\pm 20\%$  of the nominal depth on the first application day. The depth is documented in the appendix (Appendix D).

**Table 2** Sampling scheme

Day post 1. Appl.		Week post 1. Appl.	Zooplankton	Macro-invertebrates	Meiozooplankton	<i>Potamopyrgus</i> Assay	Insect emergence	Test item in water	Test item in sediment	Water level
$\geq -7$			x	x	x	intro	intro			x
$x/\div -1$			x	x	x			x	x	x
0	1.Appl.						(x)	x*	x*	x
2										

**Table 2** (continued) Sampling scheme

Day post 1. Appl.		Week post 1. Appl.	Zooplankton	Macro-invertebrates	Meiozooplankton	Potamopyrgus Assay	Insect emergence	Test item in water	Test item in sediment	Water level
3	2.Appl.							x*	x*	x*
6										
7	3.Appl.	1	x				(x)	x*	x*	x*
9										
10	4.Appl.							x*	x*	x
11								x	x	x
14	±2	2	x	x			(x)	x	x	x
21	±2	3	(x)	x			(x)			
28	±2	4	x	x	x	x	(x)	x	x	x
35	±4	5	(x)	x			(x)			
42	±4	6	x	x			(x)	(x)	(x)	x
49	±4	7	(x)				(x)			
56	±4	8	x	x	x	x	(x)	x	x	x
63	±4	9	(x)				(x)			
70	±4	10	x	x			(x)	(x)	(x)	x
77	±4	11	(x)				(x)			
84	±4	12	(x)	x	x	x	(x)	x	x	x
112	±4	16		x						
Day post 1. Appl.		Week post 1. Appl.	Phytoplankton (chl a)	Phytoplankton (cell counts)	Phytoplankton 30µm	Macrophytes (mapping)	Turbidity	Chemical parameters	Temp., DO, pH, conductivity	
≥ -7			x	(x)					x	
x/÷ -1			x	(x)	x	x	x	x	x	
0	1.Appl.						x*		x	
2			x	(x)			x			
3	2.Appl.						x*		x	
6			x	(x)			x			
7	3.Appl.	1					x*		x	
9			x	(x)			x			
10	4.Appl.						x*		x	
11							x			
14	±2	2	x	(x)		x	x		x	
21	±2	3	x	(x)			x		x	
28	±2	4	x	(x)	x		x		x	
35	±4	5	x	(x)					x	

**Table 2** (continued) Sampling scheme

Day post 1. Appl.		Week post 1. Appl..	Phytoplankton (chl a)	Phytoplankton (cell counts)	Phytoplankton 30µm	Macrophytes (mapping)	Turbidity	Chemical parameters	Temp., DO, pH, conductivity
42	±4	6	x	(x)			x	x	x
49	±4	7	x	(x)		x			x
56	±4	8	x	(x)	x		x		x
63	±4	9	x	(x)					x
70	±4	10	x	(x)					x
77	±4	11	x	(x)					x
84	±4	12	x	(x)	x	x	x	x	x
112	±4	16							
x*: sampling 120 min after application x: sampling performed (x): sampling performed, but no evaluation									

### 3.4.1 Water samples

Water samples (sieved 2 mm mesh) of each replicate of the treatments, silicate controls and one control mesocosm were taken 120 minutes after application and regularly during the study. The water samples were depth-integrated using a stainless-steel tube (minimum 80 cm water column and approximately 40 mm inner diameter). Per enclosure each six different sites were sampled and combined to form a composite sample.

Two 2 l aliquots of the water samples were taken per mesocosm in adequate brown glass bottles, one for analysis and one for backup. The samples were kept in darkness at  $\leq 5$  °C. Aluminum foil was inserted into the thread of the lid to prevent potential contamination of the water samples by particles of the plastic lid.

### 3.4.2 Sediment samples

Samples from all replicates of each treatment were taken as listed in Table 2. Samples from one alternating control mesocosm were taken at each sampling. Five sample cores per mesocosm were taken. The upper 5 cm horizons of the sediment core (diameter approx. 5 cm) were pooled. The sediment sample cores taken were collected in suitable glass bottles. All samples were stored deep-frozen at  $\leq -18$ °C until analysis.

### 3.4.3 Physicochemical parameters

Estimation of physical parameters included measurements of oxygen content [ $\text{mg L}^{-1}$ ] (FDO 925), conductivity [ $\mu\text{S cm}^{-1}$ ] (TetraCon925), pH (SenTix 940) and temperature [°C] (SenTix 940). All parameters were measured simultaneously using WTW probes and multiparameter measuring device (WTW GmbH, Weilheim, Germany, model 3630 IDS) approx. 50 cm beneath the surface in the middle of the mesocosms.

#### 3.4.4 Turbidity

The turbidity [Nephelometric Turbidity Unit, NTU] was measured photometrically using WTW pHotoFlex pocket photometer in three depths. Water was sampled 20 cm, 50 cm and 80 cm beneath the surface using a ball pipette. The turbidity was measured in a composite water sample after day of study 21.

#### 3.4.5 Chemical parameters

Chemical parameters were measured before, during and after the experiment. Ammonium, nitrate, phosphate and water hardness were measured in depth integrated water samples using WTW photolab Spektral (WTW GmbH, Weilheim, Germany) and test kits (Supelco spektroquant).

#### 3.4.6 DOC/TOC

Aliquots of 200 ml of a composite water sample were stored deep frozen until analysis. The analysis was done by CIP (Chemisches Institut Pforzheim GmbH, Schulberg 17, D-75175 Pforzheim). The results are listed in the appendix (Appendix J).

#### 3.4.7 Phytoplankton

Phytoplankton was sampled as a sieved (2 mm mesh, to remove bigger particles that potentially harm the spectrometer) aliquot of 350 ml of the depth integrated water sample. On occasion (Table 2), a finer (30  $\mu\text{m}$  mesh) sieved aliquot was taken additionally. The finer sieved samples were measured like the other samples. The samples were analyzed using a delayed-fluorescence spectrometer (Gerhardt and Bodemer 1998,2000). Each sample was adapted to dark light conditions for 15 minutes before measurement. The apparatus distinguishes between four chlorophyll color classes of primary producers (green algae, cyanobacteria, cryptophytes and diatoms). It measures the activity of photoactive pigments, meaning only alive algae are considered for evaluation.

#### 3.4.8 Macrophytes

Ground coverage of different macrophyte species was estimated visually. The percentage of coverage to total surface of the sediment was calculated using the software ImageJ (version 1.52a).

#### 3.4.9 Zooplankton

Zooplankton was sampled using a special acrylic glass tube with windows that can be closed from the outside and can sieve the entire water column through a 63  $\mu\text{m}$  mesh. Each trap was left in the respective mesocosm for at least five minutes to ensure equal distribution of the organisms after the interference with the system. The fixation with concentrated (>96%) ethanol was done stepwise to prevent the organisms from cramping during the mortification process.

### 3.4.10 Macroinvertebrates

Three different techniques were used to sample macroinvertebrates. All individuals were counted alive and reintroduced in the respective mesocosm afterwards, except days 36, 85 and 114 where all organisms were fixed for further evaluation such as biovolume.

#### *Netting*

To ensure representative sampling of e.g., Chaoboridae and Baetidae sweep netting (aperture 27 x 27 cm, mesh size 450 µm) of larvae in the water column was conducted in east-west direction and repeated three times (Figure 5,B). A water volume of approx. 155 l was sampled per sweep.

#### *Macroinvertebrate artificial substrate sampler (MASS)*

Open stainless steel systems (diameter of approx. 20 cm) containing clay beads (10-35 mm) and organic material were placed onto the sediment of each mesocosm (Figure 5,C). For better monitoring of isopods and amphipods the samplers contained decomposed leaves of *Alnus* sp. sampled near a pond on site. Additional leaves were introduced whenever needed during the study in all mesocosms equally.

#### *Macrophyte cage (Extra-MASS/EMASS)*

Stainless-steel baskets containing shoots of macrophytes (*Myriophyllum spicatum*, *Ceratophyllum demersum*) were fixed at medium water depth (Figure 5,C). During the sampling, a net was held under the basket to catch escaping organisms as well.

### 3.4.11 Emerging insects

Two stainless-steel constructions (Figure 5,B&C) covered with a conical tent were used to catch emerging insects. The individuals were led to an ecdector head box, at the apex of the trap, filled with a fixative containing water and a surfactant (e.g., TWEEN). The individuals in both traps were sampled, pooled and fixed (70% ethanol) in a seven-day interval. Both traps combined covered approx. 15% of the surface of each mesocosm. The taxonomic resolution depended on the condition of captured individuals.

### 3.4.12 *Potamopyrgus antipodarum* bio-assay

Each 40 New Zealand mud snails were exposed in baskets (100 µm mesh) 50 cm beneath the water surface of every mesocosm. The mud snails were sampled thrice on days 28, 56 and 85. Per sampling 10 individuals were sampled, narcotized in a 2.5% MgCl<sub>2</sub> solution and frozen. The individuals were measured, and the brood pouches were opened to count the amount of embryos per snail. The sampling was carried out according to OECD 242 to identify potential endocrine effects of the stressor over the course of the study.

### 3.5 Additional evaluations

#### 3.5.1 Sedimentation time and sinking velocity of particles

The sinking velocity for the particles was calculated following Stokes law (Formula 1) for spherical particles with small Reynold numbers. The calculation was done for silica and polyamide particles ranging from 2 to 55  $\mu\text{m}$  in diameter in increments of 0.01  $\mu\text{m}$ .

**Formula 1** Calculation of sinking velocity following Stokes law:  $v = \frac{2}{9} * \frac{r^2 * g * (p_p - p_f)}{n}$

with the gravitational force (g), the density of the fluid (pf), the density of the respective particle type (pp) and the dynamic viscosity of the water (n).

#### 3.5.2 Biovolume

Biovolume was calculated by approximating a geometric shape that resembles the body of the organism and multiplying length, height and width in mm of the respective organisms. Body appendages such as cerci or antennae were disregarded. Examples of the most important body groups are listed in Table 3. The biovolume of larvae was transformed with the natural logarithm. Transformed biovolume values below 1  $\text{mm}^3$  have negative signs.

**Table 3** Measurement and calculation of biovolume for different taxonomic groups

	Taxa	Approximated geometric shape	Length	Width	Height
Arthropoda (larvae and adults)	Ephemeroptera, Odonata,	Cylinder	Head base to thoracic end	Thoracic width	Thoracic height
	Coleoptera, Isopoda, Hemiptera	Rectangle			
Mollusca	Lymnaeidae	Cone	Shell opening to shell tip (opening facing upwards)	Shell edge to opposing shell edge	Shell opening to shell tip (opening facing downwards)
	Planorbidae	Cylinder	Longside, including downward facing shell opening	Shell straightened (Opening facing forward)	Left side, including downward facing shell opening

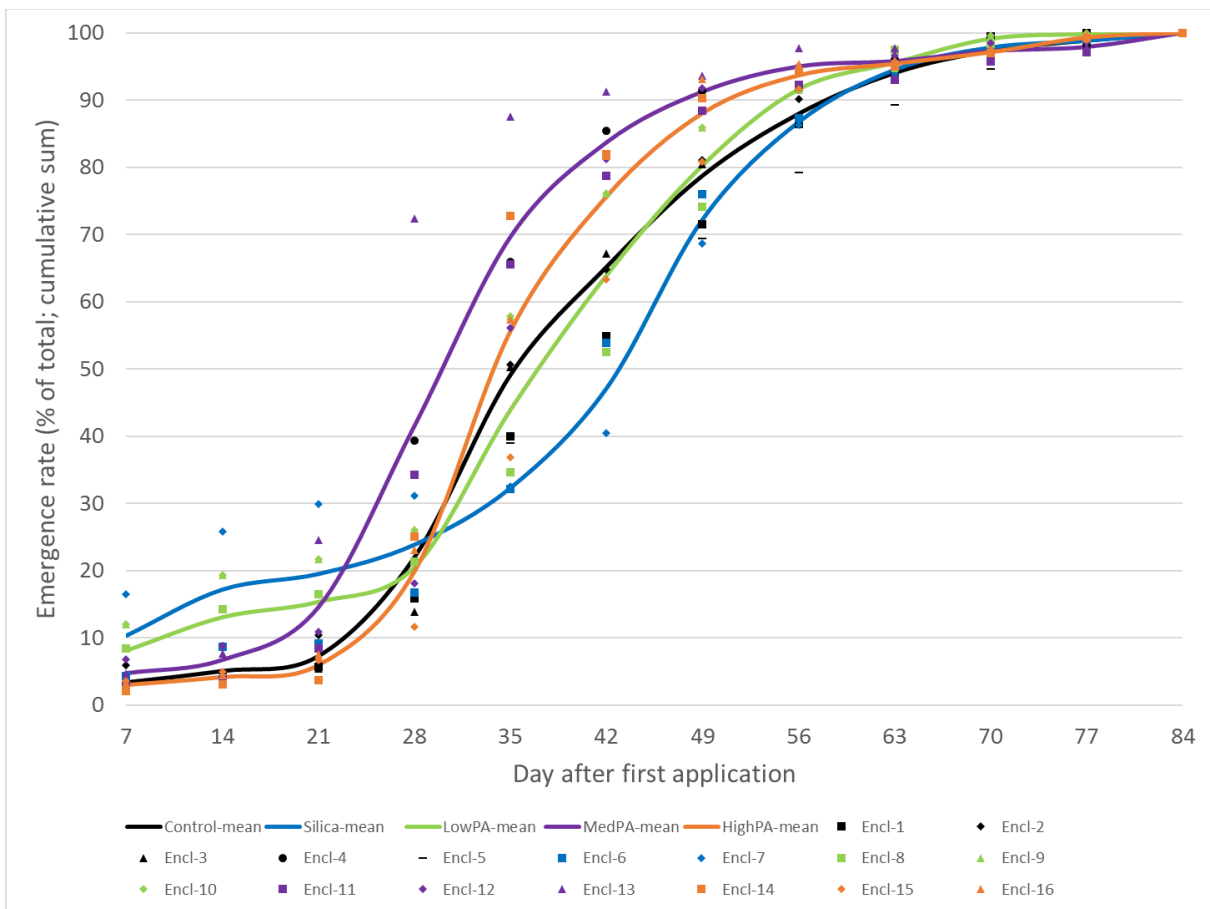
#### 3.5.3 Sexratio

Sexratio was determined for adult Chaoboridae and Baetidae. The characteristics of the male individuals of the chaoborids are the feathered antennae and the distinct gonostyli at the end of the abdomen. The male baetids are characterized by their segmented eyes.

The upper, dorsal half of the eyes, the so-called turbinate eye has a yellowish orange color in the fixed samples. Individuals, for which the sex could not be determined reliably were disregarded for this analysis.

### 3.5.4 Emergence mean Time

Emergence mean Time (EmT) was calculated for a proportion of 20% (EmT<sub>20</sub>), 50% (EmT<sub>50</sub>) and 80% (EmT<sub>80</sub>) of the most dominant taxa. To calculate the EmT, the total abundance of each taxon must be calculated for each mesocosm. Afterwards, using the total duration of the study the timepoints at which a certain proportion of the organisms emerge can be computed using a fit (linear model, R function: `lm()`). EmT was calculated for each mesocosm individually and means per treatment were calculated. By setting the EmT<sub>x</sub> of the controls as expected value the divergences were calculated. Figure 6 shows an exemplary EmT curve, which was created as the basis for all further calculations. After calculating the EmT-values per treatment they were put in relation to control values to show the treatment-related change in emergence time ( $EmT_{xTreatment}/EmT_{xControl}$ ). Values < 1 indicate a strong toxic effect without recovery, a slowly developing toxic effects or premature emergence respectively. Values > 1 indicate delayed emergence or a toxic effect with recovery. Values of 2 and 0.5 indicate the same effect strength, due to mathematical reasons



**Figure 6** Example for cumulative emergence calculation. Each mesocosm has a distinct progression. Means per treatment were calculated after fitting each mesocosm separately

### 3.5.5 Dominance ratio

The dominance ratio is the relative frequency of a species in relation to the other species on a sampling day in a certain mesocosm. The different dominance ratios can be classified as eudominant (> 10%), dominant (> 5-10%), subdominant (> 2-5%), recedent (> 1-2%) and subrecedent (< 1%) (Lira and DeSouza, 2016) Dominance ratios were calculated using the software CA (Community Analysis version 4.3.14) as a percentage of the abundance of a taxon in relation to the total abundance. Deviations of 100% total dominance are possible, due to rounding errors. The lowest taxonomical level of all taxa was used for this analysis. All sums (e.g., total sums, sum of all Diptera) were disregarded in this analysis.

### 3.5.6 Diversity indices

Diversity indices were calculated using the software CA (Community Analysis version 4.3.14). The number of taxa was calculated by counting the number of different taxa found per mesocosm and sampling day. Shannon's H was calculated with following formula (Formula 2) with  $p_i$  as the proportion of the respective species to the total abundance.

**Formula 2** Shannon H:  $H = \sum_i p_i * \ln(p_i)$

The Evenness was calculated as normalized Shannon H according to Formula 3, with  $\ln(S)$  as the maximum Shannon.

**Formula 3** Calculation of Evenness:  $E = H / \ln(S)$

The lowest taxonomical level of all taxa was used for these analyses. All sums (e.g., total sums, sum of all Diptera) were disregarded in these analyses.

## 3.6 Statistical analysis

The statistically significant effects compared to the control give an indication of effects on individual samplings. To group the effects over the entire course of the study, the effect data is categorized according to EFSA (2013) and Brock et al. (2015) (Table 4). Since the study had more than one application and lasted over 100 days all categories can be applied. The effect classes range from no effect (1) to pronounced effects without recovery (5B).

**Table 4** Effect classification based on EFSA (2013) and Brock et al. (2015)

Effect class	Description	Comment
1	No treatment-related effects demonstrated	No (statistically and/or ecologically significant) effects observed as a result of the treatment. Observed differences between treatment and controls show no clear causal relationship.
2	Slight effects	Effects concern short-term and quantitatively restricted responses usually observed at individual samplings only.

**Table 4** (continued): Effect classification based on EFSA (2013) and Brock et al. (2015)

Effect class	Description	Comment
<b>3A</b>	Pronounced short-term effects (< 8 weeks, followed by recovery)	Clear response of endpoint, but full recovery of affected endpoint within 8 weeks after the first application or, in the case of delayed responses, and repeated applications, the duration of the effect period is less than 8 weeks and followed by full recovery. Treatment-related effects demonstrated on consecutive samplings.*
<b>3B**</b>	Pronounced effects and recovery within 8 weeks post last application	Clear response of the endpoint in micro-/mesocosm experiment repeatedly treated with the test substance and that lasts longer than eight weeks (responses already start in treatment period), but full recovery of affected endpoint within eight weeks post last application.*
<b>4A</b>	Pronounced effects in short-term study	Clear effects (e.g., large reductions in densities of the population) observed, but the study is too short to demonstrate complete recovery within eight weeks after the (last) application.
<b>4B</b>	Pronounced short-term effects, but MDD too high in recovery period	Significant short-term effects demonstrated but recovery cannot be properly evaluated due to high%MDD values in recovery period or the population in the controls is declining or even absent. If significant treatment related response is demonstrated on one sampling but recovery cannot be interpreted due to high%MDD, this may be indicated as class 2 – 4B, in other case it can be 3A – 4B.
<b>5A</b>	Pronounced long-term effect followed by recovery	Clear response of sensitive endpoint, effect period longer than 8 weeks and recovery did not yet occur within 8 weeks after the last application but full recovery is demonstrated to occur in the year of application.*
<b>5B</b>	Pronounced long-term effects without recovery	Clear response of sensitive endpoints (> 8 weeks post last application) and full recovery cannot be demonstrated before termination of the experiment or before the start of the winter period.

\* Note that recovery can only be considered, if the MDDs during the recovery period were at least smaller than 100%. If this is not the case, an appropriate higher class may have to be selected.

\*\* only relevant for multiple application studies

### 3.6.1 Abundance and measurement data

A significance level of  $\alpha < 0.05$  was used for all statistical comparisons. Statistical differences between the control and all treatments were calculated using Dunnett's post hoc test. The abundance data were transformed according to (van den Brink et al., 2000). Geometric means were used to minimize the influence of outliers. Description of the graphs and data was done by using geometric means as well. In order to specify the accuracy of the geometric mean values, the geometric standard deviation is also specified as a measure of dispersion. To calculate geometric means, zero-values were replaced depending on the endpoint. For phytoplankton data the zeros were replaced with 0.001, for zooplankton with 0.01 for macroinvertebrates and for emerging insects with 0.1. The calculations were made using R (Version 4.1.1). Since data was transformed with  $\ln(Ax+1)$  normal distributed. The No Observed Effect Concentration (NOEC) and the Lowest Observed Effect Concentration (LOEC) are commonly used criteria to evaluate the toxic potential of a substance on model species. The NOEC is defined as the highest tested concentration which showed no significant differences to the control group, while the LOEC is the lowest tested concentration that already had significant influence on the endpoint. The statistical analysis used to determine the NOEC and LOEC is listed in the respective chapter.

A principal response curve (PRC) was calculated using the program CANOCO 5 (Version 5.12). The lowest taxonomical level of all taxa was used for this analysis. All sums (e.g., total sums, sum of all Diptera) were disregarded in this analysis. The y-axis shows the community level response relative to the controls. The higher the absolute value of deviation, the stronger the effect. The x-axis depicts the temporal deviation. The species score for each species shows the impact of said species on the total deviations. The higher the absolute value of the species score the more sensitive this species is. A threshold value of an absolute value of 0.5 was applied. A redundancy analysis (RDA) was performed whenever the PRC was significant. CANOCO 5 (Version 5.12) was used to calculate the RDA. All sums (e.g., total sums, sum of all Diptera) were disregarded in this analysis. RDA was performed for every sampling day separately to see how the treatments affect individual time points and whether there are certain key time points at which the differences are particularly strong.

### 3.6.2 Minimum Detectable Difference

The minimum detectable difference (MDD) is an indicator of the statistical power of an endpoint in a given study design. The MDD is represented as the percentage change from control that a treated group must show to demonstrate statistically significant differences from control. For all statistical analyses of abundance data, the MDD was used with the standard deviation as a measure of dispersion. MDD was calculated according to Brock et al. (2015). The MDD% of retransformed data ( $MDD\%_{abu}$ ) is used in this study as "MDD". Values below 100% are considered as "MDD capable". The statistical power of a higher tier experiment can be expressed by the MDD Category. For every taxon, if the MDD is at least either under 100% four times, under 90% three times, under 70% twice or under 50% once the MDD Category is 1 (direct exposition, potential effects). If none of these criteria fit, but

there are any statistically significant effects compared to the control after the first application the MDD Category is 2 (indirect exposition). If neither of these are true, the MDD Category is 3 (effects possible but high uncertainty).

### 3.6.3 Diversity indices

Statistical differences of the amount of species, the Shannon index and the evenness were calculated exactly like abundance data (3.6.1)

### 3.6.4 Reproduction (*Potamopyrgus* assay)

Statistical analysis of the reproduction of *P. antipodarum* in bio-assays was performed according to Lehmann et al., 2018. The R-Script provided by the authors was adjusted to perform under newer version of (Version 4.1.1). The closure Principal computational approach test (CPCAT) is a method to overcome limitations of generally used methods in ecotoxicology to find NOECs and LOECs especially for reproduction data. Using matrices of main and intersection hypothesis and Monte-Carlo simulations a probability of error of accepting the null hypothesis is computed. Only if all intersection hypothesis is a matrix are denied the overall hypothesis of the matrix can be denied as well. The maximal p-value of all subtests of a matrix has to be  $< 0.5$  for the difference to be significant. Due to the structure of the statistical method with Monte-Carlo simulations, the exact same maximal p-value is not determined for each run. Mean values and standard deviations of the mean were used and described.

### 3.6.5 Biovolume

The mean biovolume per mesocosm and taxon was analyzed for statistically significant changes compared to the control using a one-factorial ANOVA and a Dunnett's post-hoc test. Statistical differences of biovolume were calculated exactly like abundance data (3.6.1)

### 3.6.6 Sex ratio

Statistical differences in were determined by setting the control ratio as expected value for each point in time. A one-proportion z-test was performed. The base R function "prop.test" was used, with p as expected value, x as measured value and n as number of observations. Values with number of observations below  $n_{\text{total}} = 30$  are to be rated as uncertain. The sex ratio was calculated as  $n_{\text{males}}/n_{\text{total}}$  with not distinguishable organisms disregarded.

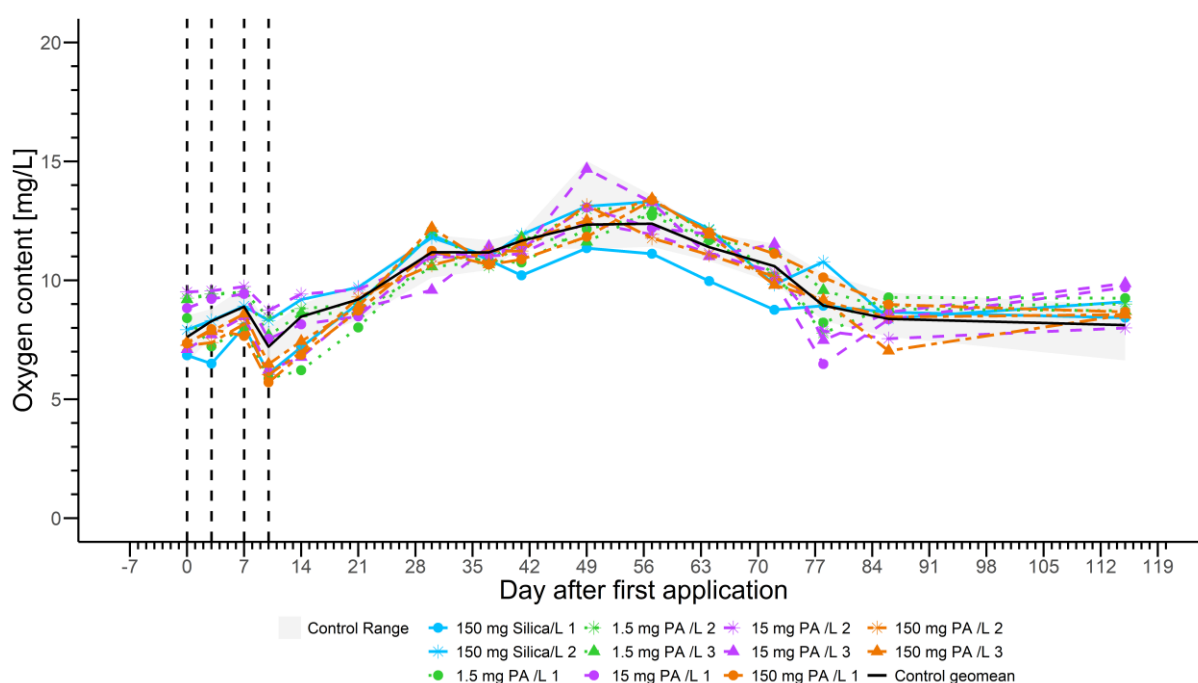
### 3.6.7 Emergence mean Time

Statistical differences of Emergence mean Time were calculated exactly like abundance data (3.6.1).



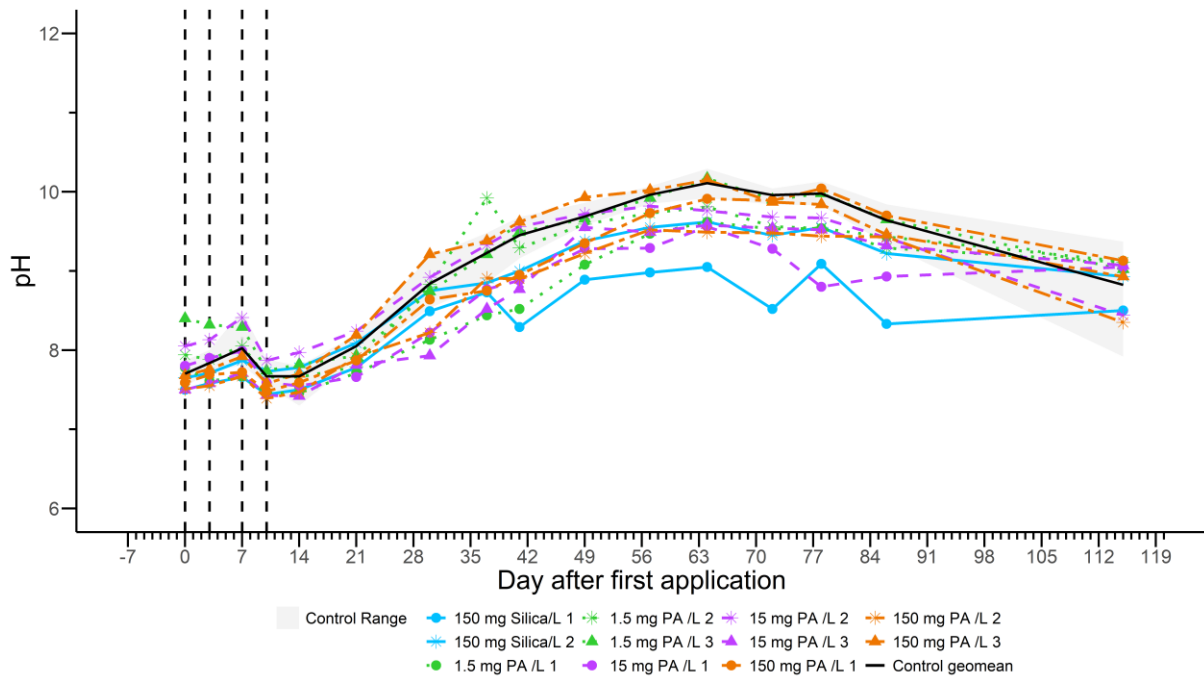
## 4. Results

The non biotic factors, such as physical and chemical parameters had an influence on and were influenced by the biological endpoints. As sum parameters, they often provide indirect information about biological dynamics. Oxygen content (Figure 7) had its peak between days 42 and 56 of the study in all mesocosms except one silica replicate where the peak occurred earlier on day 28. All mesocosms showed a comparable progression of the oxygen content throughout the study. After a slight dip before the last application on day 10 the oxygen content increased continuously to the peak of 12-15 mg L<sup>-1</sup>. After the peak the oxygen content decreased again to values comparable to the starting conditions of around 8-10 mg L<sup>-1</sup>.



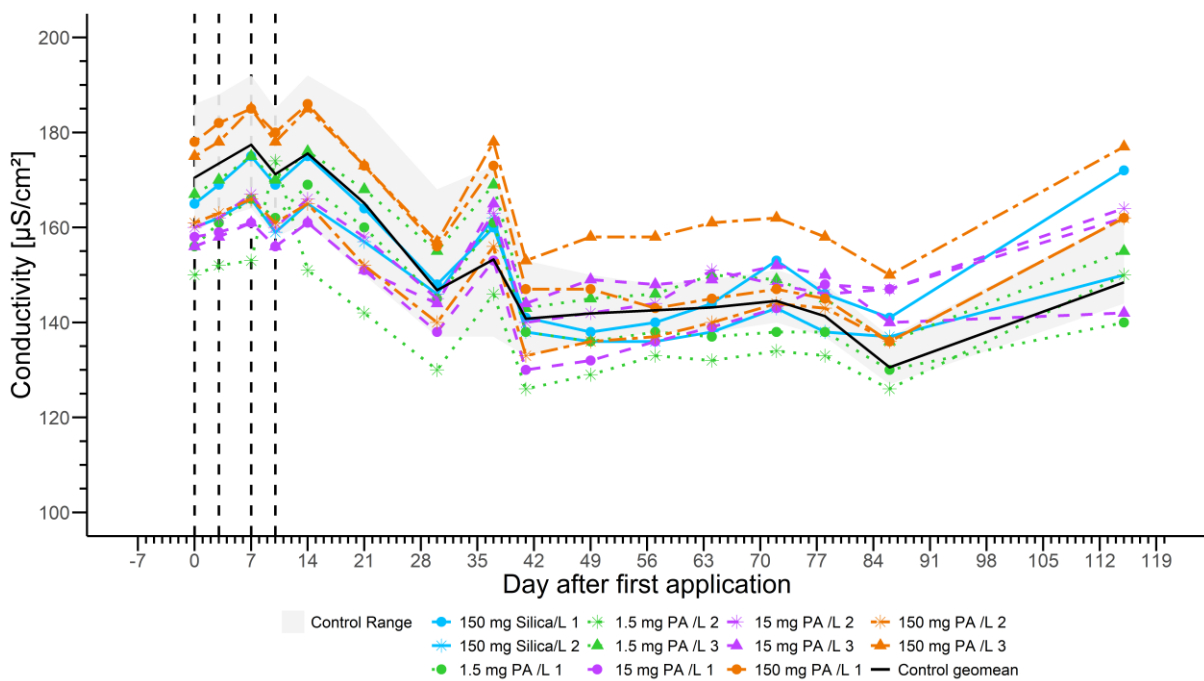
**Figure 7** Oxygen content [mg L<sup>-1</sup>] in all mesocosms

Like the oxygen content the pH (Figure 8) peaked at the halfway mark of the main study. Lowest pH values of 7.4 were measured at the day of the fourth application. Throughout the study pH was in alkaline in all mesocosms. In the Silica treatment between days 57 and 86 pH values were significantly lower in the compared to the control. The MedPA treatment had a significantly lower pH on day 78 of the study.



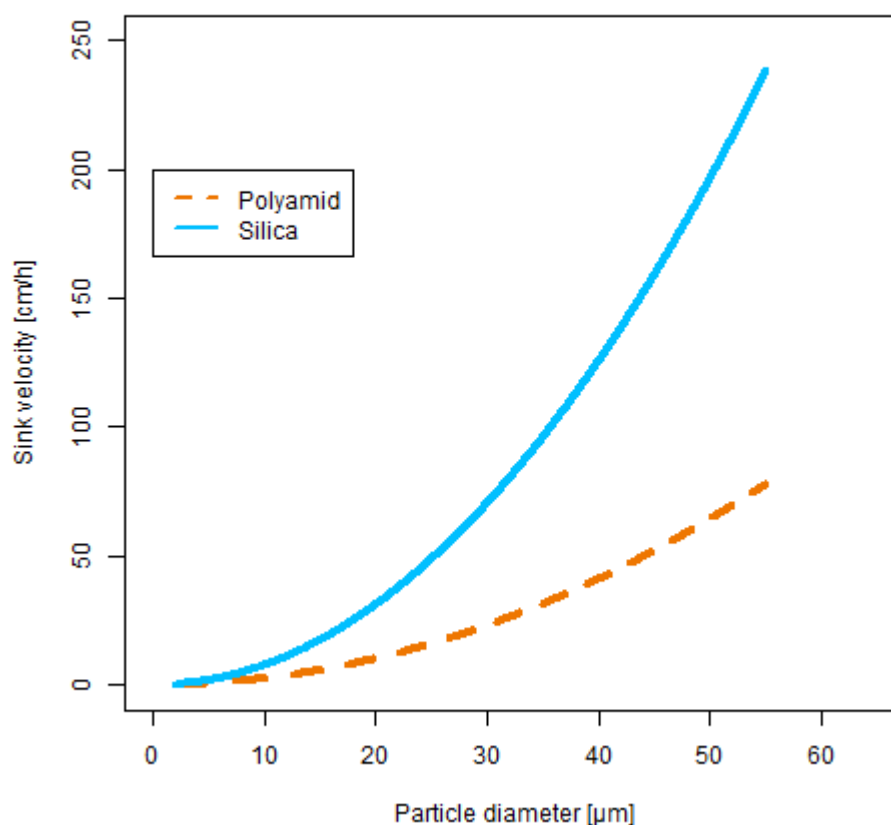
**Figure 8** pH in all mesocosms

Unlike pH and oxygen content the conductivity (Figure 9) decreased after the application procedure. The MedPA treatment had a significantly higher conductivity on day 86 of the study. No other differences were significant compared to the control throughout the study.



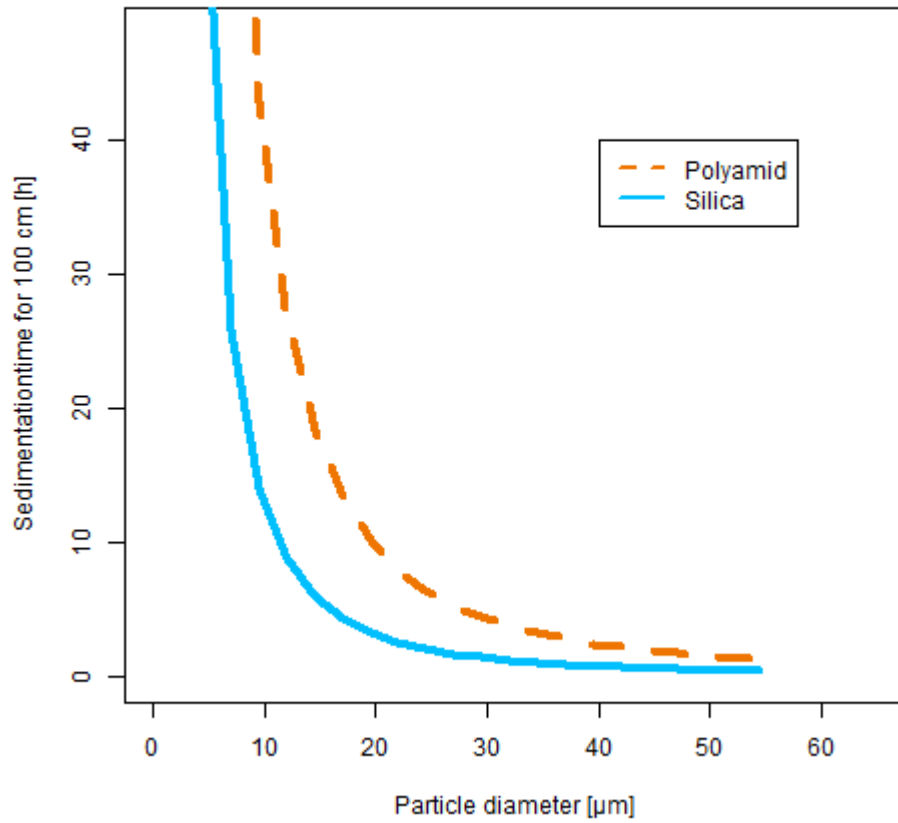
**Figure 9** Conductivity [ $\mu\text{S cm}^{-1}$ ] in all mesocosms

Chemical parameters and temperature showed no significant differences compared to the controls during the study. Most chemical parameters were below detection limits. The water temperature showed a comparable progression throughout the study in all treatments. The mesocosms differed by less than 0.3 °C on each respective sampling.



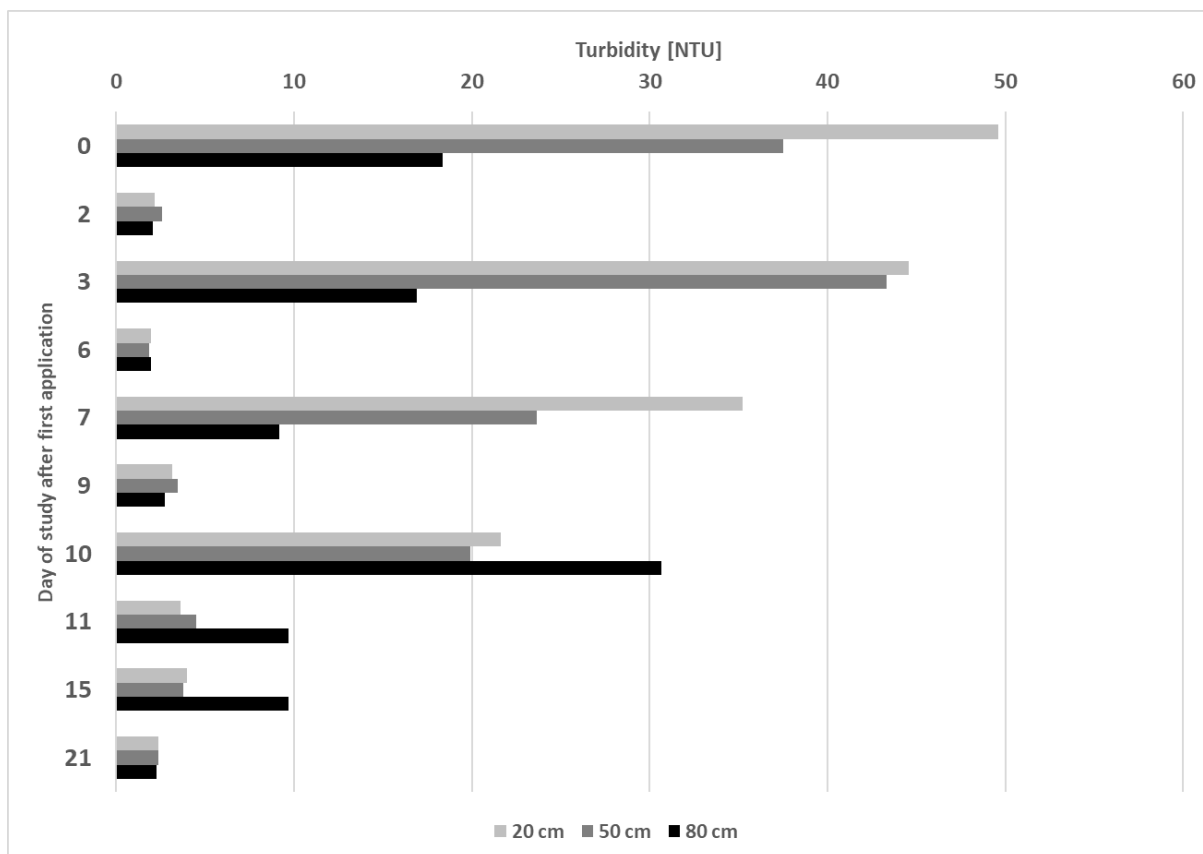
**Figure 10** Sink velocity [ $\text{cm h}^{-1}$ ] of polyamide and silica particles depending on size

The calculated sink velocity (Figure 10) and sedimentation time for 100 cm (Figure 11) for silica and microplastic particles show a size dependency. The bigger the spherical particles are the faster they sink. The calculated sink velocity is  $0.65 \text{ cm h}^{-1}$  for the smallest microplastic particles, while the biggest particles sink with a rate of  $64.6 \text{ cm h}^{-1}$ . There is no data on the smallest size of the silica particles, the biggest particles however sink  $159.6 \text{ cm h}^{-1}$  theoretically. The sedimentation time for 100 cm for the average microplastic particles is 9.7 to 17.2 hours. The average silica particles need 5.3 to 9.6 hours to sediment 100 cm.



**Figure 11** Sedimentation time for 100 cm [h] of polyamide and silica particles depending on size

The turbidity measurement (Figure 12) shows the sedimentation of the particles in the highest concentration in three depths, 20 cm, 50 cm and 80 cm below the water surface. After the first three applications the turbidity was highest near the surface. After the last application, however, the highest turbidity was measured 80 cm below the surface. The turbidity was on control level two and three days after application respectively. After the last application on study day ten the turbidity was higher compared to the control in the deeper water layer. On day 21 of the study and after the turbidity was at control level below 5 NTU.



**Figure 12** Mean turbidity [NTU] in 20 cm, 50 cm and 80 cm below water surface in HighPA treatment

In this study a total of 67 taxa were differentiated taxonomically (Table 5). They belong to two kingdoms, six tribes and 14 classes. Furthermore, 23 orders and 43 families were distinguished. Of the total of 45 orders found, 17 species could be assigned. Most of the organisms could be identified at least to genus level. Some, however, could only be identified to class, order or family level. The phytoplankton was not classified taxonomically but according to color classes. All these taxa were analyzed in at least one of the five biological endpoints: phytoplankton, macrophytes, zooplankton, macroinvertebrates and emerging insects. Some taxa such as *Chaoborus* sp. or Tanypodinae were considered in several endpoints. Most taxa and the most accurate resolution were assigned to the zooplankton endpoint.

**Table 5** Taxonomic classification of all groups and taxa found in the study. The smallest level distinguished in the study is marked in bold

Kingdom	Phylum	Class	Order	Family	Genus	Species	
Animalia	Annelida	Clitellata	Rhynchobdellida	Glossiphoniidae	<i>Helobdella</i>	<i>stagnalis</i>	
	Arthropoda	<b>Arachnida</b>	Trombidiformes				
		Branchipoda	Anomopoda	Chydoridae	<i>Alona</i>		
					<i>Alonella</i>		
					<i>Chydorus</i>	<i>sphaericus</i>	
					<i>Graptoleberis</i>		
				Daphniidae	<i>Ceriodaphnia</i>		
					<i>Daphnia</i>	<i>longispina</i>	
					<i>Daphnia</i>	<i>magna</i>	
					<i>Daphnia</i>	<i>pulex</i>	
					<i>Scapoleberis</i>		
					<i>Simocephalus</i>		
		Copepoda	Calanoida	<b>Diaptomidae</b>			
			Cyclopida	<b>Cyclopidae</b>			
		Crustacea	Isopoda	Asellidae	<i>Asellus</i>	<i>aquaticus</i>	
		Insecta	Coleoptera	<b>Curculionidae</b>			
				<b>Dytiscidae</b>	<i>Dytiscus</i>		
					<i>Hydoporus</i>		
			Hydrophilidae	<i>Helophorus</i>			
			Diptera	<b>Ceratopogonidae</b>			
				Chaoboridae	<i>Chaoborus</i>		
	<b>Chironomidae</b> (subfamilies: <b>Chironominae</b> , <b>Orthoclaadiinae</b> , <b>Podonominae</b> , <b>Tanypodinae</b> )			<i>Chironomus</i>	<i>riparius</i>		
	<b>Culicidae</b>			<i>Anopheles</i>			
				<i>Culex</i>			
	<b>Psychodidae</b>						

**Table 5** (continued) Taxonomic classification of all groups and taxa found in the study. The smallest level distinguished in the study is marked in bold

Kingdom	Phylum	Class	Order	Suborder	Family	Genus	Species			
Animalia	Insecta	Diptera	Hemiptera		<b>Corixidae</b>					
					Gerridae	<b>Gerris</b>				
					Notonectidae	<b>Notonecta</b>	<b>glauca</b>			
					Hymenoptera			<b>Mymaridae</b>		
			Odonata	<b>Zygoptera</b>	<b>Coenagrionidae</b>					
				<b>Anisoptera</b>						
			<b>Trichoptera</b>		Phryganidae					
	<b>Ostracoda</b>									
	Mollusca	Bivalvia	Sphaeriida		Sphaeriidae	<b>Musculium</b>	<b>lacustre</b>			
		Gastropoda	Pulmonata		<b>Lymnaeidae</b>	<b>Lymnaea</b>	<b>stagnalis</b>			
						<b>Radix</b>				
			Sorbeoconcha		Tateidae	<b>Potamopyrgus</b>	<b>antipodarum</b>			
	Rotifera	Bdelloidea	Bdelloida		Habrotrochidae	<b>Habrotrocha</b>				
					Philodinidae	<b>Rotaria</b>				
		Monogononta	Flosculariaceae		Hexarthridae	<b>Hexarthra</b>				
					Testudinellidae	<b>Testudinella</b>				
					Trochosphaeridae	<b>Filinia</b>				
					Asplanchnidae	<b>Asplanchna</b>				
			Ploima			Brachionidae	<b>Brachionus</b>			
							<b>Keratella</b>	<b>quadrata</b>		
						<b>Platylabus</b>	<b>quadricornis</b>			
						Euchlanidae	<b>Euchlanis</b>			
	Lecanidae	<b>Lecane</b>								
Mytilinidae	<b>Mytilinia</b>									

**Table 5** (continued) Taxonomic classification of all groups and taxa found in the study. The smallest level distinguished in the study is marked in bold

Kingdom	Phylum	Class	Order	Family	Genus	Species	Taxon
Animalia	Rotifera	Monogononta	Ploima	Notommatidae	<i>Cephalodella</i>		
				Synchaetidae	<i>Polyarhtra</i>		
					<i>Synchaeta</i>		
				Trichoceridae	<i>Trichocerca</i>		
				Trichotriidae	<i>Trichotria</i>		
Plantae	Tracheophyta	Angiosperma	Alismatales	Potamogetonaceae	<i>Potamogeton</i>	<i>perfoliatus</i>	
			Saxifragales	Haloragceae	<i>Myriophyllum</i>	<i>spicatum</i>	
		Magnoliopsida	Ceratophyllales	Ceratophyllaceae	<i>Ceratophyllum</i>	<i>demersum</i>	
							<b>Diatoms</b>
							<b>Green algae</b>
							<b>Kryptomonads</b>

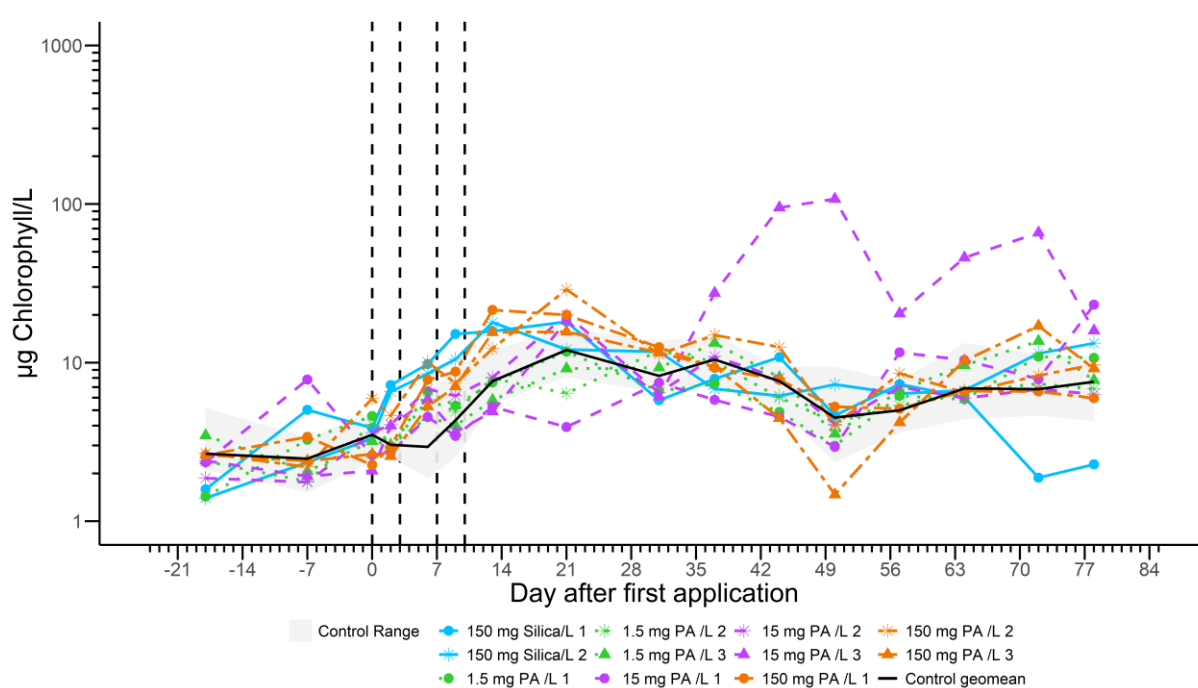
## 4.1 Primary producers



(Picture generated with OpenAI DALL·E)

#### 4.1.1 Phytoplankton chlorophyll content

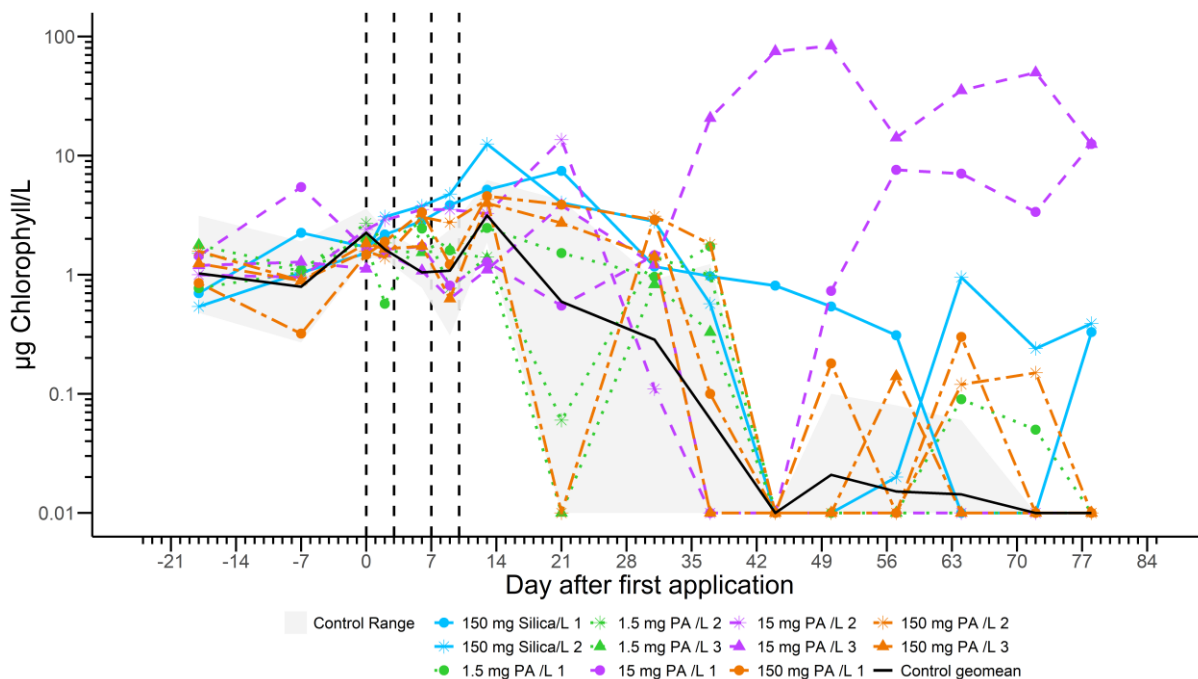
The mean of total chlorophyll (Figure 13) content ranged from  $2.48 \times/\div 1.36 \mu\text{g L}^{-1}$  to  $12.01 \times/\div 1.34 \mu\text{g L}^{-1}$  on day 21 in the control. The mean of total chlorophyll content ranged from  $1.49 \times/\div 1.09 \mu\text{g L}^{-1}$  on day -18 to  $16.82 \times/\div 1.10 \mu\text{g L}^{-1}$  on day 13 in the Silica treatment. The mean of total chlorophyll content ranged from  $2.28 \times/\div 1.56 \mu\text{g L}^{-1}$  on day -18 to  $10.88 \times/\div 1.13 \mu\text{g L}^{-1}$  on day 86 in the LowPA treatment. The mean of total chlorophyll content ranged from  $2.20 \times/\div 1.15 \mu\text{g L}^{-1}$  on day -18 to  $20.89 \times/\div 2.31 \mu\text{g L}^{-1}$  on day 86 in the MedPA treatment with one mesocosm that has a noticeably increased chlorophyll content. The mean of total chlorophyll content ranged from  $2.62 \times/\div 1.26 \mu\text{g L}^{-1}$  on day -7 to  $20.87 \times/\div 1.36 \mu\text{g L}^{-1}$  on day 21 in the HighPA treatment.



**Figure 13** Total chlorophyll content [ $\mu\text{g L}^{-1}$ ] measured with delayed fluorescence method

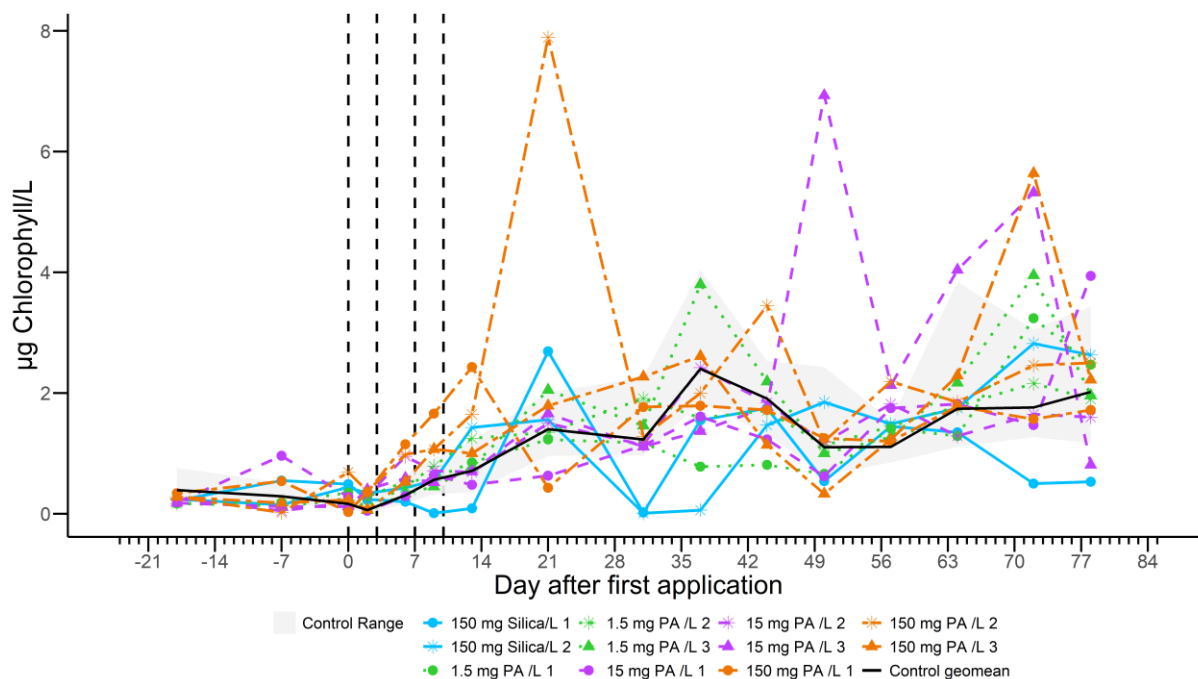
All mesocosms showed an increase in chlorophyll content in the first weeks of the experiment and a drop afterwards. The Silica treatment showed significant differences compared to the control on days 2 through 13 of the study. The MedPA and HighPA treatments showed two samplings with significant effects on nonconsecutive samplings each. The significant differences to the controls in the HighPA treatment were on days 6 and 13, a comparable time span as the Silica treatment. The LowPA treatment showed no significant changes to the control.

The mean of chlorophyll content from green algae (Figure 14) ranged from  $0 \mu\text{g L}^{-1}$  on day 44 to  $3.15 \times/\div 1.78 \mu\text{g L}^{-1}$  on day 13 in the control. The mean of chlorophyll content from green algae ranged from  $0.09 \times/\div 22.36 \mu\text{g L}^{-1}$  on day 44 to  $8.04 \times/\div 1.87 \mu\text{g L}^{-1}$  on day 13 in the Silica treatment. The mean of chlorophyll content from green algae ranged from  $0 \mu\text{g L}^{-1}$  on several days (44,50,57,78,56) to  $2.20 \times/\div 1.20 \mu\text{g L}^{-1}$  on day 0 in the LowPA treatment.



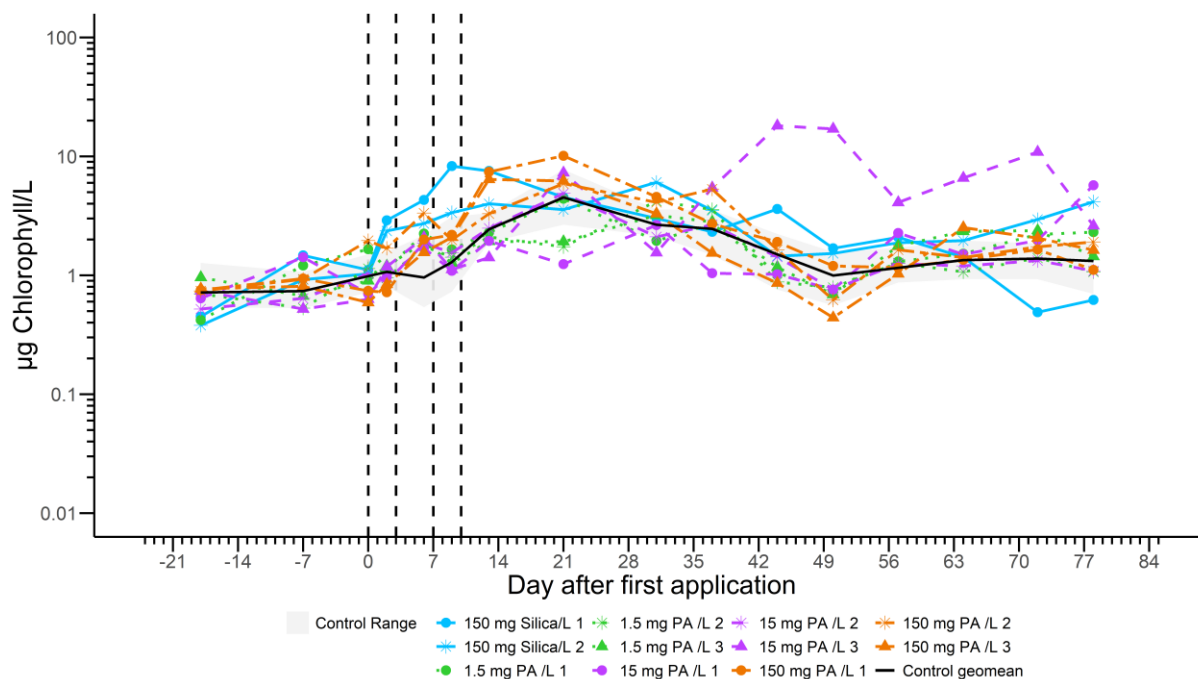
**Figure 14** Total chlorophyll content of green algae [ $\mu\text{g L}^{-1}$ ] measured with delayed fluorescence method

The mean of chlorophyll content from  $0.13 \times/\div 81.99 \mu\text{g L}^{-1}$  on day 37 to  $3.06 \times/\div 5.03 \mu\text{g L}^{-1}$  on day 21 in the MedPA treatment with two mesocosms that had a noticeably increased chlorophyll content at the end of the study. The mean of chlorophyll content from green algae ranged from  $0 \mu\text{g L}^{-1}$  on days 44, 78 and 86 to  $4.27 \times/\div 1.07 \mu\text{g L}^{-1}$  on day 13 in the HighPA treatment. The green algae had significantly higher chlorophyll content in the Silica treatment on sampling days 6 through 13 and on day 6 in the HighPA treatment. The HighPA had significantly higher chlorophyll content on day 31. All samplings after day 13, except day 31 had MDD-values  $> 100$  which makes it mathematically impossible to show direct toxic effects. The MedPA treatment however showed a strongly increased chlorophyll content in two replicates starting day 51.



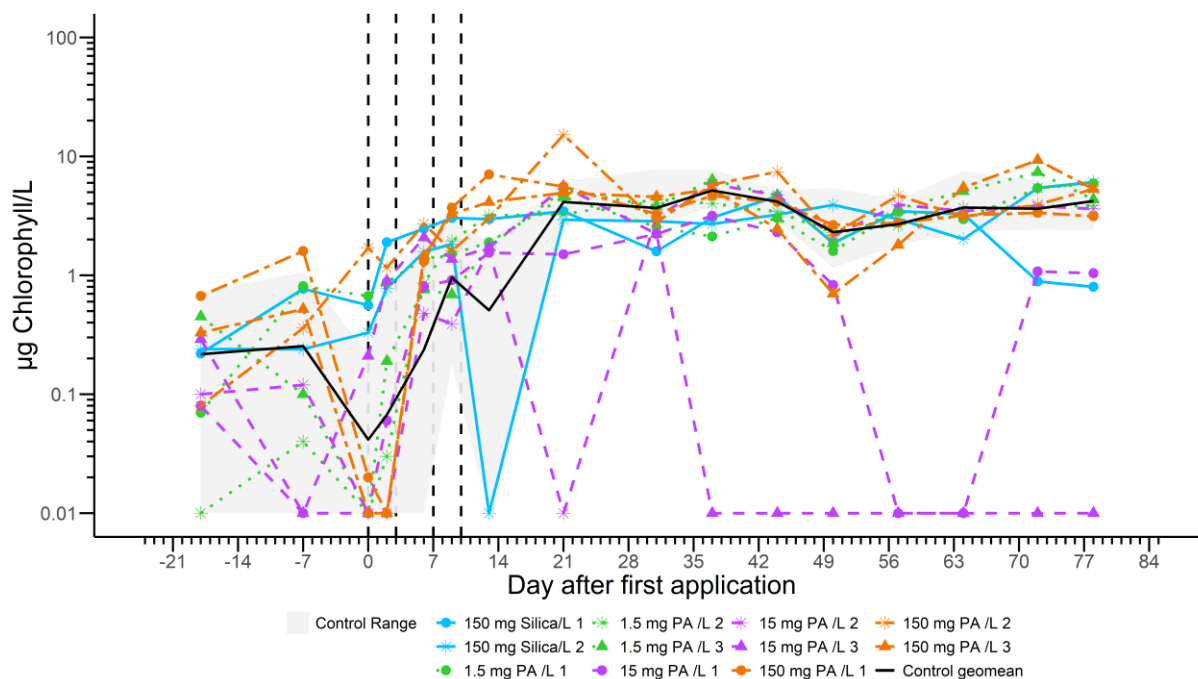
**Figure 15** Total chlorophyll content of blue algae [ $\mu\text{g L}^{-1}$ ] measured with delayed fluorescence method

The mean of chlorophyll content from blue algae (Figure 15) ranged from  $0.06 \times / \div 3.52 \mu\text{g L}^{-1}$  on day 2 to  $2.56 \times / \div 1.54 \mu\text{g L}^{-1}$  on day 86 in the control. The mean abundance of chlorophyll content from blue algae ranged from  $0.07 \times / \div 17.01 \mu\text{g L}^{-1}$  on day 9 to  $2.05 \times / \div 1.47 \mu\text{g L}^{-1}$  on day 21 in the Silica treatment with both replicates showing a severe drop in chlorophyll content three weeks after the last application. The mean of chlorophyll content from blue algae ranged from  $0.13 \times / \div 2.20 \mu\text{g L}^{-1}$  on day 2 to  $3.28 \times / \div 1.18 \mu\text{g L}^{-1}$  on day 86 in the LowPA treatment. The mean of chlorophyll content from blue algae ranged from  $0.18 \times / \div 4.56 \mu\text{g L}^{-1}$  on day -7 to  $3.05 \times / \div 1.32 \mu\text{g L}^{-1}$  on day 86 in the MedPA treatment. The mean of chlorophyll content from blue algae ranged from  $0.12 \times / \div 5.36 \mu\text{g L}^{-1}$  on day -7 to  $2.79 \times / \div 1.91 \mu\text{g L}^{-1}$  on day 72 in the HighPA treatment. After low concentrations at the begin of the study, the chlorophyll a content from blue algae rose in all treatments after the last application, with some fluctuating peaks in single mesocosms of the MedPA and HighPA treatment. The Silica treatment had significantly lower chlorophyll contents from blue algae on two samplings. The content was reduced on day 31 and 86. The MedPA treatment had significantly lower chlorophyll content on day 57 only. The HighPA treatment showed significant higher chlorophyll content on two consecutive samplings on day 6 and day 9. The following sampling on day 13 was not significant compared to the control. The p-value of that sampling however was  $p < 0.1$ .



**Figure 16** Total chlorophyll content of diatoms [ $\mu\text{g L}^{-1}$ ] measured with delayed fluorescence method

The mean of chlorophyll content from diatoms (Figure 16) ranged from  $0.72 \times/\div 1.40 \mu\text{g L}^{-1}$  on day -18 to  $4.53 \times/\div 1.63 \mu\text{g L}^{-1}$  on day 21 in the control. The mean of chlorophyll content from diatoms ranged from  $0.41 \times/\div 1.13 \mu\text{g L}^{-1}$  on day -18 to  $5.49 \times/\div 1.57 \mu\text{g L}^{-1}$  on day 13 in the Silica treatment. The mean of chlorophyll content from diatoms ranged from  $0.66 \times/\div 1.52 \mu\text{g L}^{-1}$  on day -18 to  $3.02 \times/\div 1.16 \mu\text{g L}^{-1}$  on day 37 in the LowPA treatment. The mean of chlorophyll content from diatoms ranged from  $0.63 \times/\div 1.19 \mu\text{g L}^{-1}$  on day -18 to  $4.15 \times/\div 2.88 \mu\text{g L}^{-1}$  on day 86 in the MedPA treatment with one mesocosm that has a noticeably increased chlorophyll content. The mean of chlorophyll content from diatoms ranged from  $0.69 \times/\div 1.67 \mu\text{g L}^{-1}$  on day 50 to  $7.19 \times/\div 1.34 \mu\text{g L}^{-1}$  on day 21 in the HighPA treatment. The overall course of the chlorophyll a content from diatoms was comparable in all treatments and on a similar level throughout the study. The Silica treatment had a significantly higher chlorophyll content compared to the control on sampling days 2 through day 13. The chlorophyll content in the HighPA treatment was significantly higher compared to the control on day 13.



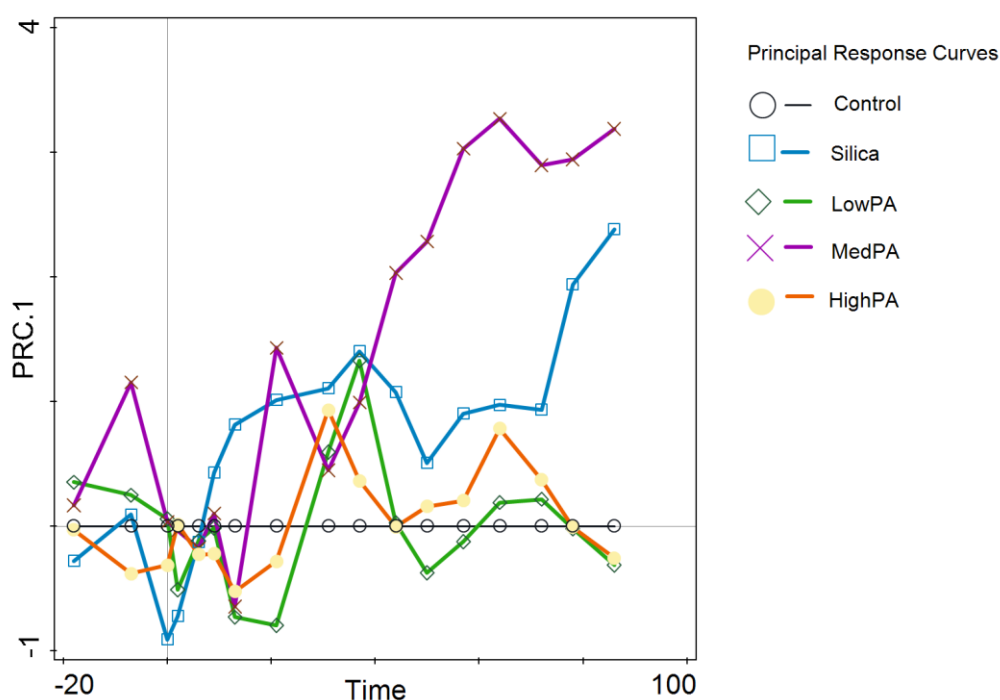
**Figure 17** Total chlorophyll content of cryptomonads [ $\mu\text{g L}^{-1}$ ] measured with delayed fluorescence method

The mean of chlorophyll content from cryptomonads (Figure 17) ranged from  $0.04 \times / \div 3.95 \mu\text{g L}^{-1}$  on day 0 to  $5.17 \times / \div 1.30 \mu\text{g L}^{-1}$  on day 37 in the control. The mean of chlorophyll content from cryptomonads ranged from  $0.17 \times / \div 56.04 \mu\text{g L}^{-1}$  on day 13 to  $3.87 \times / \div 1.29 \mu\text{g L}^{-1}$  on day 44 in the Silica treatment. The mean of chlorophyll content from cryptomonads ranged from  $0.04 \times / \div 11.33 \mu\text{g L}^{-1}$  on day 0 to  $5.97 \times / \div 1.19 \mu\text{g L}^{-1}$  on day 86 in the LowPA treatment. The mean of chlorophyll content from cryptomonads ranged from  $0.02 \times / \div 4.20 \mu\text{g L}^{-1}$  on day -7 to  $2.49 \times / \div 1.21 \mu\text{g L}^{-1}$  on day 31 in the MedPA treatment with two replicates showing lower contents than the others three weeks after the last application and after. The mean of chlorophyll content from cryptomonads ranged from  $0.05 \times / \div 15.57 \mu\text{g L}^{-1}$  on day 2 to  $7.48 \times / \div 1.85 \mu\text{g L}^{-1}$  on day 21 in the HighPA treatment. Overall, the content fluctuated strongly before and during the application period. The HighPA treatment had a significantly higher chlorophyll content on day 13.

The chlorophyll contents of the  $30 \mu\text{m}$  sieved fraction showed two significant differences compared to the control in the Silica treatment. Blue algae had significant lower abundances on day 86 and diatoms significantly higher abundances compared to the control on day 31. The total chlorophyll content of the  $30 \mu\text{m}$  fraction was between 65% on day 0 to 120% on day 57 of the whole sample.

#### 4.1.2 Phytoplankton PRC and RDA

The PRC of the phytoplankton community (Figure 18) shows the effect of the treatments on community level.



**Figure 18** Principal response curve of phytoplankton community

The Silica treatment had moderate effects with a slight increase starting day 50. The LowPA treatment has no effect on the community and shows the smallest response compared to the other treatments. The MedPA treatment showed strong positive deviations over time. The strong positive effects start at the half-way mark of the study. The HighPA treatment, like the LowPA treatment showed no real effects of the particles on the community of phytoplankton. Before the first application (vertical line; day 0) there were no differences compared to the control line. After application the Silica and MedPA treatment showed increasing effects over time.

The species score of 1.61 was the highest for green algae. Cryptomonads had the highest negative species score with -1.16. Both blue algae and diatoms had species scores with an absolute value smaller than 0.5. The changes on the community level were significant with a p-value of 0.046. A subsequent RDA, which was carried out on a sampling day basis, revealed that significant findings occurred at a total of three samplings. Two of these were during the application procedure. On day 6 ( $p = 0.018$ ) 20.21% adjusted variation was explained by the treatment and on day 9 ( $p = 0.048$ ) 12.98%. On day 31 ( $p = 0.002$ ), 27.59% of the adjusted variation was explained by the treatment.

### 4.1.3 Dominance

Table 6 shows the temporal development of the relative density [%] of the four phytoplankton color classes from day -18 to 86. A total of 17 samplings were performed per mesocosm. In the control, the green algae initially dominated with a proportion of up to 65.3% but decreased sharply after day 13 and almost disappeared from day 44. The opposite was true for cryptomonads, whose proportion increased steadily and reached values of over 50% from day 21 onwards. The diatoms initially remained relatively stable with slight fluctuations between 26.5% and 39.9% and fell below 20% from day 64. The blue algae showed a moderate increase from 14.8% to around 30.7% at the end of the experiment. In the Silica treatment, the diatoms showed an increased dominance and rose in dominance up to 51.8% (day 31). The cryptomonads increased similarly to the control and reached values above 40% after day 31. The dominance of green algae decreased faster than in the control, falling to 1.8% (day 72) before recovering slightly.

The dominance of blue algae fluctuated strongly but leveled off at the end of the study. In the LowPA treatment, the green algae were almost non-existent from day 44. The cryptomonads increased continuously and reached up to 55.9% (day 78). The dominance of diatoms remained relatively stable and only decreased slightly at the end of the study. The blue algae increased moderately from 8.6% to around 30.3% (day 86). In the MedPA treatment, the dominance of green algae differed from that in the other treatments. It increased continuously to 73.6% on day 50 and then decreased slightly. The cryptomonads remained low in dominance with an interim peak in the first third of the study. The blue algae remained largely constant with values between 7% and 17%. The diatoms remained constant with a percentage dominance between 16% and 32%. In the HighPA treatment, the dominance of green algae was similar to that of the control. The cryptomonads were also similar to the control but reached values above 50% from day 37. The blue algae increased continuously over the course of the study. The diatoms showed a similar development to the control, but at a slightly lower level.

**Table 6** Relative densities [%] of phytoplankton color classes

<b>Control</b>																	
<b>Day</b>	<b>-18</b>	<b>-7</b>	<b>0</b>	<b>2</b>	<b>6</b>	<b>9</b>	<b>13</b>	<b>21</b>	<b>31</b>	<b>37</b>	<b>44</b>	<b>50</b>	<b>57</b>	<b>64</b>	<b>72</b>	<b>78</b>	<b>86</b>
blue	14.8	12.2	4.7	3.3	10.8	12.4	9.7	11.8	15.2	23.1	25.1	24.9	22.2	26.3	26	27.2	30.7
green	44.8	38.8	65.3	54.5	32.7	28.2	45.1	13.8	6.3	3.6	0	0.6	0.3	0.2	0	0	0.2
diatoms	26.5	29.5	28	35.8	33.3	31.2	31.2	39.9	31.6	24	20.3	21.5	23.1	18.7	19.7	17.5	13.6
Crypto-monads	13.9	19.5	2	6.4	23.2	28.3	14	34.5	46.9	49.2	54.6	53.1	54.4	54.9	54.3	55.3	55.5
<b>Silica</b>																	
<b>Day</b>	<b>-18</b>	<b>-7</b>	<b>0</b>	<b>2</b>	<b>6</b>	<b>9</b>	<b>13</b>	<b>21</b>	<b>31</b>	<b>37</b>	<b>44</b>	<b>50</b>	<b>57</b>	<b>64</b>	<b>72</b>	<b>78</b>	<b>86</b>
blue	15.4	9.5	12.9	4.2	3.4	2.1	4.5	14.1	0.1	10.9	19	20	21.5	24.3	24.9	20.3	11.3
green	41.5	44.5	44.9	38.1	36	33.5	52.4	37.9	22.7	10.4	4.8	4.5	2.4	7.5	1.8	4.6	22.4
diatoms	27.8	32.3	29.8	38.2	38.4	45.4	34.2	26.8	51.8	39.6	29.8	27	29	26.5	25.8	30.7	41.4
Crypto-monads	15.4	13.7	12.4	19.5	22.1	19	8.8	21.2	25.4	39.1	46.4	48.4	47.1	41.7	47.4	44.3	24.9
<b>LowPA</b>																	
<b>Day</b>	<b>-18</b>	<b>-7</b>	<b>0</b>	<b>2</b>	<b>6</b>	<b>9</b>	<b>13</b>	<b>21</b>	<b>31</b>	<b>37</b>	<b>44</b>	<b>50</b>	<b>57</b>	<b>64</b>	<b>72</b>	<b>78</b>	<b>86</b>
blue	8.6	6.5	7	5.3	7.7	12.8	13.5	17	16.3	20	24.8	26	22.7	23.8	29.3	25	30.3
green	55.6	46.2	56.2	45.2	39.9	27.9	24.7	5.8	11.5	9.9	0	0	0.1	0.4	0.2	0	0
diatoms	28.5	33.9	31.2	37.1	34.7	30.4	29.7	29.6	33.7	29.6	16.3	20.6	23.9	22.4	18.7	19.1	14.6
Crypto-monads	7.3	13.4	5.6	12.4	17.6	29	32.1	47.7	38.5	40.5	58.8	53.5	53.3	53.4	51.8	55.9	55.1
<b>MedPA</b>																	
<b>Day</b>	<b>-18</b>	<b>-7</b>	<b>0</b>	<b>2</b>	<b>6</b>	<b>9</b>	<b>13</b>	<b>21</b>	<b>31</b>	<b>37</b>	<b>44</b>	<b>50</b>	<b>57</b>	<b>64</b>	<b>72</b>	<b>78</b>	<b>86</b>
blue	9.4	9.8	7.1	7	10.7	14.1	10.2	9	16.9	12.2	4.5	7.6	14.6	11.5	10.5	14	12.3
green	54.9	66.7	62	56	36.9	37.5	30.8	42.5	13.9	46.7	69.8	73.6	55.7	68	65.9	55.1	59.2
diatoms	28.7	22.4	28.4	28.1	32.8	28.3	32.1	31.7	31.5	20.7	19.1	16.2	19.6	14.9	17.6	20.7	22.5
Crypto-monads	7.1	1	2.5	8.9	19.6	20.1	26.8	16.8	37.7	20.4	6.5	2.6	10.1	5.6	6.1	10.3	6

**Table 6** (continued) Relative densities [%] of phytoplankton color classes

<b>HighPA</b>																	
<b>Day</b>	<b>-18</b>	<b>-7</b>	<b>0</b>	<b>2</b>	<b>6</b>	<b>9</b>	<b>13</b>	<b>21</b>	<b>31</b>	<b>37</b>	<b>44</b>	<b>50</b>	<b>57</b>	<b>64</b>	<b>72</b>	<b>78</b>	<b>86</b>
blue	11.4	9.3	8.8	7.9	11.5	16.4	10.3	15.6	15.1	19	25.5	25.8	25.9	25.3	30.3	25.9	30.4
green	46.4	26.3	45	48.6	35.1	19.8	26.1	10.2	20.9	5.7	0	1.7	0.8	1.8	0.5	0	0
diatoms	28.6	33.5	30.3	32	29.8	26.9	34.8	34.4	33.9	28.4	17.8	21	21.4	22.6	17.1	18.7	15.4
Crypto- monads	13.7	31	15.9	11.5	23.7	37	28.8	39.8	30.1	46.9	56.7	51.6	51.9	50.3	52.1	55.4	54.2

#### 4.1.4 Statistical evaluation

Table 7 shows the effects on the amount of chlorophyll in the different color classes of the algae for the total and the 30  $\mu\text{m}$  fraction. The effects were classified according to EFSA (see Table 4). Over the entire study, the Silica treatment particularly influenced the diatoms and the green algae. Both groups are assigned to class 3A+, as there were pronounced short-term indirect effects with recovery. Accordingly, the total chlorophyll was also assigned to effect class 3A+. The 30  $\mu\text{m}$  fraction showed only slight indirect effects (class 2+) for the diatoms and slight direct effects for the blue algae (class 2-). No effects according to EFSA were assigned to the LowPA treatment. The MedPA treatment only showed slight indirect effects (class 2+) for the green algae in the total fraction. In the HighPA treatment, as in all polyamide treatments, class 1 was assigned in all 30  $\mu\text{m}$  fractions. The blue algae in the HighPA treatment showed pronounced indirect effects with recovery in the HighPA treatment. The total chlorophyll content and the green algae showed slight indirect effects (class 2-).

**Table 7** Classification of the effect on phytoplankton over the entire duration of the study according to EFSA (2013).

	Silica	LowPA	MedPA	HighPA
blue	1	1	1	3A+
green	3A+	1	2+	2+
diatom	3A+	1	1	1
crypto	1	1	1	1
total chlorophyll	3A+	1	1	2+
blue (30 $\mu\text{m}$ fraction)	2-	1	1	1
green (30 $\mu\text{m}$ fraction)	1	1	1	1
diatom (30 $\mu\text{m}$ fraction)	2+	1	1	1
crypto (30 $\mu\text{m}$ fraction)	1	1	1	1
total chlorophyll (30 $\mu\text{m}$ fraction)	1	1	1	1



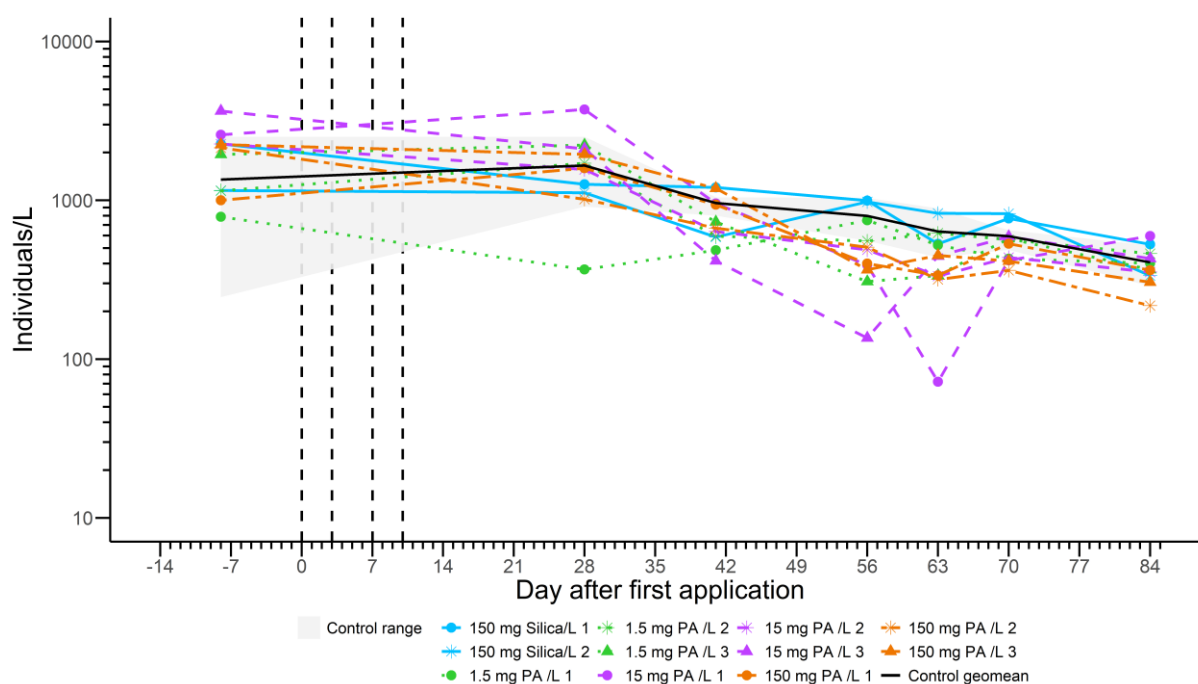
## 4.2 Zooplankton



(Picture generated with OpenAI DALL·E)

#### 4.2.1 Abundance data

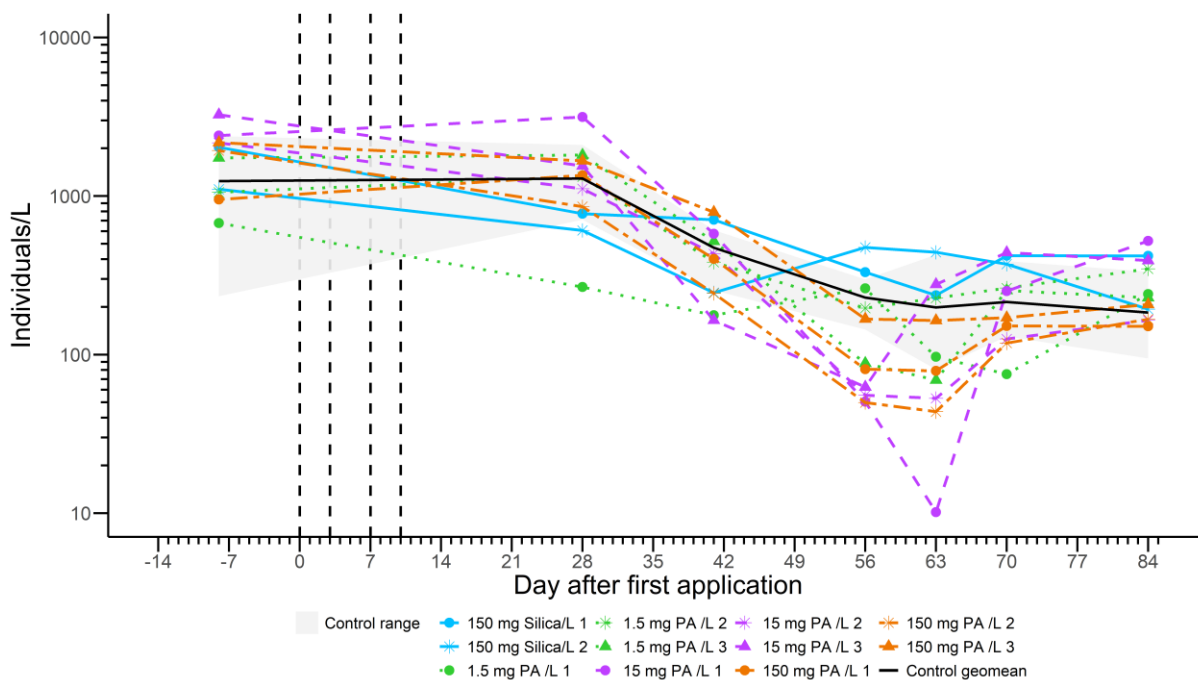
The mean abundance of all individuals (Figure 19) ranged from 406.8  $\times/\div$  1.29 Ind. L<sup>-1</sup> on day 84 to 1657.4  $\times/\div$  1.57 Ind. L<sup>-1</sup> on day 28 in the control. The mean abundance of all individuals ranged from 420.7  $\times/\div$  1.38 Ind. L<sup>-1</sup> on day 84 to 1618.2  $\times/\div$  1.61 Ind. L<sup>-1</sup> on day -8 in the Silica treatment. The mean abundance of all individuals ranged from 407.7  $\times/\div$  1.12 Ind. L<sup>-1</sup> on day 84 to 1207.6  $\times/\div$  1.57 Ind. L<sup>-1</sup> on day -8 in the LowPA treatment.



**Figure 19** Abundance of all zooplankton organisms [Ind. L<sup>-1</sup>]

The mean abundance of all individuals ranged from 220.4  $\times/\div$  2.66 Ind. L<sup>-1</sup> on day 63 to 2776.6  $\times/\div$  1.28 Ind. L<sup>-1</sup> on day -8 in the MedPA treatment. The mean abundance of all individuals ranged from 288.6  $\times/\div$  1.30 Ind. L<sup>-1</sup> on day 84 to 1687.1  $\times/\div$  1.57 Ind. L<sup>-1</sup> on day -8 in the HighPA treatment. After the application period the abundance in all treatments decreased steadily. The total number of zooplankton organisms decreased over the course of the study in all treatments comparably. The Silica treatment had significantly higher abundances in the control on day 70. The LowPA treatment showed no significant differences, while the MedPA and HighPA had significantly lower abundances on days 56 and 63 and 56 and 70 respectively.

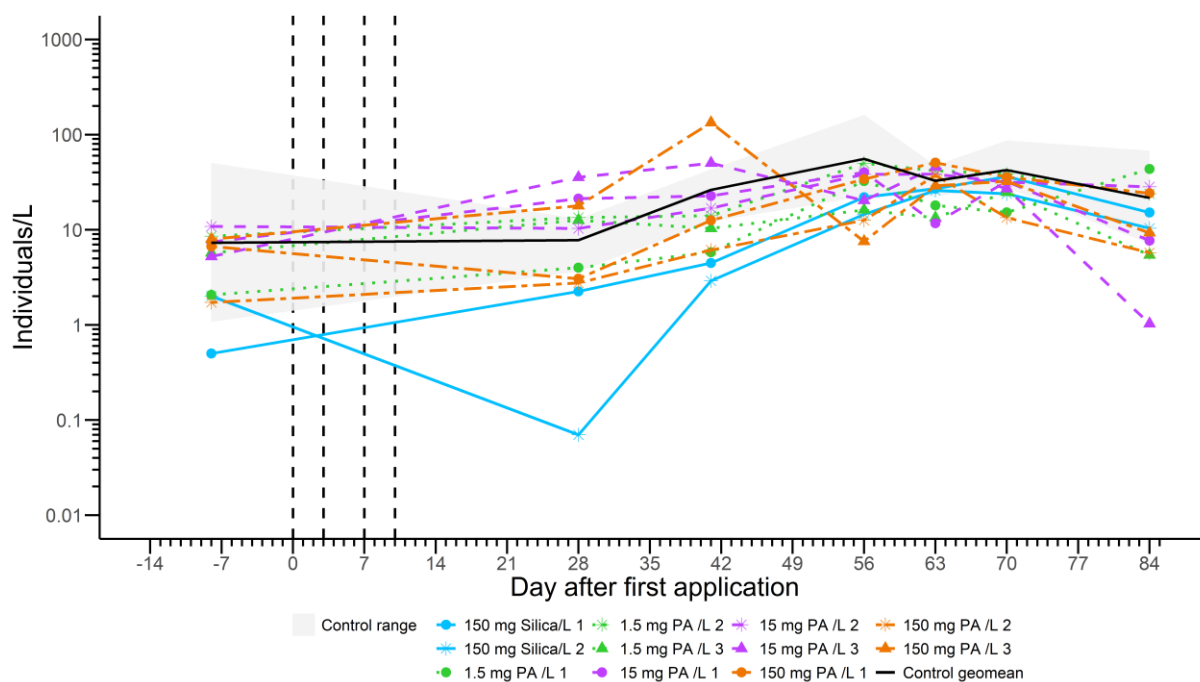
The mean abundance of all Rotifera (Figure 20) ranged from  $184.3 \times/\div 1.58$  Ind.  $L^{-1}$  on day 84 to  $1289.5 \times/\div 1.59$  Ind.  $L^{-1}$  on day 28 in the control. The mean abundance of all Rotifera ranged from  $284.8 \times/\div 1.73$  Ind.  $L^{-1}$  on day 84 to  $1498.6 \times/\div 1.54$  Ind.  $L^{-1}$  on day -8 in the Silica treatment. The mean abundance of all Rotifera ranged from  $115.0 \times/\div 1.85$  Ind.  $L^{-1}$  on day 63 to  $1075.3 \times/\div 1.61$  Ind.  $L^{-1}$  on day -8 in the LowPA treatment.



**Figure 20** Abundance of all Rotifera [Ind.  $L^{-1}$ ]

The mean abundance of all Rotifera ranged from  $53.0 \times/\div 5.22$  Ind.  $L^{-1}$  on day 63 to  $2570.1 \times/\div 1.24$  Ind.  $L^{-1}$  on day -8 in the MedPA treatment. The mean abundance of all Rotifera ranged from  $82.7 \times/\div 1.94$  Ind.  $L^{-1}$  on day 63 to  $1588.5 \times/\div 1.57$  Ind.  $L^{-1}$  on day -8 in the HighPA treatment. The abundance of Rotifera was constant during the first month after application and sunk after in all treatments. At the end of the study a tenth of the abundance before application was found. The Silica treatment had significantly higher abundance compared to the control on day 56, while the MedPA and HighPA treatments showed a significant reduction in abundance on the same day. The LowPA treatment showed no significant differences compared to the control during the whole study.

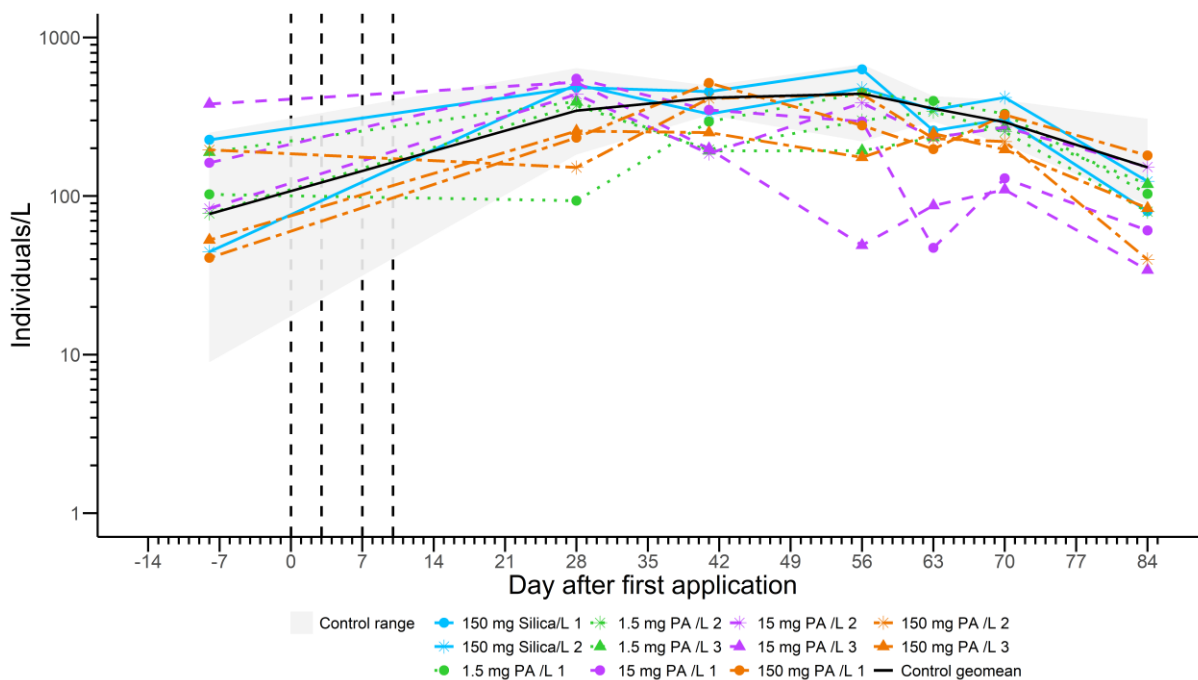
The mean abundance of all Cladocera (Figure 21) ranged from  $7.3 \times / \div 5.37 \text{ Ind. L}^{-1}$  on day -8 to  $55.5 \times / \div 2.03 \text{ Ind. L}^{-1}$  on day 56 in the control. The mean abundance of all Cladocera ranged from  $0.4 \times / \div 11.63 \text{ Ind. L}^{-1}$  on day 28 to  $29.4 \times / \div 1.34 \text{ Ind. L}^{-1}$  on day 70 in the Silica treatment with both replicates having the lowest abundances out of all mesocosms on day 28. The mean abundance of all Cladocera ranged from  $4.7 \times / \div 2.08 \text{ Ind. L}^{-1}$  on day -8 to  $29.7 \text{ Ind. L}^{-1} \times / \div 1.77$  on day 56 in the LowPA treatment.



**Figure 21** Abundance of all Cladocera [ $\text{Ind. L}^{-1}$ ]

The mean abundance of all Cladocera ranged from  $6.1 \times / \div 5.31 \text{ Ind. L}^{-1}$  on day 84 to  $31.5 \times / \div 1.46 \text{ Ind. L}^{-1}$  on day 56 in the MedPA treatment. The mean abundance of all Cladocera ranged from  $4.5 \times / \div 2.32 \text{ Ind. L}^{-1}$  on day -8 to  $38.0 \times / \div 1.32 \text{ Ind. L}^{-1}$  on day 63 in the HighPA treatment. The abundance of Cladocera increased over time. The abundance of cladocerans rose over the course of the study in the control and all treatments. The vast majority of data were in the range of the control mesocosms. There were no significant differences compared to the control group throughout the study and thus the NOEC is  $> 150 \text{ mg L}^{-1}$ .

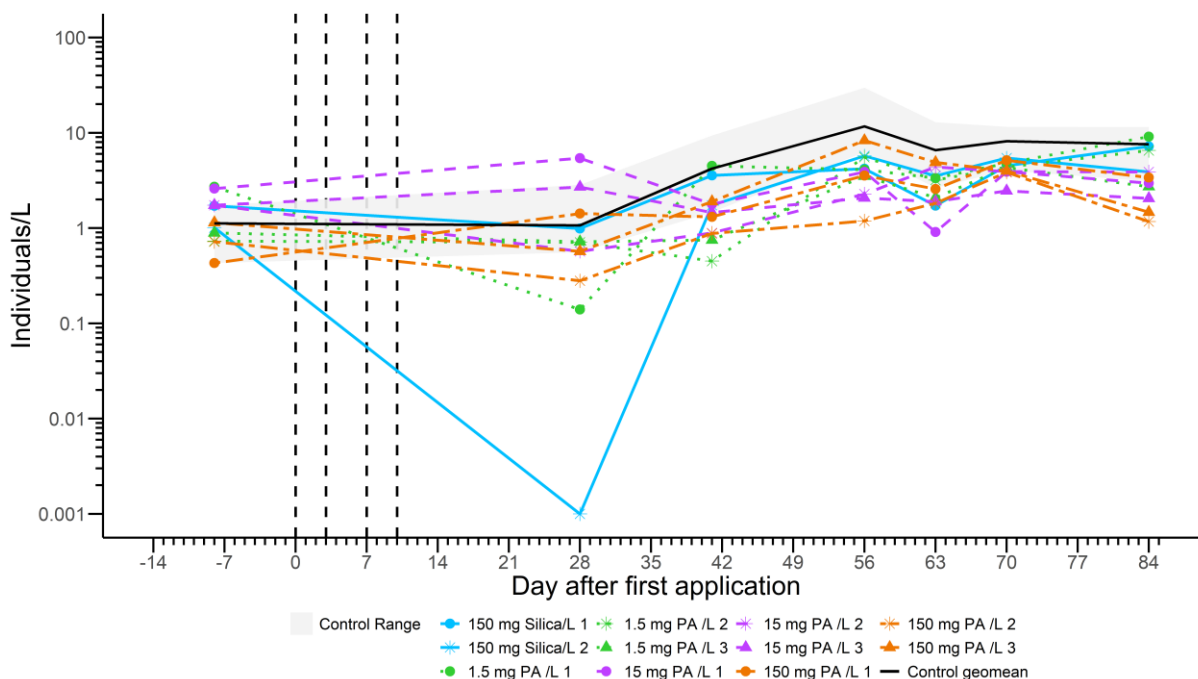
The mean abundance of all copepods (Figure 22) ranged from  $77.1 \times / \div 3.66 \text{ Ind. L}^{-1}$  on day -8 to  $442.4 \times / \div 1.55 \text{ Ind. L}^{-1}$  on day 56 in the control. The mean abundance of all copepods ranged from  $99.6 \times / \div 1.36 \text{ Ind. L}^{-1}$  on day 84 to  $549.1 \times / \div 1.21 \text{ Ind. L}^{-1}$  on day 56 in the Silica treatment. The mean abundance of all copepods ranged from  $99.2 \times / \div 1.22 \text{ Ind. L}^{-1}$  on day 84 to  $316.5 \times / \div 1.31 \text{ Ind. L}^{-1}$  on day 63 in the LowPA treatment.



**Figure 22** Abundance of all Copepoda [ $\text{Ind. L}^{-1}$ ]

The mean abundance of all copepods ranged from  $68.1 \times / \div 2.13 \text{ Ind. L}^{-1}$  on day 84 to  $501.1 \times / \div 1.13 \text{ Ind. L}^{-1}$  on day 28 in the MedPA treatment. The mean abundance of all copepods ranged from  $75.0 \times / \div 2.32 \text{ Ind. L}^{-1}$  on day -8 to  $377.2 \times / \div 1.44 \text{ Ind. L}^{-1}$  on day 41 in the HighPA treatment. The abundance of copepods rose at the beginning of the study and decreased again in the latter stages of the experiment. The Silica and HighPA treatment showed no significant differences compared to the control. The LowPA and MedPA treatment had significantly lower abundances on days 41 and 63 respectively.

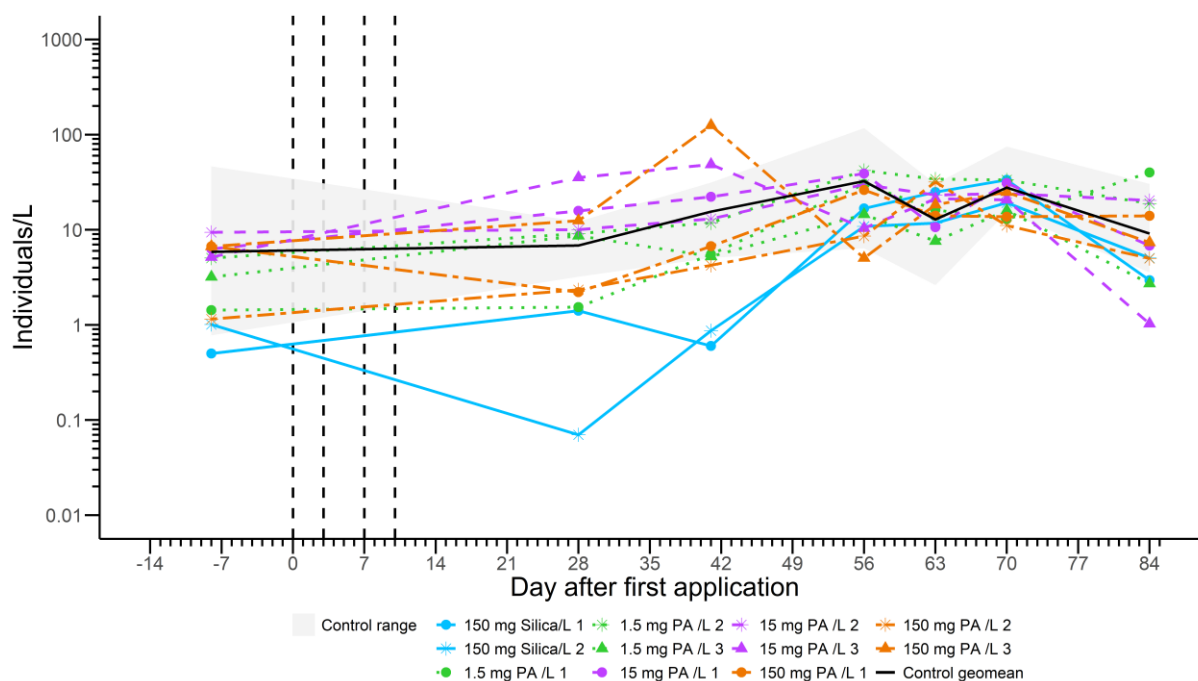
The mean abundance of ostracods (Figure 23) ranged from  $1.1 \times / \div 1.91 \text{ Ind. L}^{-1}$  on day 28 to  $11.7 \times / \div 1.8 \text{ Ind. L}^{-1}$  on day 56 in the control. The mean abundance of ostracods ranged from  $0.03 \times / \div 131.29 \text{ Ind. L}^{-1}$  on day 28 to  $5.3 \times / \div 1.55 \text{ Ind. L}^{-1}$  on day 84 in the Silica treatment. The mean abundance of ostracods ranged from  $0.4 \times / \div 2.56 \text{ Ind. L}^{-1}$  on day 28 to  $5.4 \times / \div 1.87 \text{ Ind. L}^{-1}$  on day 84 in the LowPA treatment. The mean abundance of ostracods ranged from  $1.3 \times / \div 1.43 \text{ Ind. L}^{-1}$  on day 41 to  $3.4 \times / \div 1.31 \text{ Ind. L}^{-1}$  on day 70 in the MedPA treatment.



**Figure 23** Abundance of all Ostracoda [Ind. L<sup>-1</sup>]

The mean abundance of ostracods ranged from  $0.6 \times / \div 2.26 \text{ Ind. L}^{-1}$  on day 28 to  $4.3 \times / \div 1.17 \text{ Ind. L}^{-1}$  on day 70 in the HighPA treatment. The abundance of ostracods was on a comparable low level in the first third of the study. Starting day 28 the abundance in the controls rose up to the peak value on day 56. Most of the treatment data are below control geomean after day 41. Ostracoda showed no significant differences until day 70. On day 70 all polyamide treatments had significantly lower abundances compared to the control. The HighPA treatment was still significantly reduced on the last study day (84). The Silica treatment showed no significant differences compared to the control.

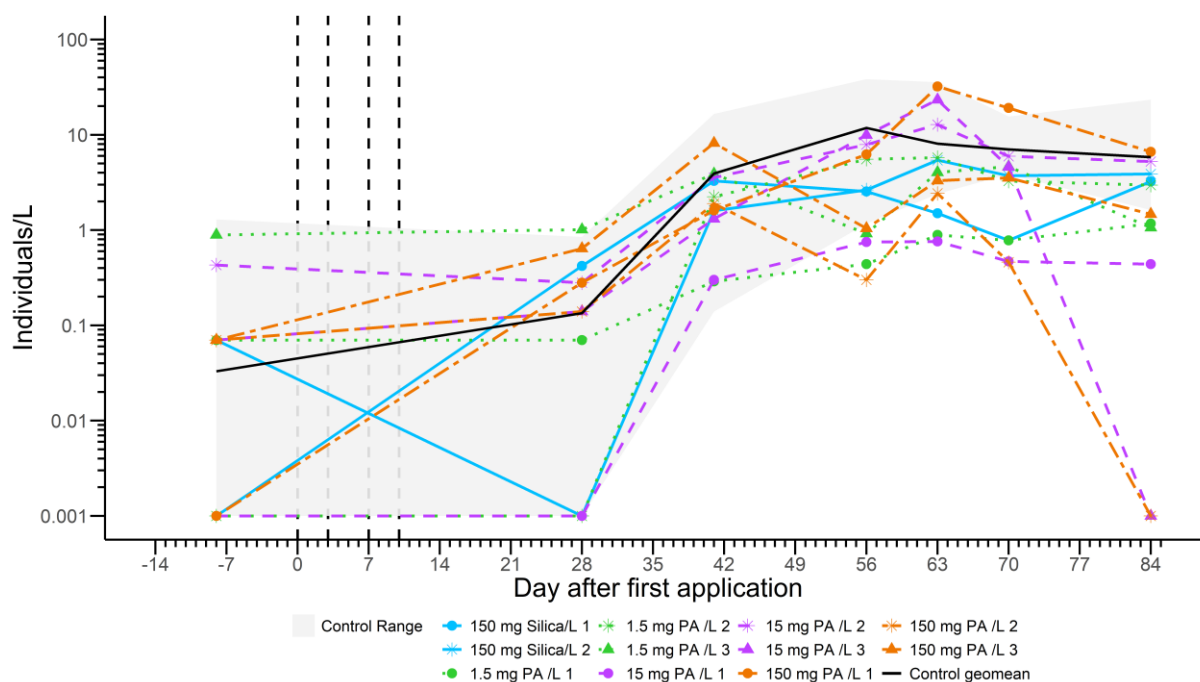
The mean abundance of *Daphnia longispina* (Figure 24) ranged from  $5.9 \times / \div 6.17 \text{ Ind. L}^{-1}$  on day -8 to  $32.4 \times / \div 2.85 \text{ Ind. L}^{-1}$  on day 56 in the control. The mean abundance of *D. longispina* ranged from  $0.3 \times / \div 8.36 \text{ Ind. L}^{-1}$  on day 28 to  $25.4 \times / \div 1.48 \text{ Ind. L}^{-1}$  on day 70 in the 150 mg L<sup>-1</sup> Silica treatment. The mean abundance of *D. longispina* ranged from  $2.8 \times / \div 1.89 \text{ Ind. L}^{-1}$  on day -8 to  $26.4 \times / \div 1.72 \text{ Ind. L}^{-1}$  on day 56 in the LowPA treatment. The mean abundance of *D. longispina* ranged from  $5.2 \times / \div 4.52 \text{ Ind. L}^{-1}$  on day 84 to  $24.9 \text{ Ind. L}^{-1} \times / \div 1.23$  on day 70 in the MedPA treatment.



**Figure 24** Abundance of *Daphnia longispina* [Ind. L<sup>-1</sup>]

The mean abundance of *D. longispina* ranged from  $3.7 \times / \div 2.77 \text{ Ind. L}^{-1}$  on day -8 to  $20.2 \times / \div 1.52 \text{ Ind. L}^{-1}$  on day 63 in the HighPA treatment. There were no significant differences compared to the control group throughout the study and thus the NOEC is  $> 150 \text{ mg L}^{-1}$ . Both replicates of the Silica treatment, however, had lower abundances than the control range in days 28 and 41. Two of three replicates of the MedPA treatment had higher abundances than all controls on day 28.

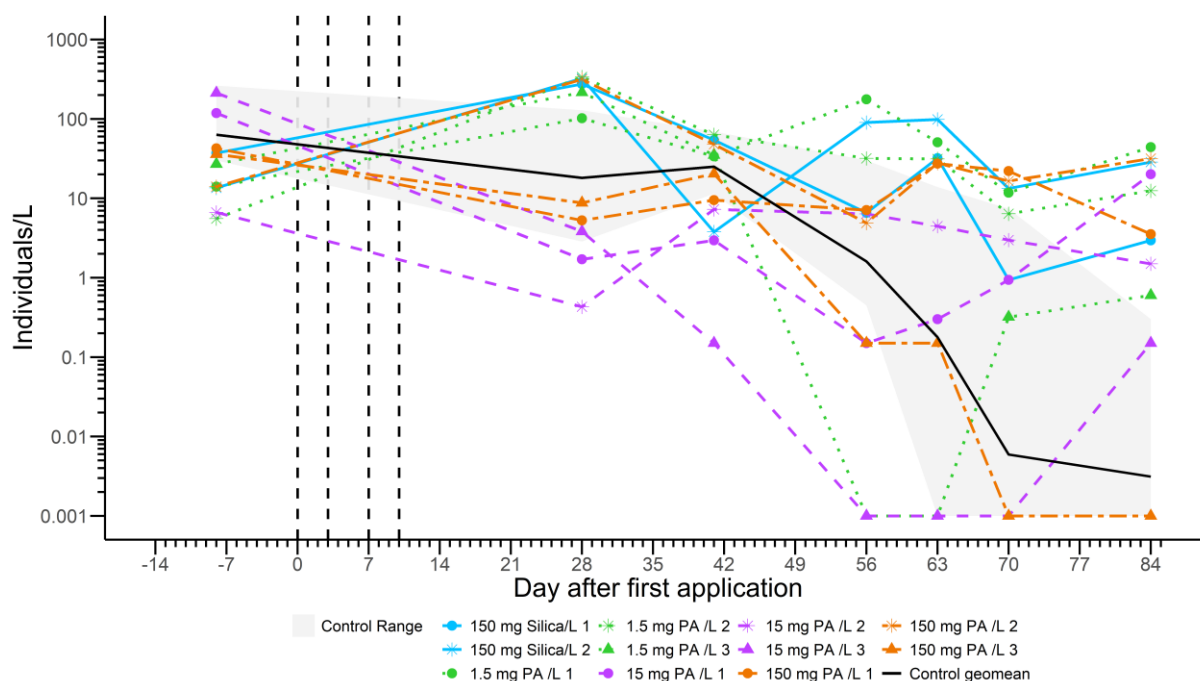
The mean abundance of *Chydorus sphaericus* (Figure 25) ranged from  $0.03 \times/\div 28.67$  Ind. L<sup>-1</sup> on day -8 to  $11.8 \times/\div 3.88$  Ind. L<sup>-1</sup> on day 56 in the control. The mean abundance of *C. sphaericus* ranged from  $0.01 \times/\div 20.17$  Ind. L<sup>-1</sup> on day -8 to  $3.5 \times/\div 1.14$  Ind. L<sup>-1</sup> on day 84 in the Silica treatment. The mean abundance of *C. sphaericus* ranged from  $0.04 \times/\div 30.91$  Ind. L<sup>-1</sup> on day -8 to  $2.7 \times/\div 2.7$  Ind. L<sup>-1</sup> on day 63 in the LowPA treatment.



**Figure 25** Abundance of *Chydorus sphaericus* [Ind. L<sup>-1</sup>]

The mean abundance of *C. sphaericus* ranged from  $0.03 \times/\div 22.47$  Ind. L<sup>-1</sup> on day -8 to  $6.1 \times/\div 6.22$  Ind. L<sup>-1</sup> on day 63 in the MedPA treatment. The mean abundance of *C. sphaericus* ranged from  $0.02 \times/\div 11.62$  Ind. L<sup>-1</sup> on day -8 to  $6.4 \times/\div 4.09$  Ind. L<sup>-1</sup> on day 63 in the HighPA treatment. The abundance of *C. sphaericus* rose after day 41 in all mesocosms. On day 56 all abundances in the treated mesocosms were below the controls geomean, but mostly in the control range. There were no significant differences compared to the control group throughout the study and thus the NOEC is  $> 150$  mg L<sup>-1</sup>.

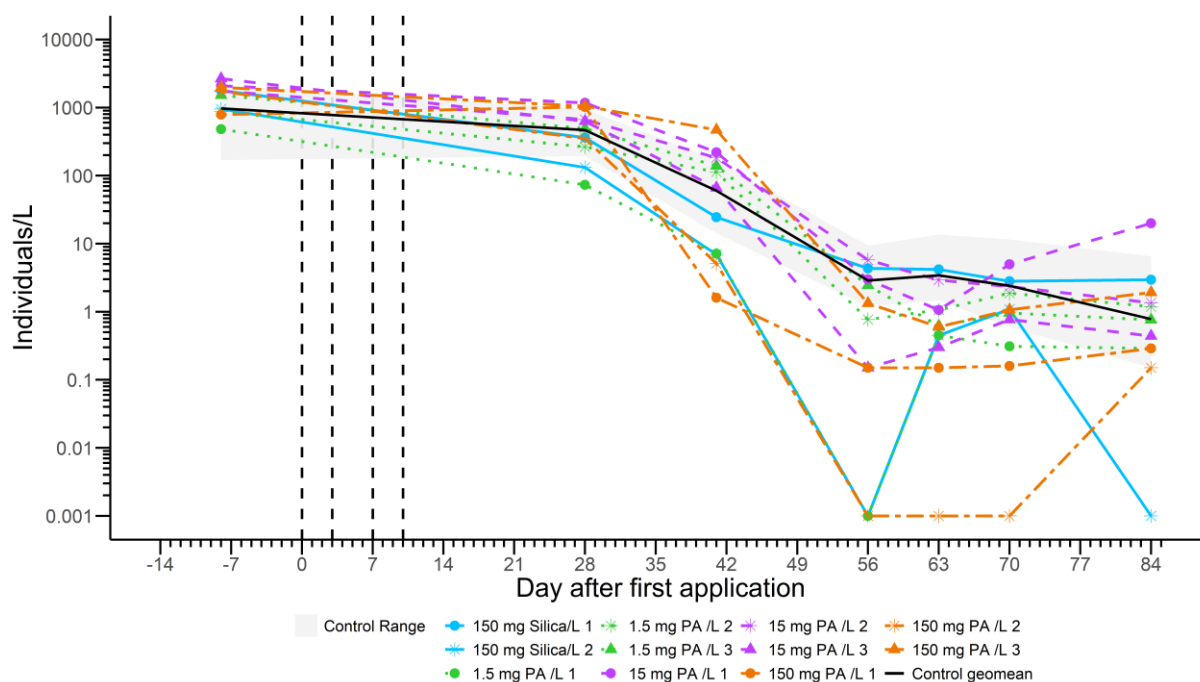
The mean abundance of *Synchaeta* sp. (Figure 26) ranged from 0.003  $\times/\div$  12.82 Ind. L<sup>-1</sup> on day 84 to 63.2  $\times/\div$  2.61 Ind. L<sup>-1</sup> on day -8 in the control. The mean abundance of *Synchaeta* sp. ranged from 3.5  $\times/\div$  6.52 Ind. L<sup>-1</sup> on day 70 to 299 Ind. L<sup>-1</sup>  $\times/\div$  1.12 on day 28 in the Silica treatment. The mean abundance of *Synchaeta* sp. ranged from 1.2  $\times/\div$  455.29 Ind. L<sup>-1</sup> on day 63 to 195.4  $\times/\div$  1.83 Ind. L<sup>-1</sup> on day 28 in the LowPA treatment. The mean abundance of *Synchaeta* sp. ranged from 0.1  $\times/\div$  81.09 Ind. L<sup>-1</sup> on day 56 to 55.1  $\times/\div$  6.39 Ind. L<sup>-1</sup> on day -8 in the MedPA treatment.



**Figure 26** Abundance of *Synchaeta* sp. [Ind. L<sup>-1</sup>]

The mean abundance of *Synchaeta* sp. ranged from 0.5  $\times/\div$  235.76 Ind. L<sup>-1</sup> on day 84 to 27.8  $\times/\div$  1.82 Ind. L<sup>-1</sup> on day -8 in the HighPA treatment. The Silica treatment had significantly higher abundances on days 28 and 63. The significant difference on day 63 had a MDD of 107%. There were no significant differences compared to the control in either polyamide treatment. On days 28 and 41, two and three replicates respectively in the MedPA treatment were under the control range (with MDD values of 97 and 94)

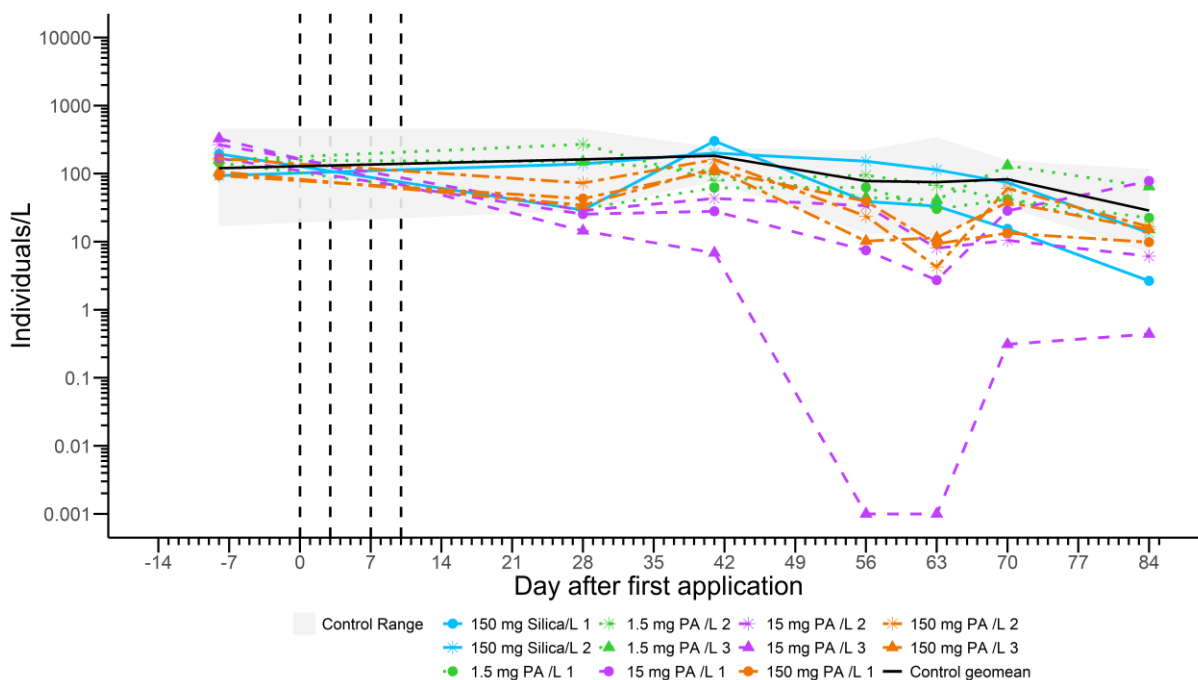
The mean abundance of *Keratella quadrata* (Figure 27) ranged from  $0.8 \times / \div 5.44 \text{ Ind. L}^{-1}$  on day 84 to  $975.3 \times / \div 2.75 \text{ Ind. L}^{-1}$  on day -8 in the control. The mean abundance of *K. quadrata* ranged from  $0.1 \times / \div 284.14 \text{ Ind. L}^{-1}$  on day 84 to  $1304.4 \times / \div 1.55 \text{ Ind. L}^{-1}$  on day -8 in the Silica treatment. The mean abundance of *K. quadrata* ranged from  $0.1 \times / \div 67.35 \text{ Ind. L}^{-1}$  on day 56 to  $860.4 \times / \div 1.77 \text{ Ind. L}^{-1}$  on day -8 in the LowPA treatment.



**Figure 27** Abundance of *Keratella quadrata* [ $\text{Ind. L}^{-1}$ ]

The mean abundance of *K. quadrata* ranged from  $1.0 \times / \div 3.13 \text{ Ind. L}^{-1}$  on day 63 to  $2142.6 \times / \div 1.23 \text{ Ind. L}^{-1}$  on day -8 in the MedPA treatment. The mean abundance of *K. quadrata* ranged from  $0.04 \times / \div 28.94 \text{ Ind. L}^{-1}$  on day 63 to  $1393.8 \times / \div 1.64 \text{ Ind. L}^{-1}$  on day -8 in the HighPA treatment. The number of *K. quadrata* declined in the second half of the experiment in all mesocosms to less than one per mille compared to preapplication. There were no significant differences compared to the control group throughout the study and thus the NOEC is  $> 150 \text{ mg L}^{-1}$ . Individual replicates of all treatments were below the control range starting day 28.

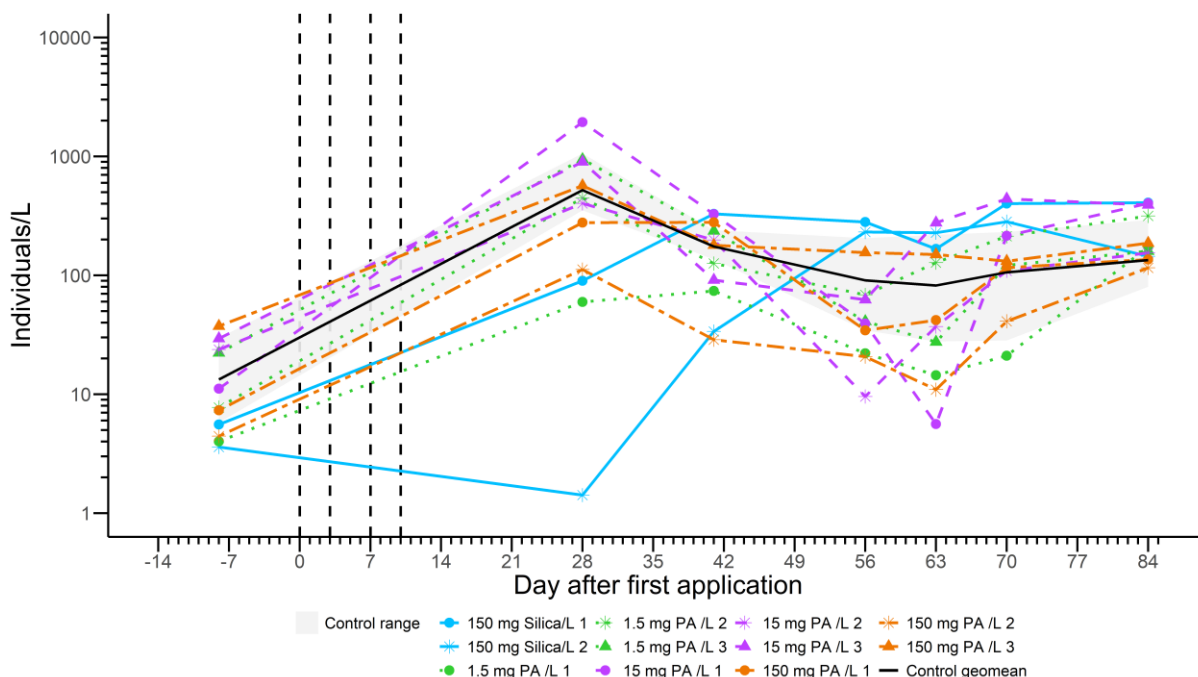
The mean abundance of *Polyarthra* sp. (Figure 28) ranged from  $28.7 \times / \div 3.29$  Ind. L<sup>-1</sup> on day 84 to  $183.7 \times / \div 1.62$  Ind. L<sup>-1</sup> on day 41 in the control. The mean abundance of *Polyarthra* sp. ranged from  $6.0 \times / \div 3.17$  Ind. L<sup>-1</sup> on day 84 to  $245.9 \times / \div 1.33$  Ind. L<sup>-1</sup> on day 41 in the Silica treatment. The mean abundance of *Polyarthra* sp. ranged from  $27.9 \times / \div 2.12$  Ind. L<sup>-1</sup> on day 84 to  $154.1 \times / \div 1.05$  Ind. L<sup>-1</sup> on day -8 in the LowPA treatment.



**Figure 28** Abundance of *Polyarthra* sp. [Ind. L<sup>-1</sup>]

The mean abundance of *Polyarthra* sp. ranged from  $0.3 \times / \div 135.47$  Ind. L<sup>-1</sup> on day 63 to  $245.3 \times / \div 1.41$  Ind. L<sup>-1</sup> on day -8 in the MedPA treatment. The mean abundance of *Polyarthra* sp. ranged from  $7.7 \times / \div 1.69$  Ind. L<sup>-1</sup> on day 63 to  $128.9 \times / \div 1.22$  Ind. L<sup>-1</sup> on day 41 in the HighPA treatment. The abundance of *Polyarthra* sp. Remained on a similar level throughout the study with one mesocosm in the MedPA treatment having clearly lower abundances in the second half of the study. On day 41 the LowPA and MedPA treatment had significantly reduced abundances compared to the control. The Silica and HighPA treatments showed no significant differences compared to the control.

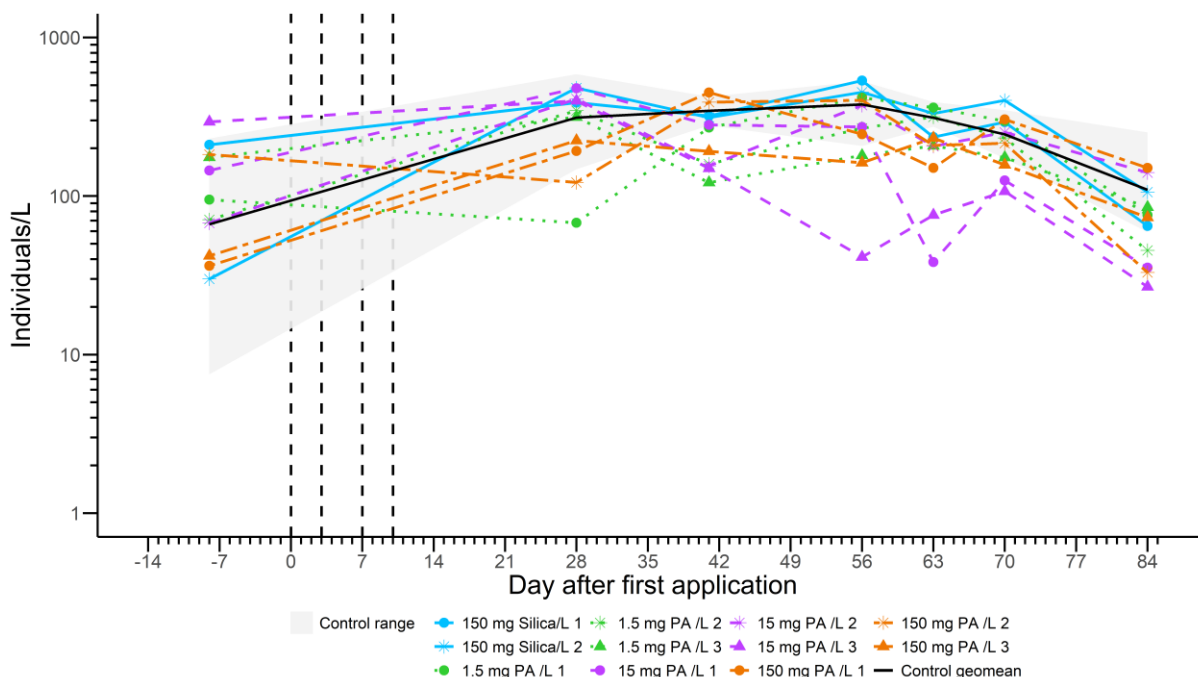
The mean abundance of *Hexarthra* sp. (Figure 29) ranged from 13.3  $\times/\div$  1.97 Ind. L<sup>-1</sup> on day -8 to 520.3  $\times/\div$  1.58 Ind. L<sup>-1</sup> on day 28 in the control. The mean abundance of *Hexarthra* sp. ranged from 4.5  $\times/\div$  1.36 Ind. L<sup>-1</sup> on day -8 to 336.6  $\times/\div$  1.28 Ind. L<sup>-1</sup> on day 70 in the Silica treatment. The mean abundance of *Hexarthra* sp. ranged from 8.8  $\times/\div$  2.38 Ind. L<sup>-1</sup> on day -8 to 293.2  $\times/\div$  4.17 Ind. L<sup>-1</sup> on day 28 in the LowPA treatment. The mean abundance of *Hexarthra* sp. ranged from 19.8  $\times/\div$  1.67 Ind. L<sup>-1</sup> on day -8 to 889.2  $\times/\div$  2.2 Ind. L<sup>-1</sup> on day 28 in the MedPA treatment.



**Figure 29** Abundance of *Hexarthra* sp. [Ind. L<sup>-1</sup>]

The mean abundance of *Hexarthra* sp. ranged from 10.7  $\times/\div$  3.05 Ind. L<sup>-1</sup> on day -8 to 260.6  $\times/\div$  2.26 Ind. L<sup>-1</sup> on day 28 in the HighPA treatment. The abundance of *Hexarthra* sp. Rose from preapplication phase until day 28 and declined again after. The Silica treatment had significantly higher abundances on day 56. On day 28 both replicates were below the control range, but the difference was not significant (MDD = 84). Neither polyamide showed significant differences with individual replicates out of the control range.

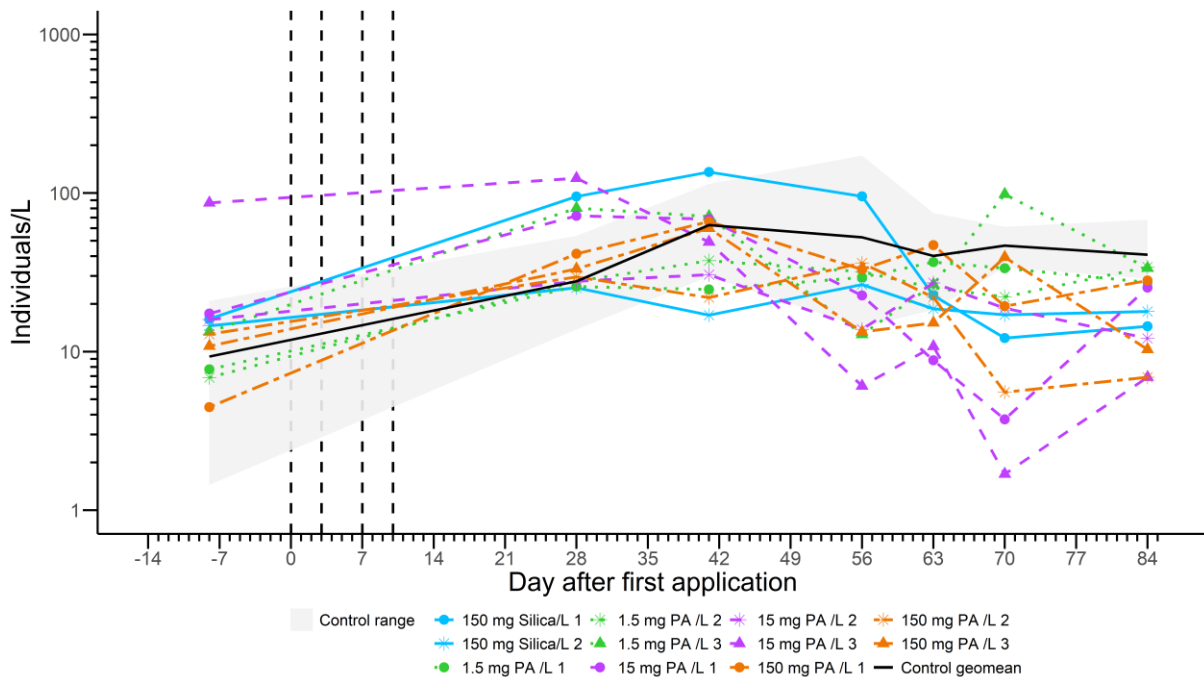
The mean abundance of nauplia (Figure 30) ranged from 66.4  $\times/\div$  3.83 Ind. L<sup>-1</sup> on day -8 to 378.4  $\times/\div$  1.49 Ind. L<sup>-1</sup> on day 56 in the control. The mean abundance of nauplia ranged from 79.4  $\times/\div$  3.96 Ind. L<sup>-1</sup> on day -8 to 491.6  $\times/\div$  1.13 on day 56 in the Silica treatment. The mean abundance of nauplia ranged from 66.4  $\times/\div$  1.4 Ind. L<sup>-1</sup> on day 84 to 287.0  $\times/\div$  1.34 Ind. L<sup>-1</sup> on day 63 in the LowPA treatment. The mean abundance of nauplia ranged from 51.0  $\times/\div$  2.43 Ind. L<sup>-1</sup> on day 84 to 427.5  $\times/\div$  1.1 Ind. L<sup>-1</sup> on day 28 in the MedPA treatment.



**Figure 30** Abundance of nauplia [Ind. L<sup>-1</sup>]

The mean abundance of nauplia ranged from 65.3  $\times/\div$  2.45 Ind. L<sup>-1</sup> on day -8 to 323.1  $\times/\div$  1.58 Ind. L<sup>-1</sup> on day 41 in the HighPA treatment. The abundance of nauplia rose after application in all mesocosms and declined at the half way mark of the study to preapplication values. The MedPA treatment had significantly lower numbers of nauplia compared to the control on day 63. The Silica, LowPA and HighPA treatments showed no significant differences compared to the control.

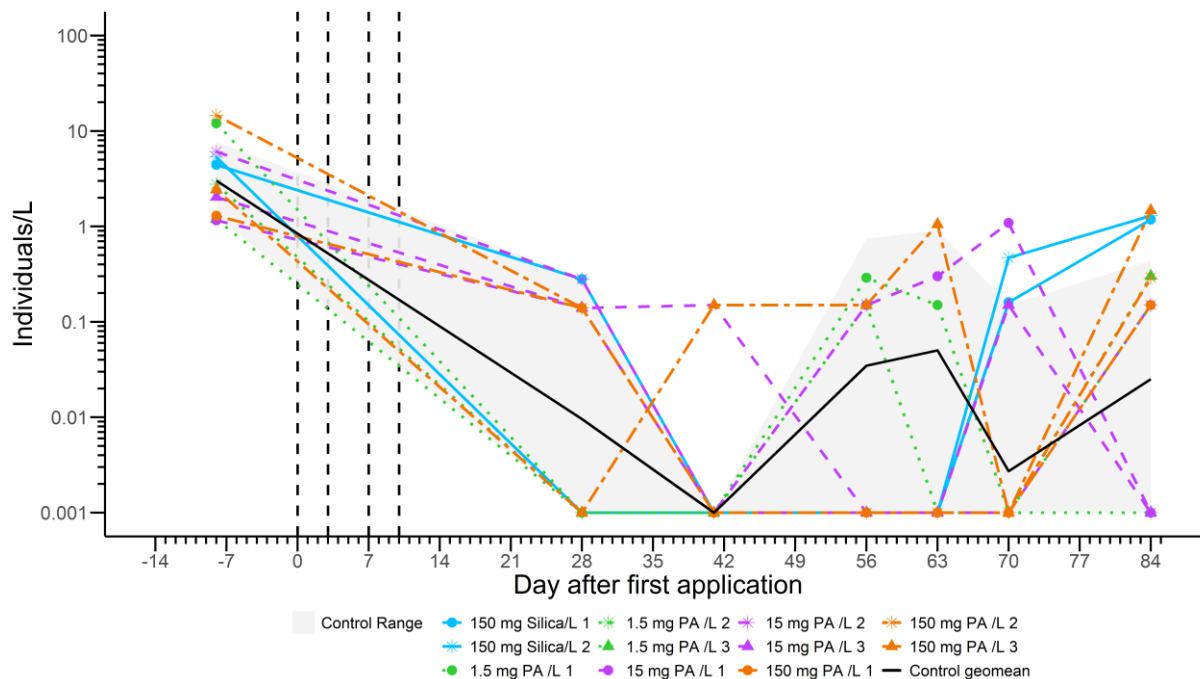
The mean abundance of Cyclopidae (Figure 31) ranged from  $9.3 \times / \div 2.94 \text{ Ind. L}^{-1}$  on day -8 to  $62.8 \times / \div 1.82 \text{ Ind. L}^{-1}$  on day 41 in the control. The mean abundance of Cyclopidae ranged from  $14.4 \times / \div 1.27 \text{ Ind. L}^{-1}$  on day 70 to  $50.3 \times / \div 2.48 \text{ Ind. L}^{-1}$  on day 56 in the Silica treatment. The mean abundance of Cyclopidae ranged from  $8.9 \times / \div 1.42 \text{ Ind. L}^{-1}$  on day -8 to  $41.8 \times / \div 2.16 \text{ Ind. L}^{-1}$  on day 70 in the LowPA treatment.



**Figure 31** Abundance of Cyclopidae [ $\text{Ind. L}^{-1}$ ]

The mean abundance of Cyclopidae ranged from  $4.9 \times / \div 3.41 \text{ Ind. L}^{-1}$  on day 70 to  $62.8 \times / \div 2.13 \text{ Ind. L}^{-1}$  on day 28 in the MedPA treatment. The mean abundance of Cyclopidae ranged from  $8.5 \times / \div 1.77 \text{ Ind. L}^{-1}$  on day -8 to  $44.3 \times / \div 1.84 \text{ Ind. L}^{-1}$  on day 41 in the HighPA treatment. On the last studyday, day 84, the MedPA and HighPA treatment had significantly reduced abundances compared to the control. The abundances of Cyclopidae increased after application in all treatments and the control. Following day 41 only singular replicates had higher abundances as the control geomean. All replicates of both treatments are below the control range. The LowPA and Silica treatments showed no significant differences compared to the control.

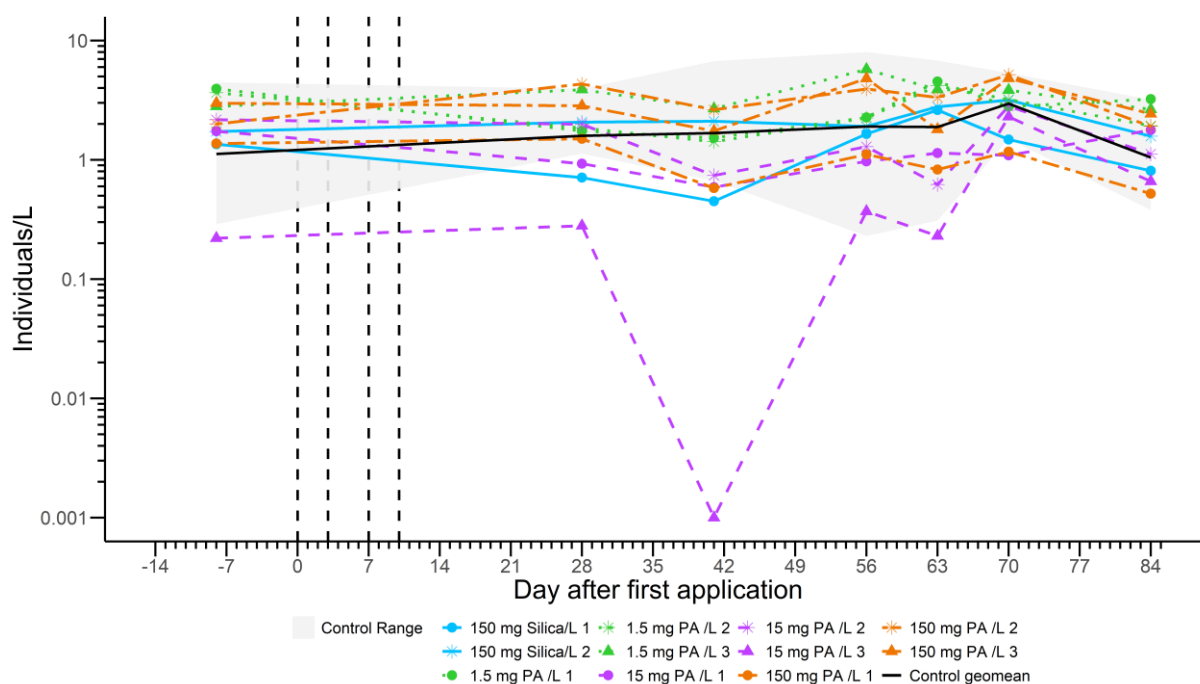
The mean abundance of *Lecane* sp. (Figure 32) ranged from 0 Ind. L<sup>-1</sup> on day 41 to 3.0 ×/÷ 2.25 Ind. L<sup>-1</sup> on day -8 in the control. The mean abundance of *Lecane* sp. ranged from 0 Ind. L<sup>-1</sup> on day 41, 56 and 63 to 4.8 ×/÷ 1.14 Ind. L<sup>-1</sup> on day -8 in the Silica treatment. The mean abundance of *Lecane* sp. ranged from 0 Ind. L<sup>-1</sup> on days 28,41 and 70 to 3.4 ×/÷ 3.22 Ind. L<sup>-1</sup> on day -8 in the LowPA treatment.



**Figure 32** Abundance of *Lecane* sp. [Ind. L<sup>-1</sup>]

The mean abundance of *Lecane* sp. ranged from 0.005 ×/÷ 18.05 Ind. L<sup>-1</sup> on days 41 and 56 to 2.4 ×/÷ 2.32 Ind. L<sup>-1</sup> on day -8 in the MedPA treatment. The mean abundance of *Lecane* sp. ranged from 0 Ind. L<sup>-1</sup> on day 70 to 3.6 ×/÷ 3.52 Ind. L<sup>-1</sup> on day -8 in the HighPA treatment. The Silica treatment had significantly higher abundances on day 84. Neither polyamide treatment showed significant differences compared to the control

The mean abundance of *Chaoborus* sp. (Figure 33) ranged from  $1.0 \times / \div 2.37$  Ind. L<sup>-1</sup> on day 84 to  $3.0 \times / \div 1.75$  Ind. L<sup>-1</sup> on day 70 in the control. The mean abundance of *Chaoborus* sp. ranged from  $1.0 \times / \div 2.98$  Ind. L<sup>-1</sup> on day 41 to  $2.7 \times / \div 1.04$  Ind. L<sup>-1</sup> on day 63 in the Silica treatment. The mean abundance of *Chaoborus* sp. ranged from  $1.8 \times / \div 1.42$  Ind. L<sup>-1</sup> on day 41 to  $4.2 \times / \div 1.08$  Ind. L<sup>-1</sup> on day 63 in the LowPA treatment.

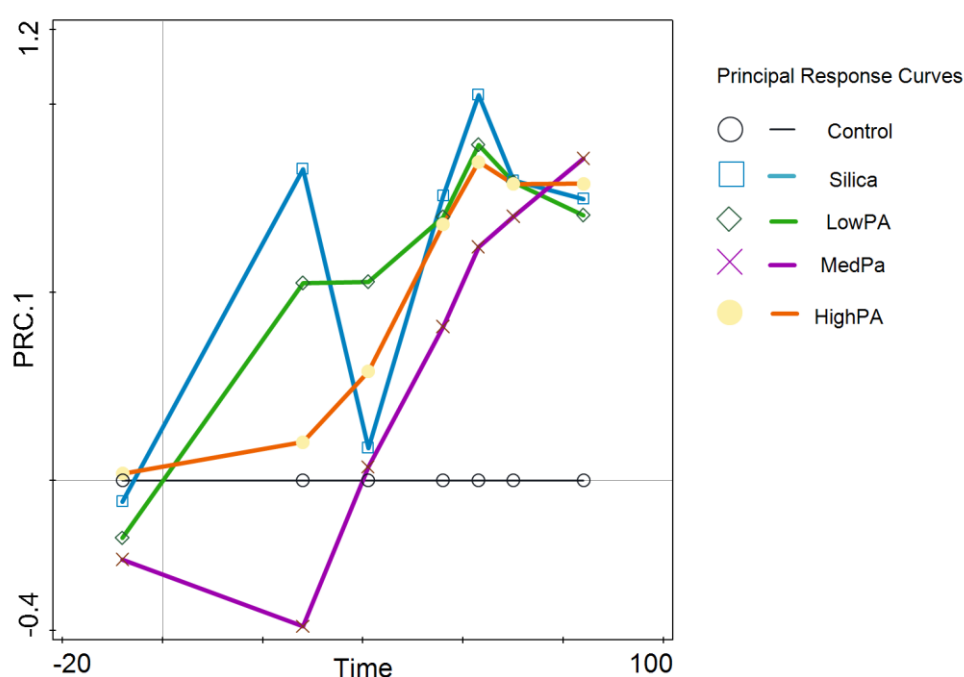


**Figure 33** Abundance of *Chaoborus* sp. [Ind. L<sup>-1</sup>] in zooplankton samples

The mean abundance of *Chaoborus* sp. ranged from  $0.1 \times / \div 42.55$  Ind. L<sup>-1</sup> on day 41 to  $1.9 \times / \div 1.65$  Ind. L<sup>-1</sup> on day 70 in the MedPA treatment. The mean abundance of *Chaoborus* sp. ranged from  $1.3 \times / \div 2.29$  Ind. L<sup>-1</sup> on day 84 to  $3.1 \times / \div 2.31$  Ind. L<sup>-1</sup> on day 70 in the HighPA treatment. There were no significant differences compared to the control group throughout the study and thus the NOEC is = 150 mg L<sup>-1</sup>. The abundances of *Chaoborus* sp. in the zooplankton traps was relatively constant except for one replicate in the MedPA treatment, which was below the control range before the first application and had a drop of abundance on day 41.

#### 4.2.2 PRC of zooplankton community

The PRC of the zooplankton community (Figure 34) shows the effect of the treatments on community level. The Silica treatment had moderate effects with a slight increase on day 21, a dip in effect on day 41 and an increase again starting day 56. The LowPA treatment showed a similar curve like the Silica treatment without the dip on day 21. The MedPA treatment showed a negative effect at the start and an increase after. The positive effects started on day 56. HighPA treatment, like the LowPA treatment showed an increasing effect of the particles on the community over time. Before the first application (vertical line; day 0) there were no differences compared to the control line. The MedPA treatment, however, started with the lowest value before application.



**Figure 34** Principal response curve of zooplankton community

The changes on the zooplankton community were not significant ( $p$ -value = 0.058). 14 of 32 taxa had a species score with an absolute value  $> 0.5$ . 12 of these 14 species scores were negative. *Synchaeta* sp. had the highest absolute value of the species scores with 3.74 and a positive sign. The other taxon with a positive sign was *Rhinoglena* sp. *Keratella quadrata*, *Scapholeberis* sp., *Graptoleberis* sp., and Chironomidae larvae had the highest species scores with negative sign. All four values ranged from -1.47 (*Keratella quadrata*) to -1.45 (Chironomidae larvae). Three other taxa, *Chydorus sphaericus*, Ostracoda and *Simocephalus* sp., had a negative value of -1 or greater. Cyclopidae, *Polyarthra* sp., *Daphnia longispina*, *Alonella* sp. and *Hexarthra* sp. had species scores between -0.5 and -1.0.

### 4.2.3 Dominance

Table 8 shows the temporal development of the relative density [%] of the most abundant and relevant zooplankton taxa. All taxa, that exceeded a dominance of over 1% at least on one sampling are presented. In the control, *Keratella quadrata* was eudominant initially with 73.6% before application and decreased sharply over the course of the study until less than 1% of all individuals belonged to this species on day 56. In contrast, the genus *Hexarthra* showed a strong increase from day 28 and reached the highest relative density of 35.9% on day 84. The dominance of nauplii increased continuously and reached the highest value of 49.2% after day 56, before decreasing slightly. Cyclopidae between day 41 and 84, *Polyarthra* sp. throughout the study were classified as dominant taxa. *Daphnia longispina*, *Simocephalus* sp. and *Chydorus sphaericus* were classified as subdominant starting day 41 until the end of the experiment. The ostracods were mainly recedent and subrecedent with one exception on day 84 (2%). In the control *Mytilinia* sp. was subrecedent continuously. In the Silica treatment most taxa were less dominant than in the control except the nauplia which were eudominant after the application. *Synchaeta* sp. (25.2%) on day 28 and *Polyarthra* (28.0%) on day 41 peaked and were the second most dominant species on the respective days. *Hexarthra* sp. was classified as eudominant on day 41 and after, with a dominance of 64.1% on day 84. In the LowPA treatment nauplia were eudominant from day 41 to 70 and *Hexarthra* sp. in all samplings after application, except day 56 with 8.2%. *Keratella quadrata* was eudominant until day 41 and subrecedent after. Cyclopidae were dominant and *Daphnia longispina* subdominant occasionally. *Chydorus sphaericus*, *Simocephalus* sp., *Mytilinia* sp. and *Graptoleberis* sp. were mainly subrecedent

**Table 8:** Relative densities [%] of most abundant zooplankton taxa

<b>Control</b>							
<b>Day</b>	<b>-8</b>	<b>28</b>	<b>41</b>	<b>56</b>	<b>63</b>	<b>70</b>	<b>84</b>
<i>Chydorus sphaericus</i>	0	0	0.9	2.3	1.8	1.3	2
Cyclopidae	0.7	1.7	7.4	9	6.9	8	10.4
<i>Daphnia longispina</i>	0.9	0.4	2	5.7	2.5	5.5	3
<i>Graptoleberis</i> sp.	0	0	0	0.3	0.6	0.7	1.3
<i>Hexarthra</i> sp.	0.9	31.9	18.5	13.4	15.7	21.9	35.9
<i>Keratella quadrata</i>	73.6	29.9	8.4	0.5	0.7	0.7	0.5
<i>Mytilinia</i> sp.	0.7	0.2	0	0	0	0	0.1
Nauplia	6.2	19.3	36.2	49.2	47.6	42.2	30.9
Ostracoda	0.1	0.1	0.6	1.7	1.1	1.4	2
<i>Polyarthra</i> sp.	10.7	13.8	20.5	14.1	19.4	15.8	11.2
<i>Simocephalus</i> sp.	0.1	0.1	0.8	2	1.7	0.7	1.5
<i>Synchaeta</i> sp.	5.4	2.4	3.7	0.8	0.4	0.2	0

**Table 8** (continued) Relative densities [%] of most abundant zooplankton taxa

<b>Silica</b>							
<b>Day</b>	<b>-8</b>	<b>28</b>	<b>41</b>	<b>56</b>	<b>63</b>	<b>70</b>	<b>84</b>
<i>Chydorus sphaericus</i>	0	0	0.3	0.3	0.5	0.3	0.8
Cyclopidae	0.9	5.1	8.5	6.2	3	1.8	3.7
<i>Daphnia longispina</i>	0	0.1	0.1	1.4	2.7	3.3	0.9
<i>Graptoleberis</i> sp.	0	0	0.1	0	0.7	0.2	0.9
<i>Hexarthra</i> sp.	0.3	3.8	20.2	26	29.1	42.9	64.1
<i>Keratella quadrata</i>	79.9	20.8	1.8	0.2	0.3	0.2	0.3
<i>Mytilina</i> sp.	0.8	0.3	0	0	0	0	0.1
Nauplia	7	36.5	35.3	50	41.7	43.6	19.8
Ostracoda	0.1	0	0.3	0.5	0.4	0.6	1.3
<i>Polyarthra</i> sp.	8.4	7.1	28	9.7	10.8	5.6	1.9
<i>Simocephalus</i> sp.	0	0.1	1.9	0.4	0.5	0.1	0.5
<i>Synchaeta</i> sp.	1.5	25.2	3.2	4.9	9.6	0.9	3.7
<b>LowPA</b>							
<b>Day</b>	<b>-8</b>	<b>28</b>	<b>41</b>	<b>56</b>	<b>63</b>	<b>70</b>	<b>84</b>
<i>Chydorus sphaericus</i>	0	0	0.4	0.4	0.7	0.6	0.4
Cyclopidae	0.7	3.1	7.4	4.6	6	9.8	7.7
<i>Daphnia longispina</i>	0.2	0.4	1.2	5.4	3.9	4	5
<i>Graptoleberis</i> sp.	0	0	0	0.1	0.1	0.3	0.4
<i>Hexarthra</i> sp.	0.9	33.7	24.1	8.2	11.5	22.7	52.8
<i>Keratella quadrata</i>	73.9	19.3	14.3	0.2	0.1	0.2	0.2
<i>Mytilina</i> sp.	0.6	0.3	0	0	0	0	0.1
Nauplia	8.8	16.9	30.4	53.7	59.7	44.9	16.8
Ostracoda	0.1	0	0.3	0.8	0.6	0.9	1.5
<i>Polyarthra</i> sp.	11.9	10.5	13.7	12.6	9.3	13.7	8.3
<i>Simocephalus</i> sp.	0.1	0.1	0.4	0.1	1.3	0.9	0.7
<i>Synchaeta</i> sp.	1.2	15.2	7.3	12.9	5.5	1.2	4.6
<b>MedPA</b>							
<b>Day</b>	<b>-8</b>	<b>28</b>	<b>41</b>	<b>56</b>	<b>63</b>	<b>70</b>	<b>84</b>
<i>Chydorus sphaericus</i>	0	0	0.3	1.8	4.3	0.8	0.4
Cyclopidae	1.4	3	7.4	4.2	5.5	1.7	3.2
<i>Daphnia longispina</i>	0.2	0.8	4.2	7.8	6.5	5.2	2
<i>Graptoleberis</i> sp.	0	0	0	0.1	0.3	0.2	0.2
<i>Hexarthra</i> sp.	0.8	43.8	30.7	11	37.6	52.8	68.7
<i>Keratella quadrata</i>	76.7	33.3	23.4	0.9	0.5	0.6	1.6
<i>Mytilina</i> sp.	1.3	0.3	0	0	0	0	0
Nauplia	6	17.4	29.1	67.8	37.7	33.6	14.7
Ostracoda	0.1	0.1	0.2	0.8	0.8	0.7	0.6
<i>Polyarthra</i> sp.	9	0.9	3.9	4.1	1.3	2.7	6.1
<i>Simocephalus</i> sp.	0.1	0.1	0.2	0.3	4.3	0.7	0.3
<i>Synchaeta</i> sp.	4	0.1	0.5	0.6	0.6	0.3	1.6

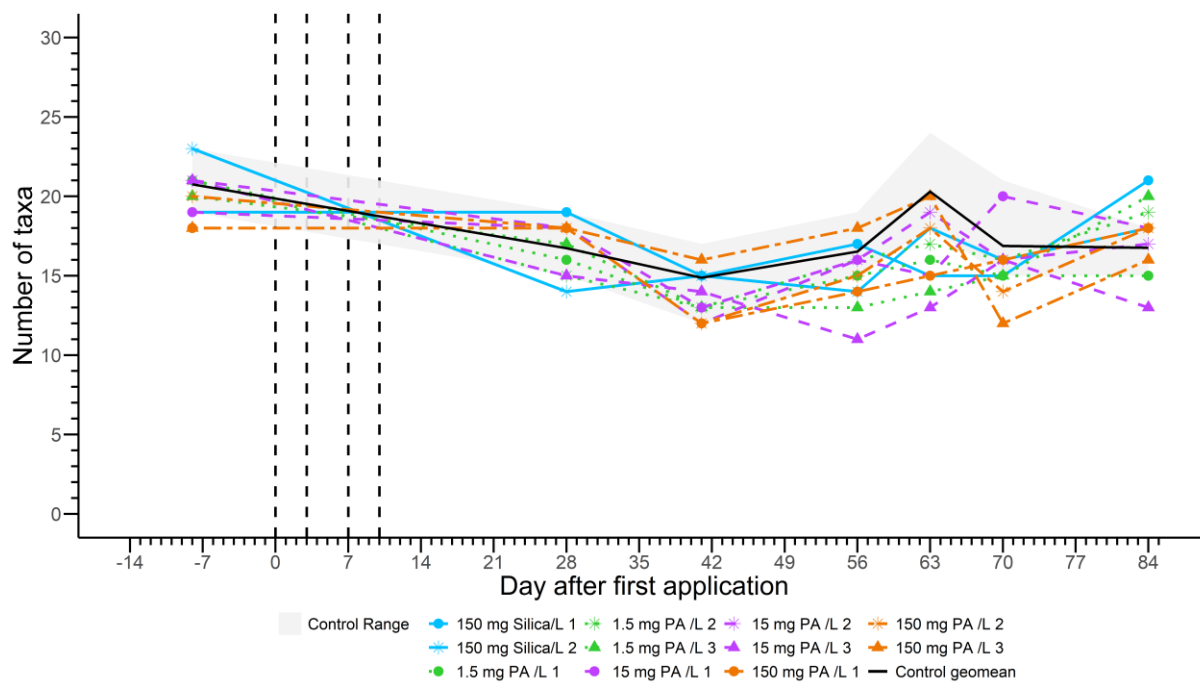
**Table 8** (continued) Relative densities [%] of most abundant zooplankton taxa

HighPA							
Day	-8	28	41	56	63	70	84
<i>Chydorus sphaericus</i>	0	0	0.4	0.6	3.4	1.8	0.9
Cyclopidae	0.5	2.3	5.3	6.5	7.6	4.9	5.1
<i>Daphnia longispina</i>	0.3	0.4	4.9	3.1	5.8	3.8	3
<i>Graptoleberis</i> sp.	0	0	0	0.1	0.7	0.6	0.5
<i>Hexarthra</i> sp.	0.9	21	17.4	16.5	18.3	22.1	49.4
<i>Keratella quadrata</i>	83.6	53.3	17.1	0.1	0.1	0.1	0.3
<i>Mytilina</i> sp.	0.4	0.2	0	0	0.1	0	0.3
Nauplia	4.8	11.8	36.9	63.7	53.5	51.7	29
Ostracoda	0	0	0.1	1	0.8	1	0.7
<i>Polyarthra</i> sp.	6.8	3.3	14	5.7	2.3	8.6	4.7
<i>Simocephalus</i> sp.	0	0	0.4	0.3	0.8	1.1	0.6
<i>Synchaeta</i> sp.	1.7	7.2	2.8	1	5	2.9	4

In the MedPA treatment *Keratella quadrata* until day 28, nauplia and *Hexarthra* sp. starting day 28 were eudominant. *Polyarthra* sp. were dominant on day -8 and *Daphnia longispina* on days 56 through 70. Ostracods were subrecedent throughout the study in the MedPA treatment. In the HighPA treatment *Keratella quadrata* had the highest proportion in dominance on day -8 with 83.6%. *Synchaeta* sp. were dominant on days 28 and 63. *Polyarthra* sp. were eudominant on day 41 and otherwise dominant or subdominant. The course of dominance of the other taxa is comparable to that of the control.

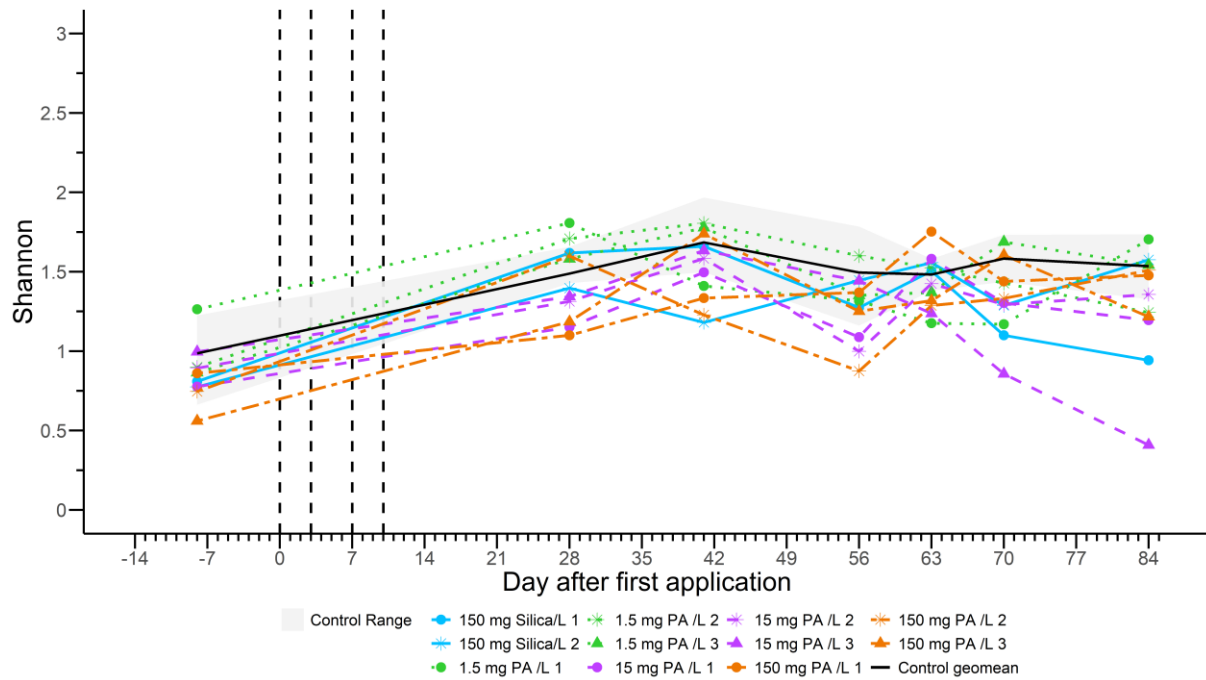
#### 4.2.4 Diversity indices

The number of taxa in the zooplankton samples (Figure 35) had no significant differences between the controls and treatments. The number of taxa ranged from 14.9  $\times/\div$  1.16 on day 41 to 20.8  $\times/\div$  1.07 on day -8 in the control. The mean number of taxa ranged from 15.0  $\times/\div$  1.00 on day 41 to 20.9  $\times/\div$  1.14 on day -8 in the Silica treatment. The mean number of taxa ranged from 13.0  $\times/\div$  1 on day 41 to 20.7  $\times/\div$  1.03 on day -8 in the LowPA treatment. The mean number of taxa ranged from 13.0  $\times/\div$  1.08 on day 41 to 20.3  $\times/\div$  1.06 on day -8 in the MedPA treatment. The mean number of taxa ranged from 13.2  $\times/\div$  1.18 on day 41 to 18.6  $\times/\div$  1.06 on day -8 in the HighPA treatment. The number of taxa was constant, with a slight decline throughout the study, but dispersion was higher in the second half of the study. There were no statistically significant differences compared to the control.



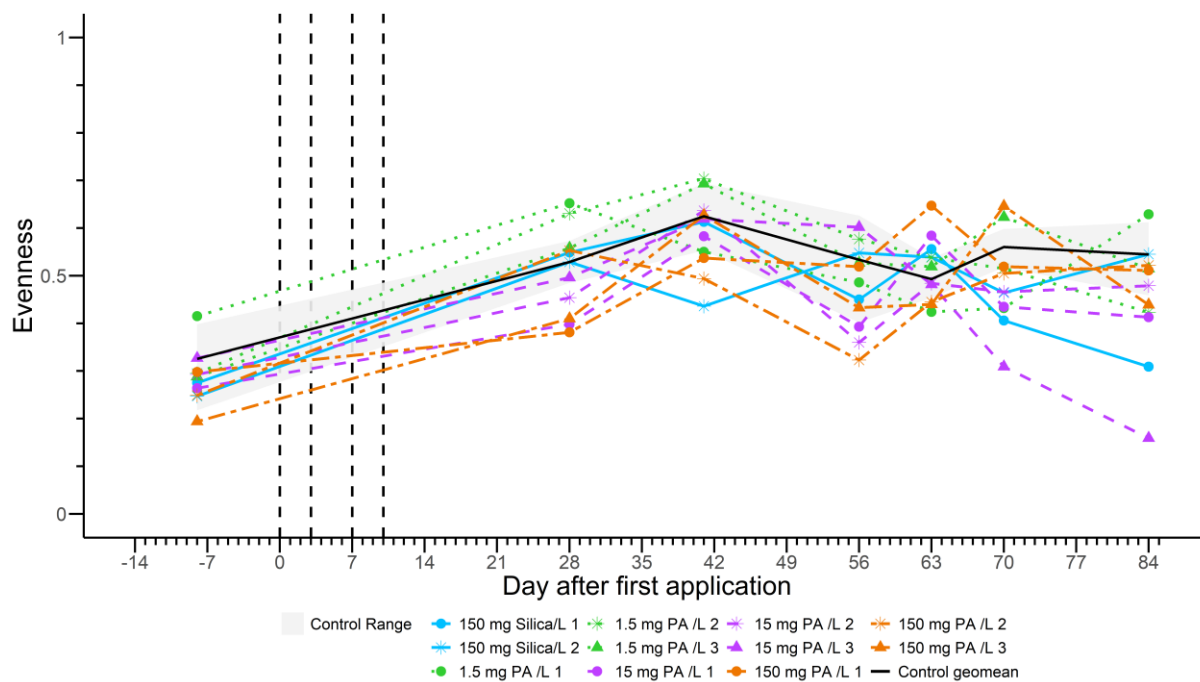
**Figure 35** Number of zooplankton taxa

The mean Shannon index (Figure 36) ranged from  $1.0 \times/\div 1.27$  on day -8 to  $1.7 \times/\div 1.11$  on day 41 in the control. The mean Shannon index ranged from  $0.8 \times/\div 1.03$  on day -8 to  $1.5 \times/\div 1.02$  on day 63 in the Silica treatment. The mean Shannon index ranged from  $1.0 \times/\div 1.23$  on day -8 to  $1.7 \times/\div 1.07$  on day 28 in the LowPA treatment. The mean Shannon index ranged from  $0.9 \times/\div 1.93$  on day 84 to  $1.6 \times/\div 1.05$  on day 41 in the MedPA treatment. The mean Shannon index ranged from  $0.7 \times/\div 1.24$  on day -8 to  $1.5 \times/\div 1.1$  on day 70 in the HighPA treatment. Contrary to the number of taxa the Shannon index rose in the first half of the study, but the dispersion increased later in the study as well. The MedPA treatment had a significantly lower Shannon index compared to the control on day 70.



**Figure 36** Shannon index of zooplankton community

The mean evenness (Figure 37) ranged from  $0.33 \times / \div 1.27$  on day -8 to  $0.62 \times / \div 1.09$  on day 41 in the control. The mean evenness ranged from  $0.26 \times / \div 1.08$  on -8 to  $0.55 \times / \div 1.02$  on day 63 in the Silica treatment. The mean evenness ranged from  $0.33 \times / \div 1.23$  on day -8 to  $0.64 \times / \div 1.15$  on day 41 in the LowPA treatment. The mean evenness ranged from  $0.29 \times / \div 1.11$  on day -8 to  $0.61 \times / \div 1.05$  on day 41 in the MedPA treatment. The mean evenness ranged from  $0.24 \times / \div 1.24$  on day -8 to  $0.55 \times / \div 1.14$  on day 70 in the HighPA treatment. The MedPA treatment had a significantly lower evenness compared to the control on day 70.



**Figure 37** Evenness of zooplankton community

#### 4.2.5 Statistical evaluation

Table 9 shows the effects on the zooplankton taxa. The effects were classified according to EFSA (see Table 4). In the Silica treatment all taxa showed no relevant effects and were classified as class 1, except *Rhinoglena* sp. This genus had pronounced indirect effects with recovery and were assigned to class 3A-. The only taxon which had slight direct effects in all polyamide treatments were the ostracods. The LowPA treatment as well as the MedPA and HighPA treatment were assigned effect class 2-. The Cyclopidae and the total sum both showed slight effects in the MedPA and HighPA treatments (class 2-). All other taxa and sums of taxa were assigned effect class 1 due to not showing effects compared to the control. Overall 31 taxa, including sums are classified as MDD Category 1 and one as MDD Category 2.

**Table 9** Classification of the effect on zooplankton over the entire duration of the study according to EFSA (2013)

MDD Category	Taxon	Silica	LowPA	MedPA	HighPA
1	<i>Alonella</i> sp.	1	1	1	1
1	<i>Ascomorpha</i> sp.	1	1	1	1
1	<i>Asplanchna</i> sp.	1	1	1	1
1	<i>Brachionus</i> sp.	1	1	1	1
1	<i>Cephalodella</i> sp.	1	1	1	1
1	<i>Chaoborus</i> sp.	1	1	1	1
1	<i>Chaoborus</i> sp. pupa	1	1	1	1
1	Chironomidae	1	1	1	1
1	<i>Chydorus sphaericus</i>	1	1	1	1
1	Cyclopidae	1	1	2-	2-
1	<i>Daphnia pulex</i>	1	1	1	1
1	<i>Daphnia</i> sp.	1	1	1	1
1	Diaptomidae	1	1	1	1
1	<i>Filinia</i> sp.	1	1	1	1
1	<i>Graptoleberis</i> sp.	1	1	1	1
1	<i>Hexarthra</i> sp.	1	1	1	1
1	<i>Keratella quadrata</i>	1	1	1	1
1	<i>Lecane</i> sp.	1	1	1	1
1	<i>Lepadella</i> sp.	1	1	1	1
1	<i>Mytilina</i> sp.	1	1	1	1
1	Nauplia	1	1	1	1
1	Ostracoda	1	2-	2-	2-4B-
1	<i>Platyias quadricornis</i>	1	1	1	1
1	<i>Polyarthra</i> sp.	1	1	1	1
1	<i>Rhinoglena</i> sp.	3A+	1	1	1
1	<i>Scapholeberis</i> sp.	1	1	1	1
1	Sum Cladocera	1	1	1	1
1	Sum Rotaria	1	1	1	1
1	<i>Synchaeta</i> sp.	1	1	1	1
1	<i>Testudinella</i> sp.	1	1	1	1
1	Total Sum	1	1	2-	2-
2	Sum Copepoda	1	1	1	1
3	Bdelloidae	1	1	1	1
3	<i>Daphnia longispina</i>	1	1	1	1
3	<i>Daphnia magna</i>	1	1	1	1
3	<i>Euchlanis</i> sp.	1	1	1	1
3	<i>Simocephalus</i> sp.	1	1	1	1

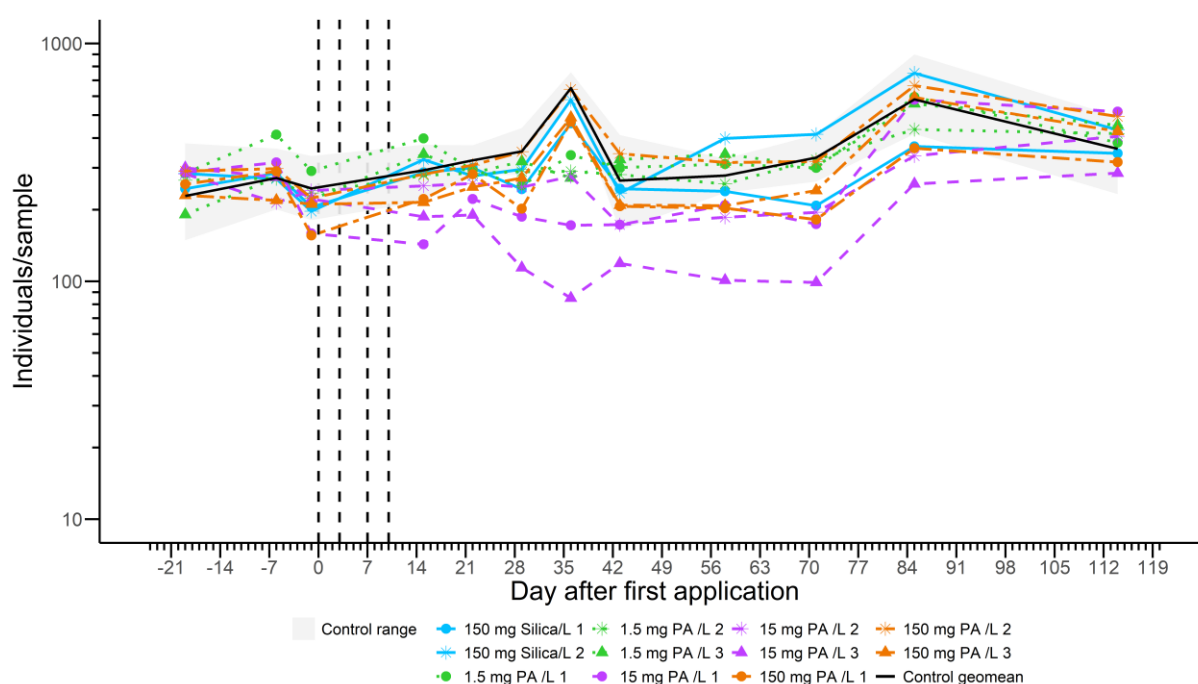
## 4.3 Macroinvertebrates



(Picture generated with OpenAI DALL-E)

### 4.3.1 Abundance data

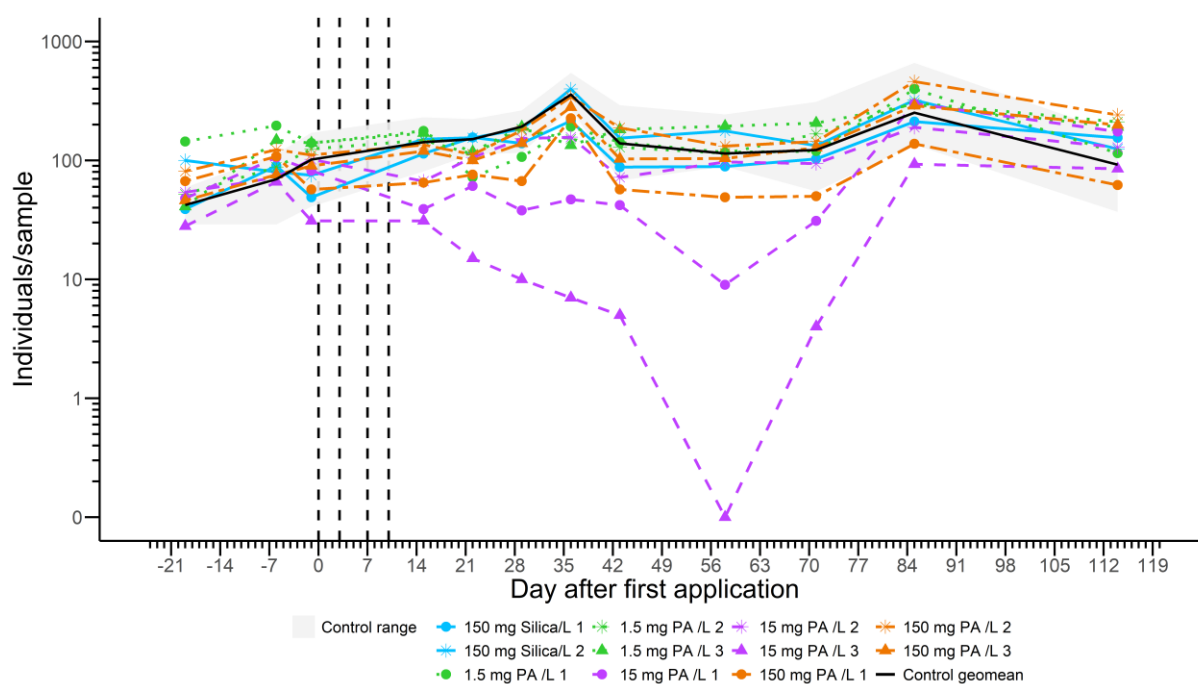
The mean abundance of all macroinvertebrates (Figure 38) ranged from 228.4  $\times/\div$  1.59 Ind. sample<sup>-1</sup> on day -19 to 649.4  $\times/\div$  1.09 Ind. sample<sup>-1</sup> on day 36 in the control. The mean abundance of all macroinvertebrates ranged from 201.0  $\times/\div$  1.03 Ind. sample<sup>-1</sup> on day -1 to 526.4  $\times/\div$  1.65 Ind. sample<sup>-1</sup> on day 85 in the Silica treatment. The mean abundance of all macroinvertebrates ranged from 243.7  $\times/\div$  1.24 Ind. sample<sup>-1</sup> on day -19 to 525 Ind. sample<sup>-1</sup>  $\times/\div$  1.18 on day 85 in the LowPA treatment.



**Figure 38** Abundance of all macroinvertebrates [Ind. sample<sup>-1</sup>]

The mean abundance of all macroinvertebrates ranged from 149.8  $\times/\div$  1.44 Ind. sample<sup>-1</sup> on day 71 to 391.4  $\times/\div$  1.35 Ind. sample<sup>-1</sup> on day 114 in the MedPA treatment. The mean abundance of all macroinvertebrates ranged from 194.6  $\times/\div$  1.21 Ind. sample<sup>-1</sup> on day -1 to 527.7  $\times/\div$  1.19 Ind. sample<sup>-1</sup> on day 36 in the HighPA treatment. The total amount of macroinvertebrates stayed relatively constant throughout the study, with peaks on day 35 and 84. In general, the total abundances showed an increasing trend towards the end of the experiment. The Silica and HighPA treatments showed no significant differences compared to the control. The LowPA treatment had significantly lower abundances of macroinvertebrates on day 36. The MedPA treatment had significantly reduced numbers compared to the control on days 22, 29, 36 and 71, with one replicate having lower abundances as all controls from day 14 until the second to last sampling on day 85.

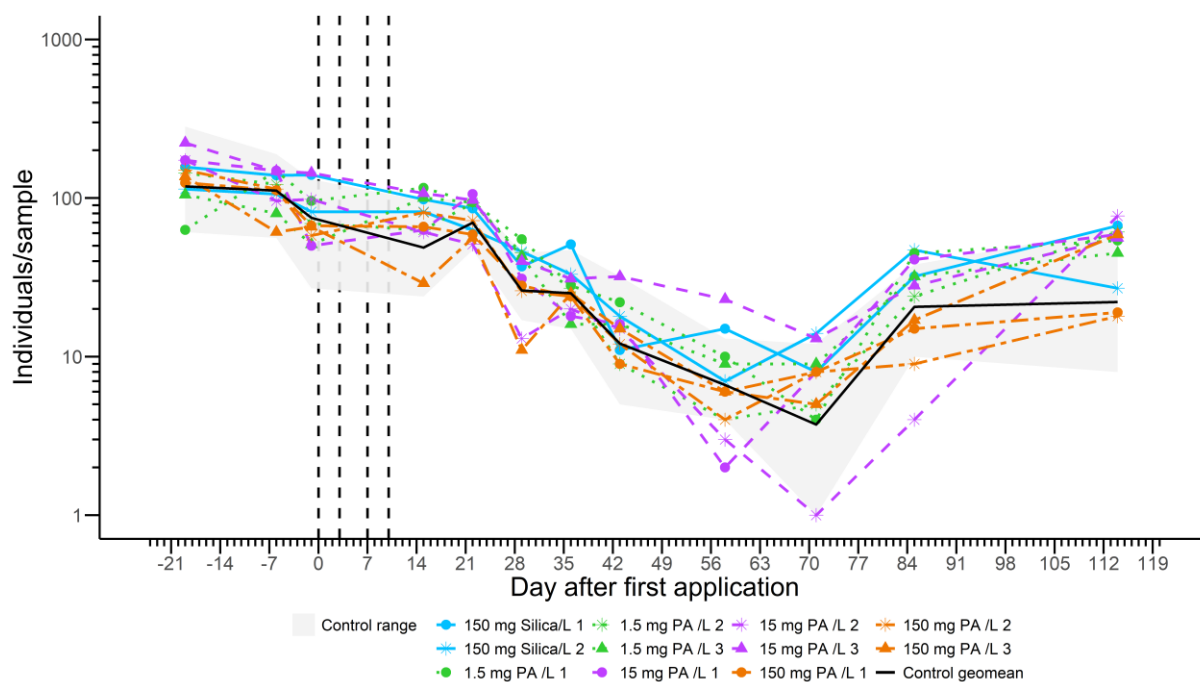
The mean abundance of *Chaoborus* sp. (Figure 39) ranged from  $42.1 \times/\div 1.34$  Ind. sample<sup>-1</sup> on day -19 to  $357.5 \times/\div 1.3$  Ind. sample<sup>-1</sup> on day 36 in the control. The mean abundance of *Chaoborus* sp. ranged from  $60.6 \times/\div 1.35$  Ind. sample<sup>-1</sup> on day -1 to  $292.9 \times/\div 1.55$  Ind. sample<sup>-1</sup> on day 36 in the Silica treatment. The mean abundance of *Chaoborus* sp. ranged from  $67.5 \times/\div 1.95$  Ind. sample<sup>-1</sup> on day -19 to  $330.0 \times/\div 1.18$  Ind. sample<sup>-1</sup> on day 85 in the LowPA treatment.



**Figure 39** Abundance of *Chaoborus* sp. larvae [Ind. sample<sup>-1</sup>]

The mean abundance of *Chaoborus* sp. ranged from  $4.4 \times/\div 32.88$  Ind. sample<sup>-1</sup> on day 58 to  $174.4 \times/\div 1.8$  Ind. sample<sup>-1</sup> on day 85 in the MedPA treatment. The mean abundance of *Chaoborus* sp. ranged from  $63.0 \times/\div 1.33$  Ind. sample<sup>-1</sup> on day -19 to  $278.7 \times/\div 1.23$  Ind. sample<sup>-1</sup> on day 36 in the HighPA treatment. The abundance of chaoborids stayed constant with a light increase throughout the study. Two of the three MedPA replicates had abundances clearly below the control range after the last application until day 84. The Silica and HighPA treatments showed no significant differences compared to the control. The LowPA treatment had significantly lower abundances of macroinvertebrates on day 36. The MedPA treatment had significantly reduced numbers compared to the control on days 15 through 36.

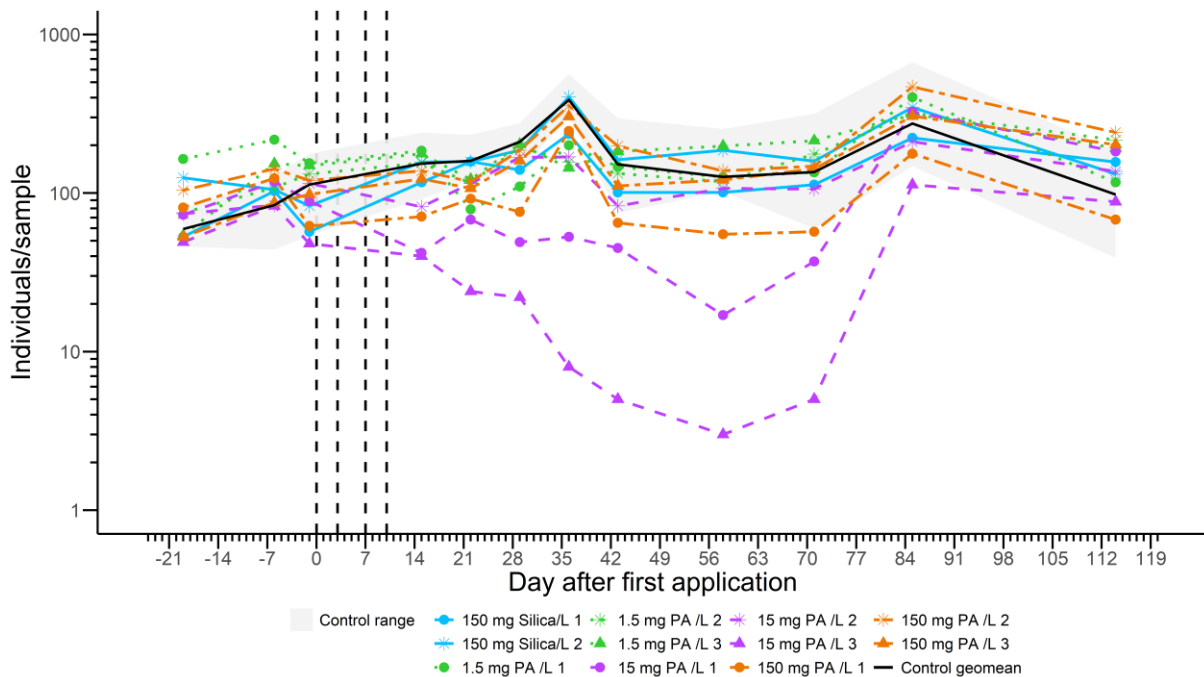
The mean abundance of *Cloeon dipterum* (Figure 40) ranged from  $3.7 \times / \div 2.46$  Ind. sample<sup>-1</sup> on day 71 to  $118.4 \times / \div 2.08$  Ind. sample<sup>-1</sup> on day -19 in the control. The mean abundance of *C. dipterum* ranged from  $10.2 \times / \div 1.71$  Ind. sample<sup>-1</sup> on day 58 to  $133.8 \times / \div 1.25$  Ind. sample<sup>-1</sup> on day -19 in the Silica treatment.



**Figure 40** Abundance of *Cloeon dipterum* larvae [Ind. sample<sup>-1</sup>]

The mean abundance of *C. dipterum* ranged from  $5.6 \times / \div 1.52$  Ind. sample<sup>-1</sup> on day 71 to  $113.3 \times / \div 1.37$  Ind. sample<sup>-1</sup> on day -6 in the LowPA treatment. The mean abundance of *C. dipterum* ranged from  $4.7 \times / \div 3.91$  Ind. sample<sup>-1</sup> on day 71 to  $188.3 \times / \div 1.16$  Ind. sample<sup>-1</sup> on day -19 in the MedPA treatment. The mean abundance of *C. dipterum* ranged from  $5.2 \times / \div 1.26$  Ind. sample<sup>-1</sup> on day 58 to  $137.0 \times / \div 1.1$  Ind. sample<sup>-1</sup> on day -19 in the HighPA treatment. All mesocosms in the treatments and control have their minimum between day 58 and 71 and increased afterwards again. There were no significant differences compared to the control group throughout the study and thus the NOEC is  $> 150$  mg L<sup>-1</sup>.

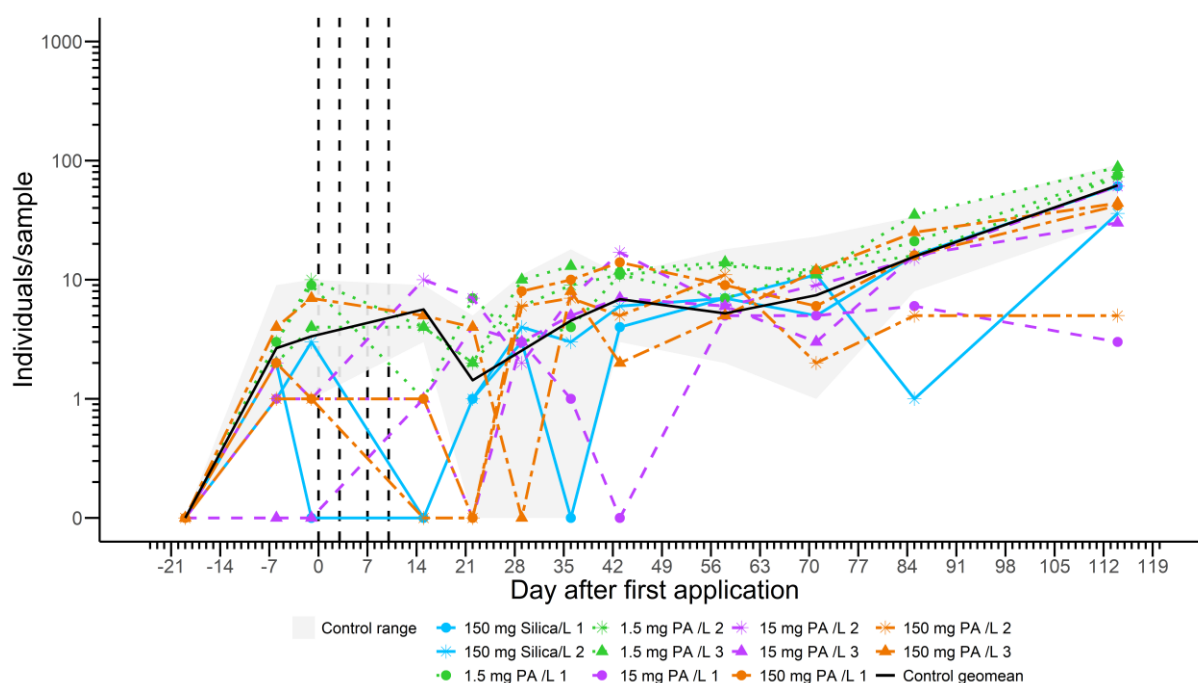
The mean abundance of the sum of all Diptera (Figure 41) ranged from  $59.3 \times/\div 1.26$  Ind. sample<sup>-1</sup> on day -19 to  $387.8 \times/\div 1.29$  Ind. sample<sup>-1</sup> on day 36 in the control. The mean abundance of all Diptera ranged from  $68.8 \times/\div 1.3$  Ind. sample<sup>-1</sup> on day -1 to  $308.8 \times/\div 1.46$  Ind. sample<sup>-1</sup> on day 36 in the Silica treatment.



**Figure 41** Summed abundance of Diptera larvae [Ind. sample<sup>-1</sup>]

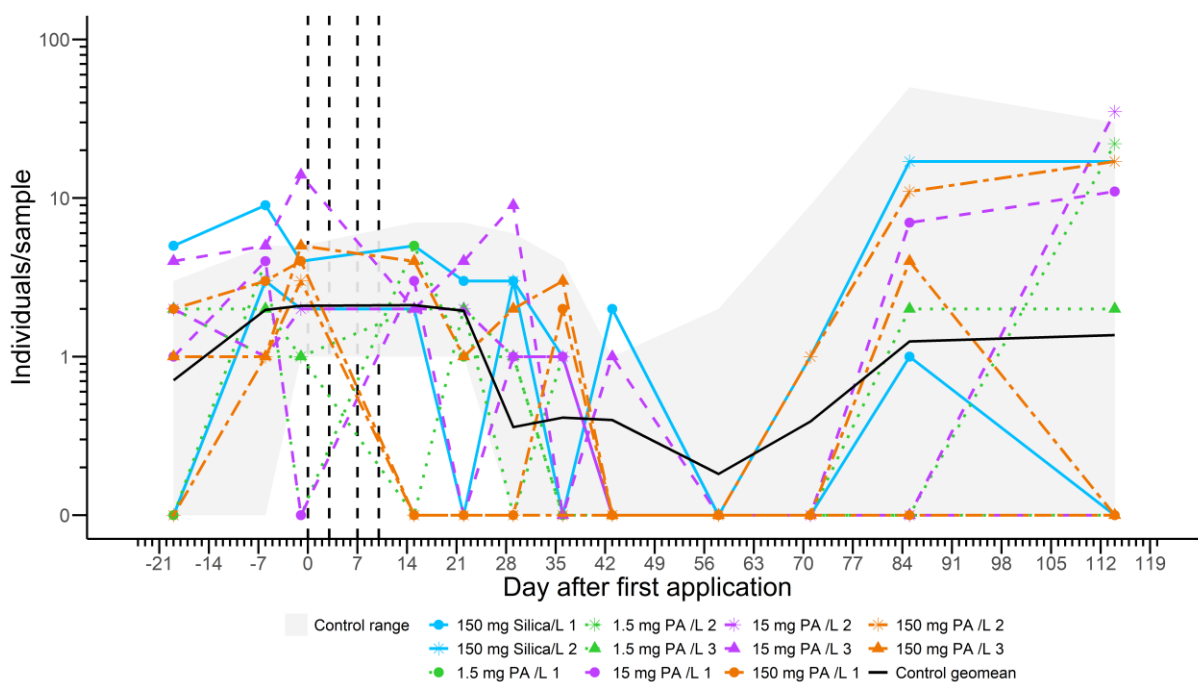
The mean abundance of all Diptera ranged from  $87.3 \times/\div 1.77$  Ind. sample<sup>-1</sup> on day -19 to  $343.5 \times/\div 1.15$  Ind. sample<sup>-1</sup> on day 85 in the LowPA treatment. The mean abundance of all Diptera ranged from  $17.6 \times/\div 5.97$  Ind. sample<sup>-1</sup> on day 58 to  $198.1 \times/\div 1.7$  Ind. sample<sup>-1</sup> on day 85 in the MedPA treatment. The mean abundance of all Diptera ranged from  $76.4 \times/\div 1.41$  Ind. sample<sup>-1</sup> on day -19 to  $298.3 \times/\div 1.2$  Ind. sample<sup>-1</sup> on day 36 in the HighPA treatment. The Silica and HighPA treatments showed no significant differences compared to the control. The LowPA treatment had significantly lower abundances of macroinvertebrates on day 36. The MedPA treatment had significantly reduced numbers compared to the control on days 15 through 36. Two replicates of the MedPA are below control range through days 15 to 71 mainly influenced by *Chaoborus* abundance.

The mean abundance of *Asellus aquaticus* (Figure 42) ranged from 0 Ind. sample<sup>-1</sup> on day -19 to 62.1  $\times/\div$  1.56 Ind. sample<sup>-1</sup> on day 114 in the control. The mean abundance of *Asellus aquaticus* ranged from 0 Ind. sample<sup>-1</sup> on day -19 to 46.9  $\times/\div$  1.45 Ind. sample<sup>-1</sup> on day 114 in the Silica treatment. The mean abundance of *Asellus aquaticus* ranged from 0 Ind. sample<sup>-1</sup> on day -19 to 78.4  $\times/\div$  1.11 Ind. sample<sup>-1</sup> on day 114 in the LowPA treatment. The mean abundance of *Asellus aquaticus* ranged from 0 Ind. sample<sup>-1</sup> on day -19 to 17.6  $\times/\div$  4.83 Ind. sample<sup>-1</sup> on day 114 in the MedPA treatment. The mean abundance of *Asellus aquaticus* ranged from 0 Ind. sample<sup>-1</sup> on day -19 to 21.0  $\times/\div$  3.46 Ind. sample<sup>-1</sup> on day 114 in the HighPA treatment. The abundance of the isopods fluctuated strongly in the first half of the study and became more constant after day 56. Most mesocosms, with few exceptions, were in the control range afterwards. There were no significant differences compared to the control group throughout the study and thus the NOEC is > 150 mg L<sup>-1</sup>.



**Figure 42** Abundance of *Asellus aquaticus* [Ind. sample<sup>-1</sup>]

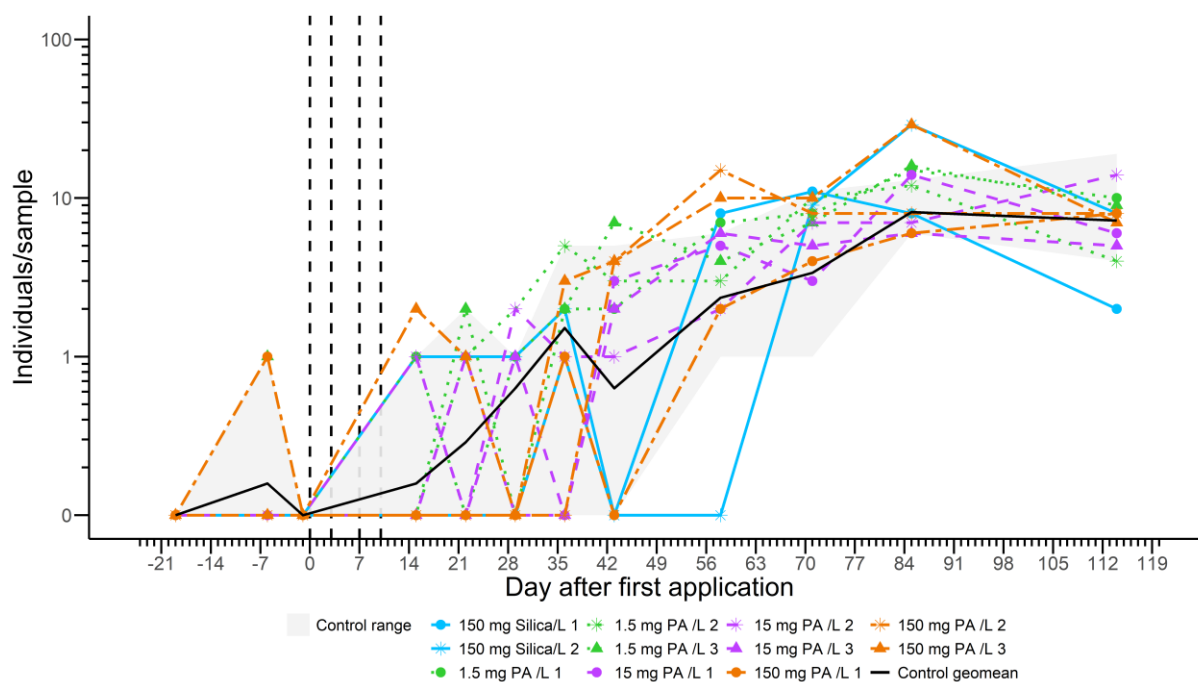
The mean abundance of Zygotera (Figure 43) ranged from  $0.2 \times/\div 3.82$  Ind. sample<sup>-1</sup> on day 58 to  $2.1 \times/\div 2.79$  Ind. sample<sup>-1</sup> on day 15 in the control. The mean abundance of Zygotera ranged from 0 Ind. sample<sup>-1</sup> on day 58 to  $5.2 \times/\div 2.17$  Ind. sample<sup>-1</sup> on day -6 in the Silica treatment.



**Figure 43** Abundance of Zygotera larvae [Ind. sample<sup>-1</sup>]

The mean abundance of Zygotera ranged from 0 Ind. sample<sup>-1</sup> on day 43 to  $2.0 \times/\div 2.0$  Ind. sample<sup>-1</sup> on day -6 in the LowPA treatment. The mean abundance of Zygotera ranged from  $0.1 \times/\div 1.0$  Ind. sample<sup>-1</sup> on day 58 to  $3.4 \times/\div 22.25$  Ind. sample<sup>-1</sup> on day 114 in the MedPA treatment. The mean abundance of Zygotera ranged from 0 Ind. sample<sup>-1</sup> on day 43 to  $3.9 \times/\div 1.29$  Ind. sample<sup>-1</sup> on day -1 in the HighPA treatment. There were no significant differences compared to the control group throughout the study, the MDD, however was only below 100 once after application on day 22 in the polyamide treatments.

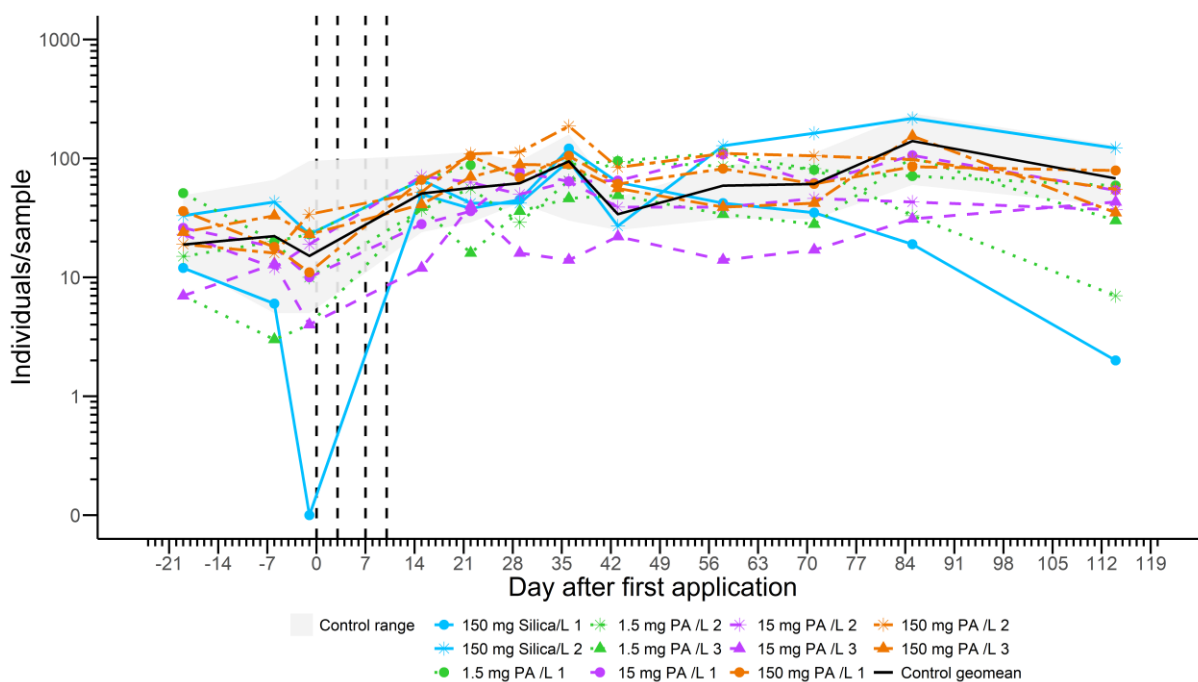
The mean abundance of *Lymnaea stagnalis* (Figure 44) ranged from 0 Ind. sample<sup>-1</sup> on day -19 to 8.2  $\times/\div$  1.43 Ind. sample<sup>-1</sup> on day 85 in the control. The mean abundance of *L. stagnalis* ranged from 0 Ind. sample<sup>-1</sup> on day -19 to 15.2  $\times/\div$  2.49 Ind. sample<sup>-1</sup> on day 85 in the Silica treatment. The mean abundance of *L. stagnalis* ranged from 0 Ind. sample<sup>-1</sup> on day -19 to 14.2  $\times/\div$  1.16 Ind. sample<sup>-1</sup> on day 85 in the LowPA treatment.



**Figure 44** Abundance of *Lymnaea stagnalis* [Ind. sample<sup>-1</sup>]

The mean abundance of *L. stagnalis* ranged from 0 Ind. sample<sup>-1</sup> on day -19 to 8.4  $\times/\div$  1.57 Ind. sample<sup>-1</sup> on day 85 in the MedPA treatment. The mean abundance of *L. stagnalis* ranged from 0 Ind. sample<sup>-1</sup> on day -19 to 11.2  $\times/\div$  2.31 Ind. sample<sup>-1</sup> on day 85 in the HighPA treatment. The abundance of the gastropods fluctuated strongly in the first half of the study and became more constant after day 56. There were no significant differences compared to the control group throughout the study and thus the NOEC is  $> 150 \text{ mg L}^{-1}$ . The offspring belonging to the family of Lymneidae, which is, in this study, composed of the genera *Lymnaea* and *Radix* showed effects in the LowPA and MedPA treatment on day 36 and in the Silica and MedPA treatment on day 58.

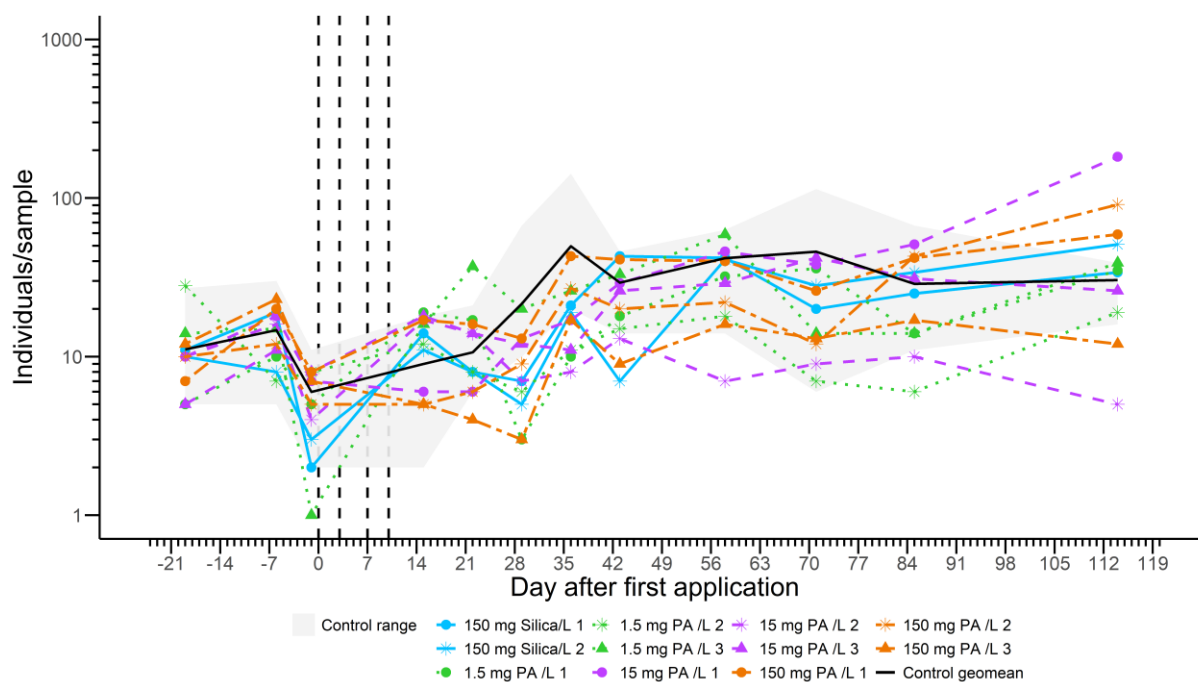
The mean abundance of Planorbidae (Figure 45) ranged from  $15.1 \times / \div 3.42$  Ind. sample<sup>-1</sup> on day -1 to  $139.9 \times / \div 1.71$  Ind. sample<sup>-1</sup> on day 85 in the control. The mean abundance of Planorbidae ranged from  $1.5 \times / \div 46.77$  on day -1 to  $107.2 \times / \div 1.19$  Ind. sample<sup>-1</sup> on day 36 in the Silica treatment.



**Figure 45** Abundance of Planorbidae [Ind. sample<sup>-1</sup>]

The mean abundance of Planorbidae ranged from  $9.7 \times / \div 2.4$  Ind. sample<sup>-1</sup> on day -1 to  $75.9 \times / \div 1.46$  Ind. sample<sup>-1</sup> on day 43 in the LowPA treatment. The mean abundance of Planorbidae ranged from  $9.1 \times / \div 2.19$  Ind. sample<sup>-1</sup> on day -1 to  $52.1 \times / \div 1.89$  Ind. sample<sup>-1</sup> on day 85 in the MedPA treatment. The mean abundance of Planorbidae ranged from  $20.5 \times / \div 1.77$  Ind. sample<sup>-1</sup> on day -1 to  $120.0 \times / \div 1.48$  Ind. sample<sup>-1</sup> on day 36 in the HighPA treatment. The abundance of Planorbidae rose after the application phase in all mesocosm. Some individual treated replicates had abundances below the control range occasionally. On day 43 the LowPA treatment had significantly lower abundances compared to the control. The Silica, MedPA and HighPA treatments had no significant differences compared to the control.

The mean abundance of Naididae (Figure 46) ranged from  $6.0 \times / \div 1.99$  Ind. sample<sup>-1</sup> on day -1 to  $49.7 \times / \div 2.29$  Ind. sample<sup>-1</sup> on day 36 in the control. The mean abundance of Naididae ranged from  $2.4 \times / \div 1.33$  Ind. sample<sup>-1</sup> on day -1 to  $41.6 \times / \div 1.33$  Ind. sample<sup>-1</sup> on day 114 in the Silica treatment.

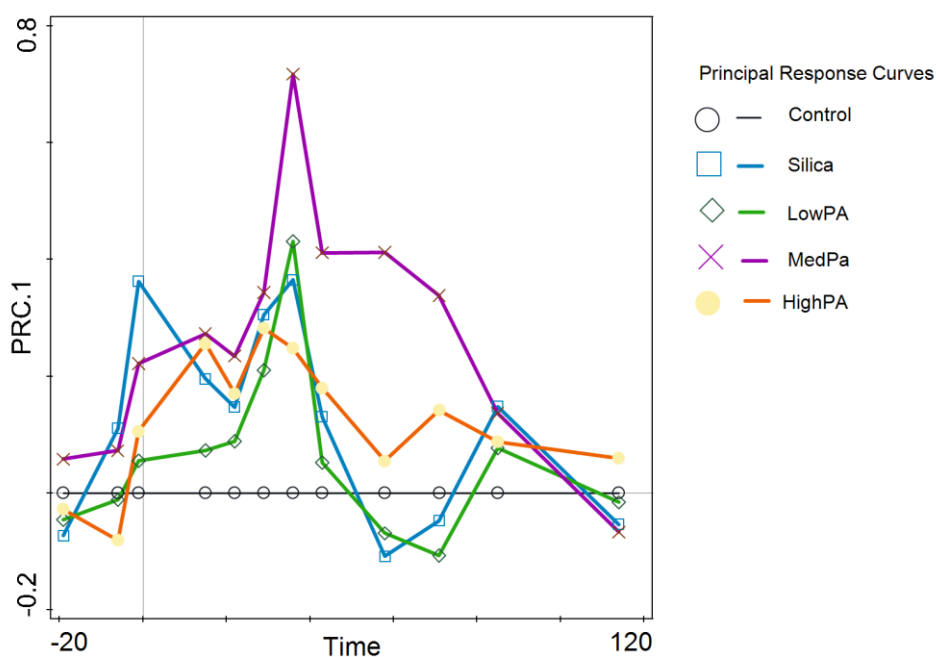


**Figure 46** Abundance of Naididae [Ind. sample<sup>-1</sup>]

The mean abundance of Naididae ranged from  $3.4 \times / \div 2.98$  Ind. sample<sup>-1</sup> on day -1 to  $32.4 \times / \div 1.81$  Ind. sample<sup>-1</sup> on day 58 in the LowPA treatment. The mean abundance of Naididae ranged from  $6.1 \times / \div 1.44$  Ind. sample<sup>-1</sup> on day -1 to  $28.7 \times / \div 6.05$  Ind. sample<sup>-1</sup> on day 114 in the MedPA treatment. The mean abundance of Naididae ranged from  $6.5 \times / \div 1.27$  Ind. sample<sup>-1</sup> on day -1 to  $40.1 \times / \div 2.91$  Ind. sample<sup>-1</sup> on day 114 in the HighPA treatment. The abundance of Naididae declined shortly before the first application and rose again afterwards. The abundance of the oligochaetes remained mostly in the control range over the course of the study. There were no significant differences compared to the control group throughout the study and thus the NOEC is  $> 150$  mg L<sup>-1</sup>.

#### 4.3.2 PRC of macroinvertebrate community

The PRC of the macroinvertebrate community (Figure 47) showed the effect of the treatments on community level. All treatments had moderate effects at the start of the study. On day 36 all treatments showed the highest positive effect, with MedPA having the highest positive effect on the community of macroinvertebrates. After day 36 the positive effects decreased steadily until the end of the study. On days 58 and 71 the Silica and LowPA treatment showed slight negative effects.

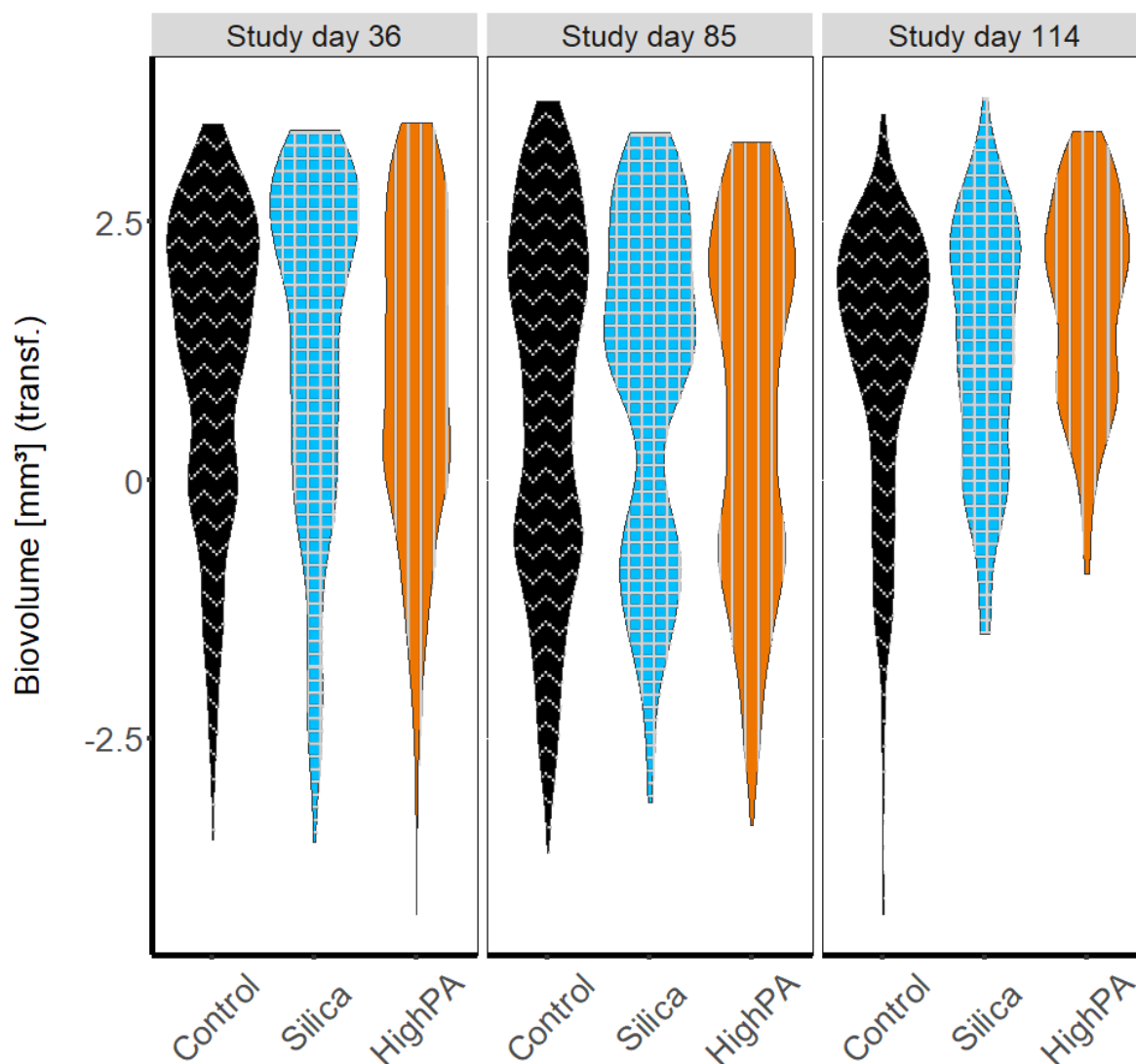


**Figure 47** Principal response curve of macroinvertebrate community

The changes in the macroinvertebrate community were not significant ( $p$ -value = 0.186). Out of the 30 taxa 10 had a species score over 0.5 in absolute value. The only positive species score over 0.5 was 1.52 for Coleoptera. *Chaoborus* sp. had the biggest negative species score with -3.54, followed by *Radix* sp. with -1.75, Tanypodinae larvae with -1.69 and *Asellus aquaticus* with -1.53. Naididae, Chironomidae and Planorbidae had species scores between -1.0 and -1.5. The small individuals of the family Lymnaeidae and *Gerris* sp. had species scores smaller than -1.0 but greater than -0.5.

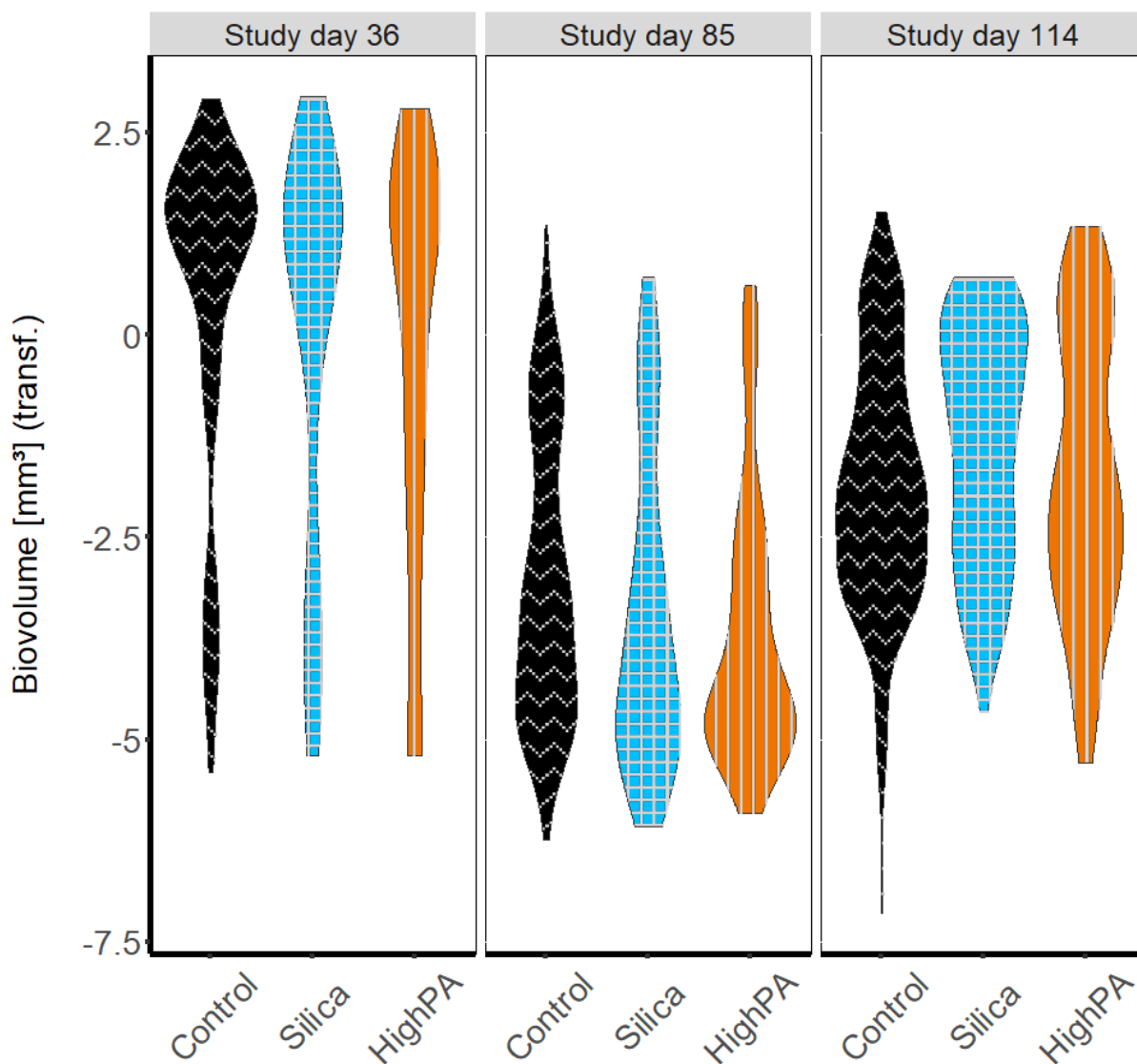
#### 4.3.3 Biovolume

The transformed ( $\ln$ ) biovolume of *Chaoborus* sp. larvae (Figure 48) on day 36 showed no significant differences compared to the control, neither in the Silica nor HighPA treatment. In the controls and the Silica treatment most larvae had a biovolume between 2 and 3 mm<sup>3</sup>. The median of the control was lower with 1.5 mm<sup>3</sup> compared to 1.9 mm<sup>3</sup> in the Silica treatment. The HighPA treatment showed no real maximum distribution and a uniformly distributed biovolume above 0 mm<sup>3</sup>. The median was 1.2 mm<sup>3</sup> in this treatment. On day 85 there were no significant differences between the controls and the treatments. The violin plots had a similar shape with maxima around 1 to 2.5 mm<sup>3</sup> and around -1 to 0 mm<sup>3</sup> of transformed biovolume in all treatments. The medians of the control and the HighPA treatment were 1.0 mm<sup>3</sup> and in the Silica treatment the median was at 1.4 mm<sup>3</sup>. On the last day of the study there were fewer small organisms in all treatments. The course of the violin plots is unimodal in all treatments. The peaks were around 2 mm<sup>3</sup> with median values of 1.6 mm<sup>3</sup> in the control, 1.4 mm<sup>3</sup> in the Silica treatment and 1.9 mm<sup>3</sup> in the HighPA treatment.



**Figure 48** Transformed (LN) biovolume of *Chaoborus* sp. larvae on days 36, 85 and 114

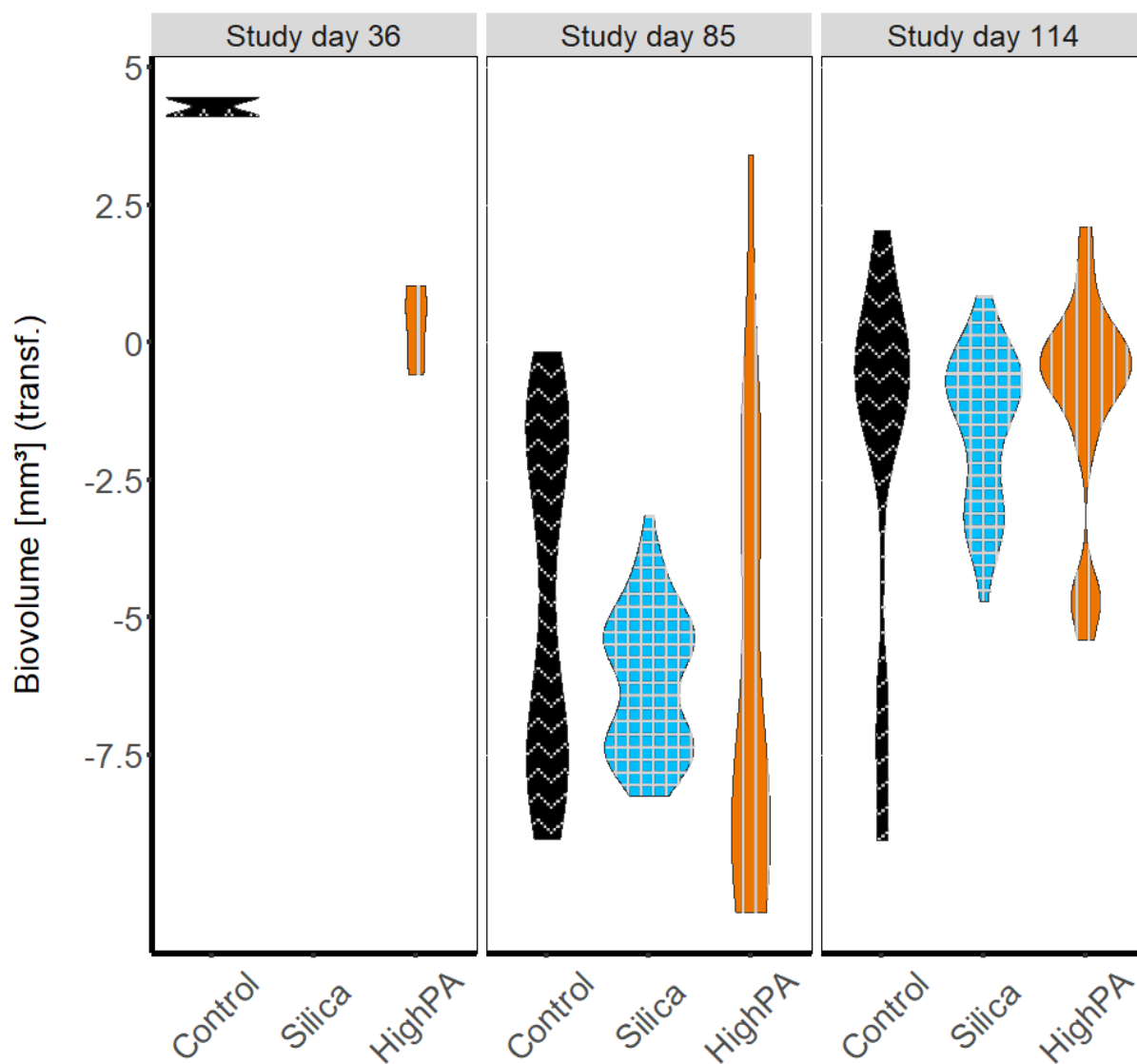
The transformed values of the biovolume of *Cloeon dipterum* (Figure 49) show a different course over the study than the *Chaoborus* sp. larvae. On day 36 the mayfly larvae had the biggest individuals in all treatments. There was no significant difference between the controls and the treatments. In all treatments and the control, the peak value of the unimodal course was between 1 and 2 mm<sup>3</sup>. The Silica and HighPA treatments have slightly wider violin plots and single individuals with higher biovolume than in the control. The median in the control of 1.3 mm<sup>3</sup> was higher than in the Silica treatment and the HighPA treatment with 1.1 mm<sup>3</sup> and 1.0 mm<sup>3</sup> respectively. On day 85 the organisms were much smaller compared to day 36. This picture can be seen in all the treatments considered.



**Figure 49** Transformed (LN) biovolume of *Cloeon dipterum* larvae on days 36, 85 and 114

The biggest organisms were found in the controls. The median in the control was  $-4.0 \text{ mm}^3$  and in the Silica and HighPA treatments in a similar region of  $-4.5 \text{ mm}^3$ . The low biovolume values suggest that the organisms that were found were in the lower larval stages on day 85 and in higher larval stages on day 36. On study day 114 the organisms that were found were bigger than on day 85 but smaller compared to day 36. The Silica treatment had the biggest organisms with a median of  $-1.1 \text{ mm}^3$  and a bimodal course with peaks around  $0.5 \text{ mm}^3$  and  $-2.0 \text{ mm}^3$ . The HighPA treatment had a bimodal course with the largest peak at  $-2.5 \text{ mm}^3$  and a smaller peak at  $1.0 \text{ mm}^3$  and a median of  $-1.9 \text{ mm}^3$ . The control had only one peak around  $-2$  to  $-3 \text{ mm}^3$  and a median of  $-2 \text{ mm}^3$ .

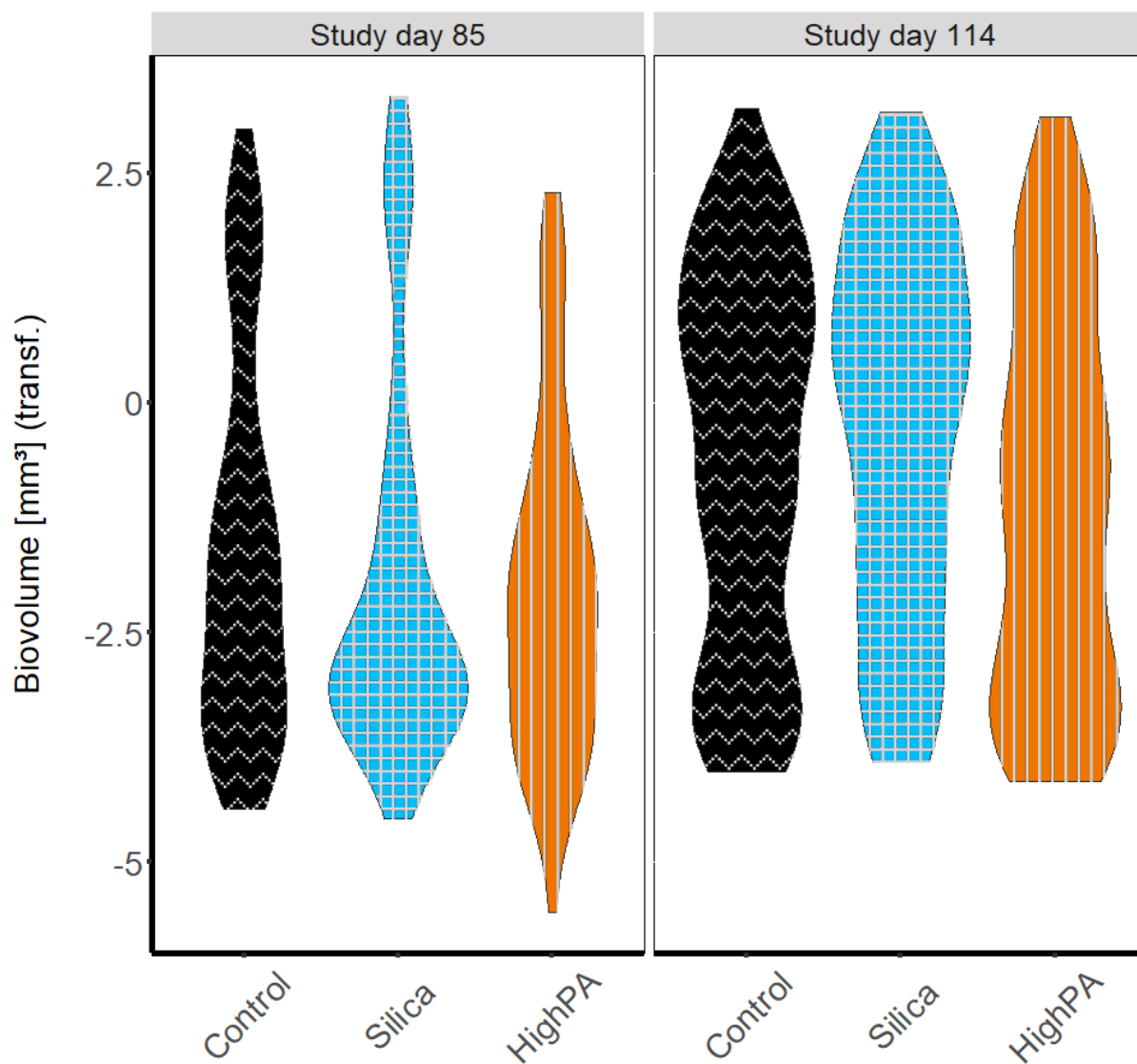
On study day 26 there were no Zygoptera larvae in the Silica treatment (Figure 50). The number of larvae in the control and HighPA treatment was low as well.



**Figure 50** Transformed (LN) biovolume of Zygoptera larvae on days 36, 85 and 114

The organisms in the control were bigger than the ones in the HighPA treatment with a median of  $4.3 \text{ mm}^3$  compared to  $0.4 \text{ mm}^3$ . On day 85 more organisms were found. The individuals in the Silica treatment were smaller with a bimodal course. The controls showed a bimodal course as well with both smaller and bigger organisms than in the Silica treatment. The HighPA treatment had the biggest and smallest organisms and a bimodal course at around the same scale as the control. The violin plots on day 114 show comparable, but upwards shifted shapes as day 85. All treatments had a similar peak at  $1.5 - 2 \text{ mm}^3$  of transformed biovolume.

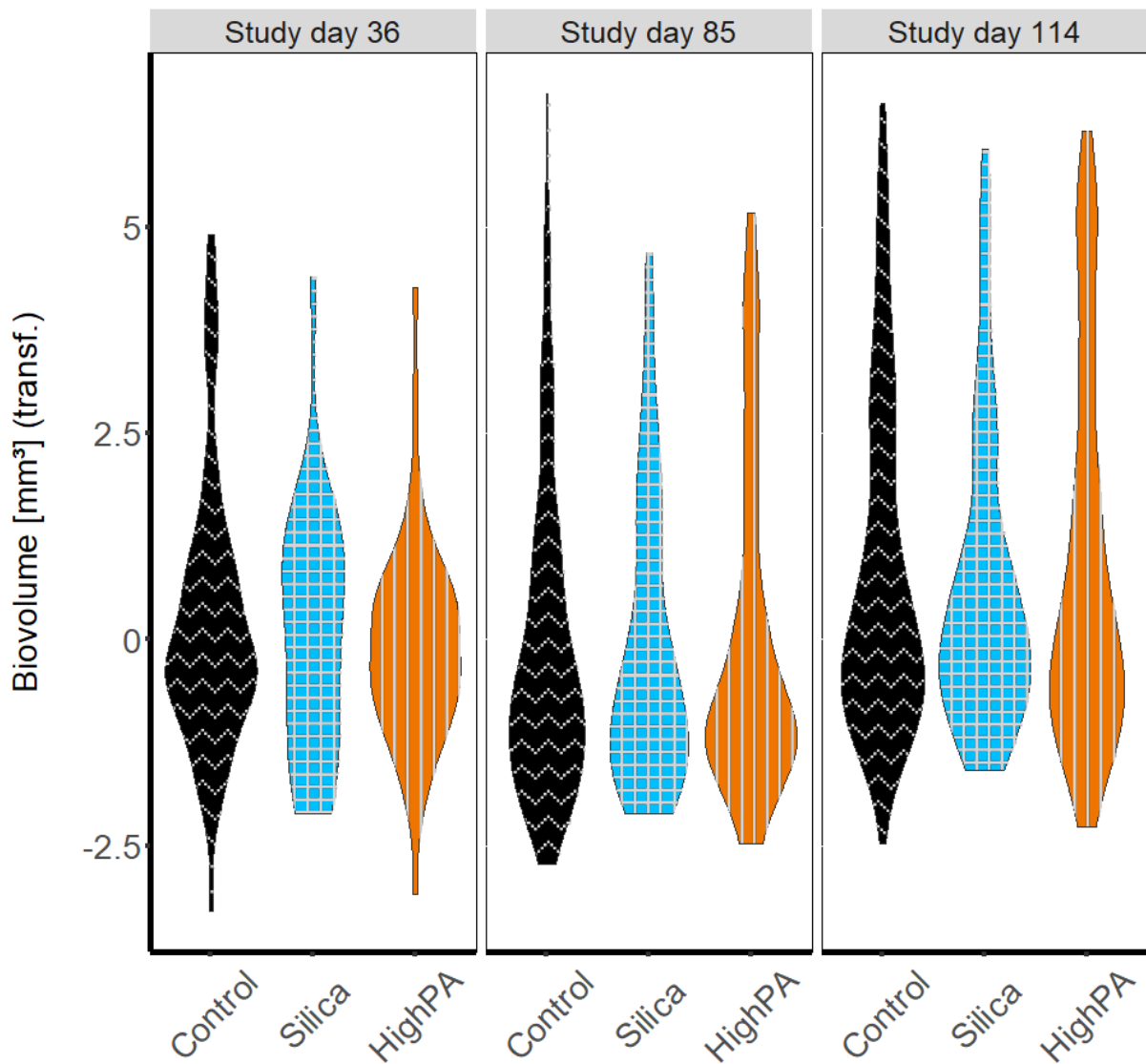
On day 36 no *Asellus aquaticus* were present in any of the samples (Figure 51). On day 85 the unimodal violin plots of all treatments had their maximum between  $-2 \text{ mm}^3$  and  $-4 \text{ mm}^3$  of transformed biovolume. There was no significant difference between the control and the treatments.



**Figure 51** Transformed (LN) biovolume of *Asellus aquaticus* on days 85 and 114

The median of the control was  $-2.1 \text{ mm}^3$  and  $-2.3 \text{ mm}^3$  in the HighPA treatment. In the Silica treatment the median was the lowest at  $-2.8 \text{ mm}^3$ . On day 114 the organisms were bigger in all treatments. The medians increased to  $-0.5 \text{ mm}^3$  in the control, to  $0.2 \text{ mm}^3$  in the Silica treatment and to  $-1.1 \text{ mm}^3$  in the HighPA treatment. The isopods had a broad spectrum of biovolume on day 114 ranging from  $-4.0 \text{ mm}^3$  to  $3.2 \text{ mm}^3$  in the control.

The biovolume of the organisms of the family of Lymnaeidae, consisting of the genera *Radix* and *Lymnaea* in this study ranged from 0 mm<sup>3</sup> to 5 mm<sup>3</sup> in the control on day 36 (Figure 52). The peak in the control was between 0.5 mm<sup>3</sup> to 1 mm<sup>3</sup>. In the Silica treatment the peak is higher, but the maximum transformed biovolume is lower at 2.5 mm<sup>3</sup>. The peak in the HighPA treatment was lower compared to the control at 0.25 mm<sup>3</sup> to 0.5 mm<sup>3</sup>. On day 85 the range of biovolume in the control and treatments was broader. Smaller and bigger individuals were present.

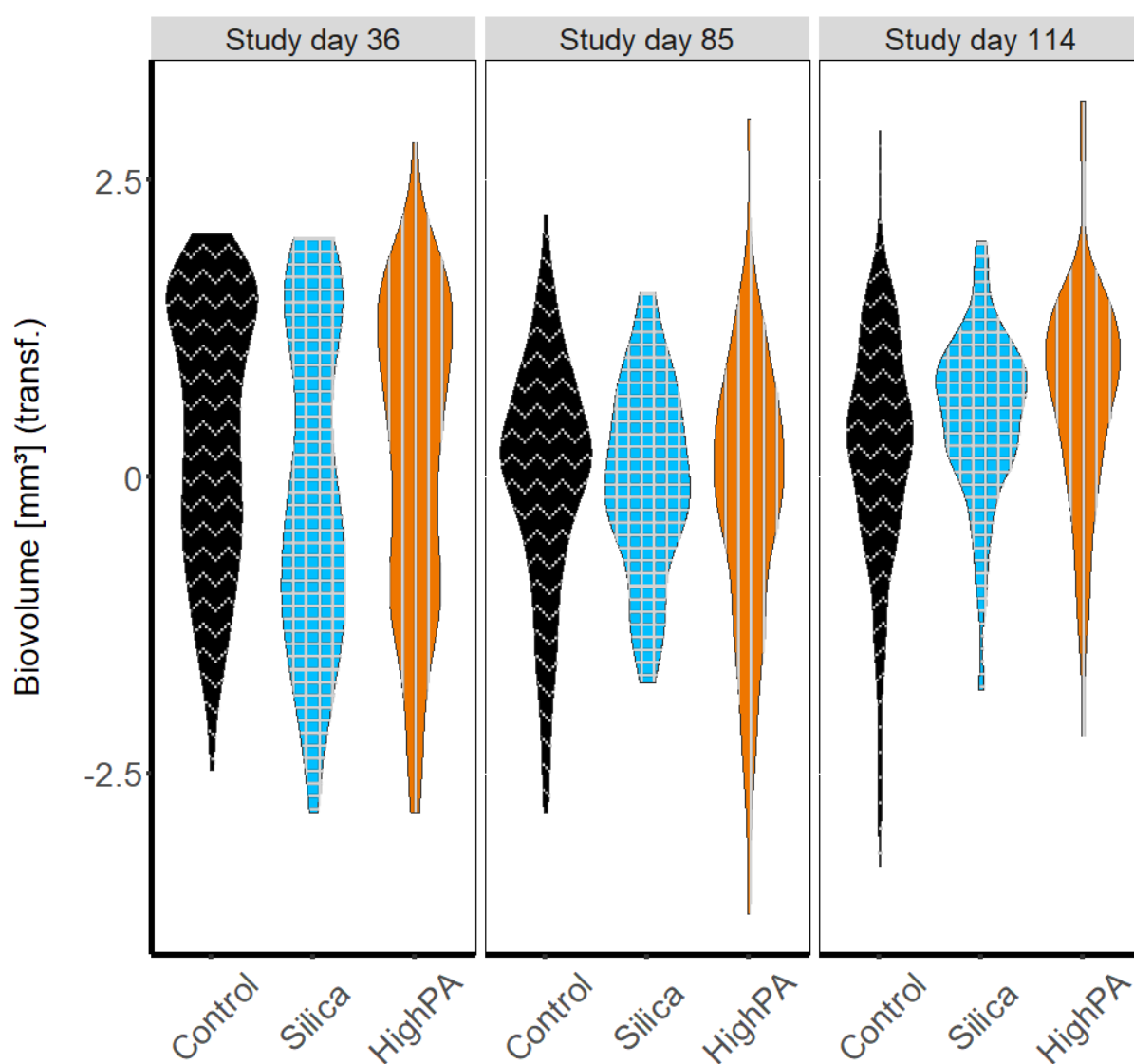


**Figure 52** Transformed (LN) biovolume of Lymnaeidae on days 36, 85 and 114

In the control the peak was comparable to sampling day 36, while mean of biovolume of the individuals in the Silica treatment was lower at -0.6 mm<sup>3</sup>. The HighPA treatment showed no peak in biovolume. The different sizes were evenly distributed. The median of -0.9 mm<sup>3</sup>

was lower than in the Silica treatment and the control. On day 114 there were larger and more large individuals in all mesocosms. The shape of the violin plots are comparable to day 85 with an increased number of large individuals.

The transformed biovolume of the family Planorbidae on day 36 (Figure 53) showed no significant differences between the control and the treatments. All violin plots on this day show a bimodal course of transformed biovolume. The biggest peak in the control and the HighPA treatment were in the positive value range between 1.3 mm<sup>3</sup> and 1.6 mm<sup>3</sup>. The biggest peak in the Silica treatment was in the negative value range at around -1 mm<sup>3</sup>. On day 85 less large organisms were sampled.



**Figure 53** Transformed (LN) biovolume of Planorbidae on days 36, 85 and 114

The shape of the violin plots is unimodal in all observed mesocosms. The peaks are close to each other around 0 mm<sup>3</sup>. On day 114 the shape of the violin plots and the distribution of the biovolume had a similar course as on day 85. The peak values are higher at 0.3 mm<sup>3</sup> in the control, 0.7 mm<sup>3</sup> in the Silica treatment and 1.1 mm<sup>3</sup> in the HighPA treatment. The biovolume of all species did not differ significantly from the control on any day. An ANOVA was performed with the transformed ( $y^*=\ln(2y+1)$ ) values for each taxon and day separately. None of the p-values were below 0.2, which means that there is no indication of significant differences.

#### 4.3.4 Dominance

Table 10 shows the temporal development of the relative density [%] of the most abundant and relevant macroinvertebrate taxa. In the control, *Cloeon dipterum* was initially eudominant with almost 60%. The dominance decreased continuously and increased again slightly at the end. *Chaoborus* sp. showed a contrary course. The dominance ratios increased steadily until day 36 and then slowly decreased to 28.6% (eudominant) by the end of the study. The Naididae started with a dominance of 5.3% on the verge of being classified as dominant and rose to just under 20% (eudominant) on day 71, after which the classification fell again to dominant (8.6%). The dominance of *Asellus aquaticus* increased continuously and reached a high of 18.0% (eudominant) on the last day. The dominance of Planorbidae fluctuated between dominant and eudominant (8% and 25% respectively). The other gastropods, dragonflies and chironomids were subdominant or recedent had dominance ratios below 5%. The Silica treatment showed a similar pattern. The most dominant taxa were *Cloeon dipterum*, *Chaoborus* and Planorbidae. in the LowPA treatment, the relative dominance of *Chaoborus* larvae increased to a high of 63.1% on day 85. *Cloeon dipterum* larvae were less dominant than in the control but showed a similar pattern. Anisoptera were no longer present at the time of application. All other taxa showed a similar trend to the control. In the MedPA treatment, mayflies were also eudominant at the beginning, accounting for around a third of the total number. In this treatment, too, the percentage dominance fell sharply and rose again at the end. The chaoborids increased in their dominance from 15% to 40.3% on day 36 and then decreased, with one exception of 50.4% on day 85. In this treatment, Naididae were more dominant (19.2%) on day 71 than in any other treatment except the control. The course of the HighPA treatment was similar to that of the control. At 7.5% on day 71, juvenile lymnaeids were more dominant than ever in the control. *Asellus aquaticus* was less dominant than in all other treatment groups and the control but still subdominant to dominant at the end of the study.

**Table 10** Relative densities [%] of most abundant macroinvertebrate taxa

Control												
Day	-19	-6	-1	15	22	29	36	43	58	71	85	114
Anisoptera	2.2	1.9	0.2	0.1	0.1	0	0	0.1	0	0	0	0.1
<i>Asellus aquaticus</i>	0	1.4	1.8	2	0.8	1.4	1.6	2.8	2.6	3.4	2.9	18
Ceratopogonidae	5.3	3.4	2.1	0.9	0.7	0.5	0.3	0.1	0.3	0.2	0.6	0

**Table 10** (continued) Relative densities [%] of most abundant macroinvertebrate taxa

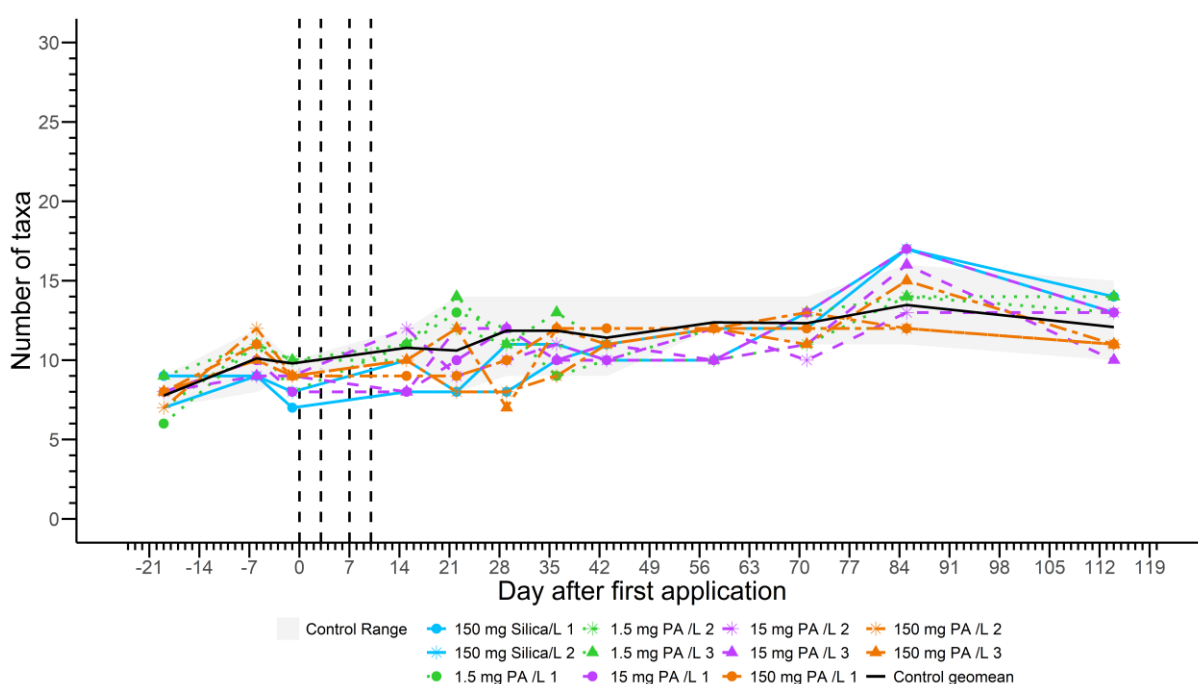
<b>Control</b>												
<b>Day</b>	<b>-19</b>	<b>-6</b>	<b>-1</b>	<b>15</b>	<b>22</b>	<b>29</b>	<b>36</b>	<b>43</b>	<b>58</b>	<b>71</b>	<b>85</b>	<b>114</b>
<i>Chaoborus</i> sp	17.6	28.3	44.8	50.8	48.1	57.5	58.4	57.5	45.4	45.8	49.8	28.6
Chironomidae	0.3	0	0.4	2.3	1	1.1	0.5	1.6	1.5	1.9	1.5	1.1
<i>Cloeon dipterum</i>	59.2	43.2	33.7	18.1	22.6	7.9	4.3	5.7	2.6	1.5	3.9	7.9
<i>Lymnaea stagnalis</i>	0	0.1	0	0.1	0.2	0.2	0.4	0.6	1.1	1.6	1.4	2.3
Lymnaeidae <0.5 cm	0	0	0	0	0	0.1	4.7	4.9	3.4	3.2	3.5	4.2
Naididae	5.3	6.2	2.8	3.8	3.6	7.9	10.2	11.6	17.2	19.8	6	8.6
Planorbidae	8.8	12.1	11.3	19.3	19.7	18.4	17.2	12.9	22.8	20.2	25.9	19.9
<i>Radix</i> sp.	0	1.7	1.5	1	1.6	2.6	0.6	0.9	0.5	0.2	0.1	0
Zygoptera	0.6	1.2	1	1.1	0.8	0.4	0.2	0.2	0.1	0.6	1.8	2.2
<b>Silica</b>												
<b>Day</b>	<b>-19</b>	<b>-6</b>	<b>-1</b>	<b>15</b>	<b>22</b>	<b>29</b>	<b>36</b>	<b>43</b>	<b>58</b>	<b>71</b>	<b>85</b>	<b>114</b>
Anisoptera	1.5	1.3	0.3	0.2	0	0	0	0.2	0	0	0	0
<i>Asellus aquaticus</i>	0	0.5	0.8	0	0.3	1.3	0.3	2.1	2.2	2.6	1.5	12.5
Ceratopogonidae	4.4	6.3	3	1	0.5	0.2	0.3	0.2	0.2	1.3	0.4	0
<i>Chaoborus</i> sp	26.9	30.8	31.2	43.4	54	59.9	60	51.8	42.2	38.4	48.3	36
Chironomidae	0.8	0	0	0.3	0.2	0.2	0.4	1.5	2.1	3.1	1.3	0.9
<i>Cloeon dipterum</i>	52.4	44.4	55.8	29.5	25.9	15.4	8.2	6.2	3.5	3.6	7.2	12.1
<i>Lymnaea stagnalis</i>	0	0	0	0.2	0.2	0.2	0.3	0	1.3	3.3	3.4	1.3
Lymnaeidae <0.5 cm	0	0	0	0	0	0	4.9	5.6	6	6.4	4.1	3.9
Naididae	4.1	4.9	1.3	4.1	2.8	2.2	3.9	10.7	13.2	7.8	5.3	10.9
Planorbidae	8.7	8.9	5.8	19	13.9	16.1	21.1	19.3	26.9	32.2	21.4	16
<i>Radix</i> sp.	0	0.5	0	1.1	1.7	2.6	0.2	1.3	1.9	0.5	0.1	0.4
Zygoptera	1	2.2	1.5	1.1	0.5	1.1	0.1	0.4	0	0.2	1.6	2.2
<b>LowPA</b>												
<b>Day</b>	<b>-19</b>	<b>-6</b>	<b>-1</b>	<b>15</b>	<b>22</b>	<b>29</b>	<b>36</b>	<b>43</b>	<b>58</b>	<b>71</b>	<b>85</b>	<b>114</b>
Anisoptera	1.5	1.8	0.4	0	0	0	0	0	0	0	0	0
<i>Asellus aquaticus</i>	0	0.8	3.1	0.9	1.3	2.2	2.9	3.8	3.8	3.8	4.5	18.8
Ceratopogonidae	6	4	3	1.2	0.7	0.4	0.1	0.2	0.1	0.9	1.3	0
<i>Chaoborus</i> sp	32.5	45.4	54.8	48.6	35.1	56.8	53.9	50.2	47.3	53.3	63.1	41.2
Chironomidae	0	0	0	0.5	0.7	0.9	0.6	0.3	0.3	2.1	0.8	0.6
<i>Cloeon dipterum</i>	42.7	36.9	29.2	27.4	32.6	16.9	8	5.2	2.6	1.9	6.4	12.8
<i>Lymnaea stagnalis</i>	0	0.1	0	0.1	0.3	0.4	1	1.3	1.6	2.7	2.7	1.8
Lymnaeidae <0.5 cm	0	0	0	0	0	0	2.7	3.5	3.9	6.2	2.5	1.4
Naididae	6.4	3.4	1.9	4.6	7.2	3.4	5.3	7.3	12.1	6.2	2.1	7.4

**Table 10** (continued) Relative densities [%] of most abundant macroinvertebrate taxa

<b>LowPA</b>												
<b>Day</b>	<b>-19</b>	<b>-6</b>	<b>-1</b>	<b>15</b>	<b>22</b>	<b>29</b>	<b>36</b>	<b>43</b>	<b>58</b>	<b>71</b>	<b>85</b>	<b>114</b>
Planorbidae	10	4.5	5	13.9	18.5	16.2	22.6	26.4	25.6	20.5	13	7.7
<i>Radix</i> sp.	0.1	1.6	1.6	1.2	1	1.4	0.7	0.9	1.2	0.6	0.1	0.1
Zygoptera	0.5	0.7	0.3	0.7	0.6	0.2	0.1	0	0	0	0.1	1.9
<b>MedPA</b>												
<b>Day</b>	<b>-19</b>	<b>-6</b>	<b>-1</b>	<b>15</b>	<b>22</b>	<b>29</b>	<b>36</b>	<b>43</b>	<b>58</b>	<b>71</b>	<b>85</b>	<b>114</b>
Anisoptera	1.3	1.6	0.3	0	0	0	0	0	0	0	0	0
<i>Asellus aquaticus</i>	0	0.4	0.3	2.1	1.7	1.5	2.5	5.2	3.5	3.7	3.2	7.8
Ceratopogonidae	6.8	4.4	4.4	1.4	0.6	1.1	0.6	0	1	0.4	1	0
<i>Chaoborus</i> sp	15	30.6	35.1	23.7	27.5	37.9	40.3	25.9	21.6	27.8	50.4	32.2
Chironomidae	0.1	0.1	0.2	2.8	2.1	2.1	1.1	1.7	2.4	2.6	2.7	1.2
<i>Cloeon dipterum</i>	65.9	49.2	47.6	39.9	38.3	15.8	13.2	13.7	5.7	4.7	6.3	15.9
<i>Lymnaea stagnalis</i>	0	0	0	0.2	0.2	0.6	0.2	1.3	2.6	3.2	2.3	2.1
Lymnaeidae <0.5 cm	0	0	0	0	0	0.6	3.4	7.4	10.4	7.8	2.7	3.9
Naididae	2.9	5.6	3.1	7.1	5.1	6	6.9	14.8	16.7	19.2	8	17.6
Planorbidae	6.5	5.4	5.4	19.2	21	26.9	27.1	27.4	32.8	26.9	15.6	11.1
<i>Radix</i> sp.	0	1.3	0.5	2.1	1.5	3.4	1.9	0.2	1.2	0	0.6	0.2
Zygoptera	0.8	1.3	2.6	1.2	0.9	2.1	0.4	0.2	0	0	0.6	3.8
<b>HighPA</b>												
<b>Day</b>	<b>-19</b>	<b>-6</b>	<b>-1</b>	<b>15</b>	<b>22</b>	<b>29</b>	<b>36</b>	<b>43</b>	<b>58</b>	<b>71</b>	<b>85</b>	<b>114</b>
Anisoptera	1.7	1.6	0.3	0.1	0	0	0	0.1	0	0	0	0
<i>Asellus aquaticus</i>	0	0.9	1.6	0.8	0.5	1.8	1.6	2.8	3.5	2.7	2.9	7.4
Ceratopogonidae	4.9	4.1	1.2	0.6	0.7	0.3	0.4	0.1	0.6	0.4	0.9	0
<i>Chaoborus</i> sp	25.1	38.3	44.6	44.6	34.7	48.4	54.5	46.8	39.6	43.9	55.2	40.6
Chironomidae	0.1	0.1	0.3	0.6	1.6	0.8	0.3	0.9	2.1	1.8	1.7	0.7
<i>Cloeon dipterum</i>	53.4	35.8	33	24.5	22.5	8.2	4.7	4.8	2.2	2.9	2.6	7.8
<i>Lymnaea stagnalis</i>	0	0.1	0	0.3	0.1	0	0.3	1.1	3.8	3	2.7	1.9
Lymnaeidae <0.5 cm	0	0	0	0	0	0	5.9	4.6	2.6	7.5	1.6	4.7
Naididae	3.8	6.9	3.5	3.8	3.1	3.1	5.6	9.4	10.8	7	6.4	13.1
Planorbidae	10.2	8.4	11.8	22.2	34.2	34	24.4	27.1	32.1	28.4	21	13.7
<i>Radix</i> sp.	0	2.1	1	1	1.1	2.8	0.6	0.5	0.6	0.5	0.1	0
Zygoptera	0.4	0.6	2.1	0.6	0.1	0.3	0.3	0	0	0.1	0.9	1.4

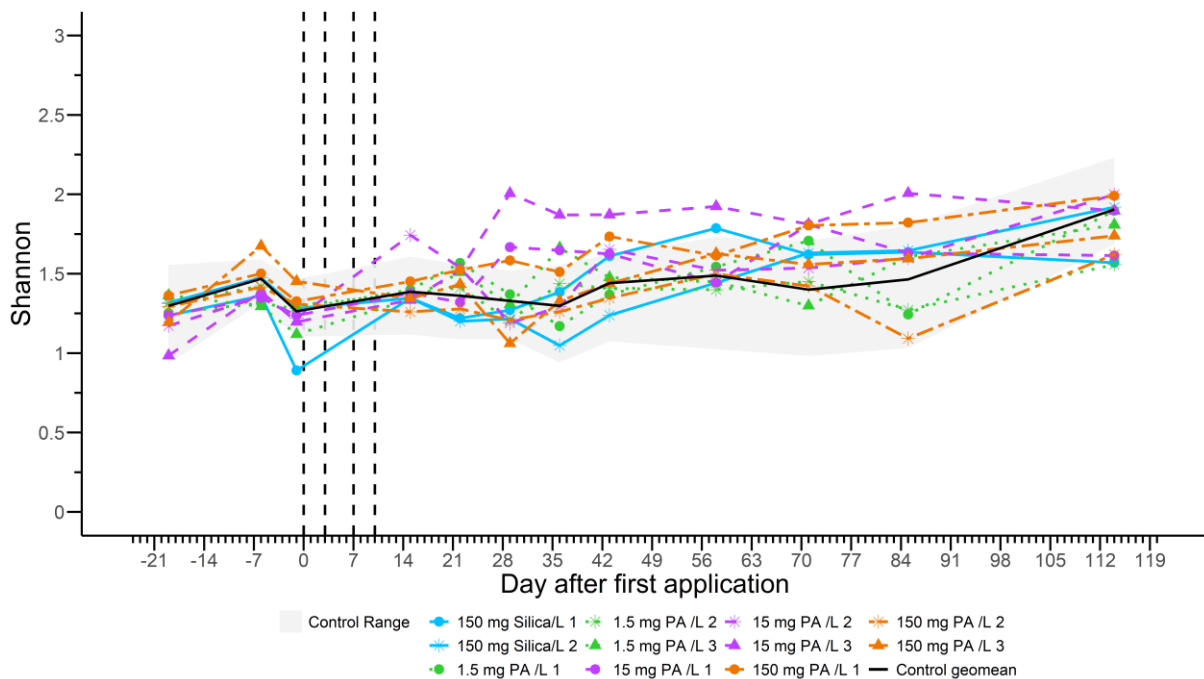
### 4.3.5 Diversity indices

The mean number of taxa (Figure 54) ranged from 7.8  $\times/\div$  1.11 on day -19 to 13.5  $\times/\div$  1.17 on day 85 in the control. The mean number of taxa ranged from 7.5  $\times/\div$  1.10 on day -1 to 17.0  $\times/\div$  1.00 on day 85 in the Silica treatment. The mean number of taxa ranged from 7.2  $\times/\div$  1.23 on day -19 to 13.3  $\times/\div$  1.09 on day 85 in the LowPA treatment. The mean number of taxa ranged from 8.0  $\times/\div$  1.00 on day -19 to 15.2  $\times/\div$  1.15 on day 85 in the MedPA treatment. The mean number of taxa ranged from 7.7  $\times/\div$  1.08 on day -19 to 12.9  $\times/\div$  1.14 on day 85 in the HighPA treatment. The number of taxa rose gradually in all mesocosms throughout the study. All treated mesocosms, with single exceptions remained in the control range. On day -1 the Silica treatment had a significantly lower number of species compared to the control. The number of species was significantly lower in the HighPA treatment on day 29.



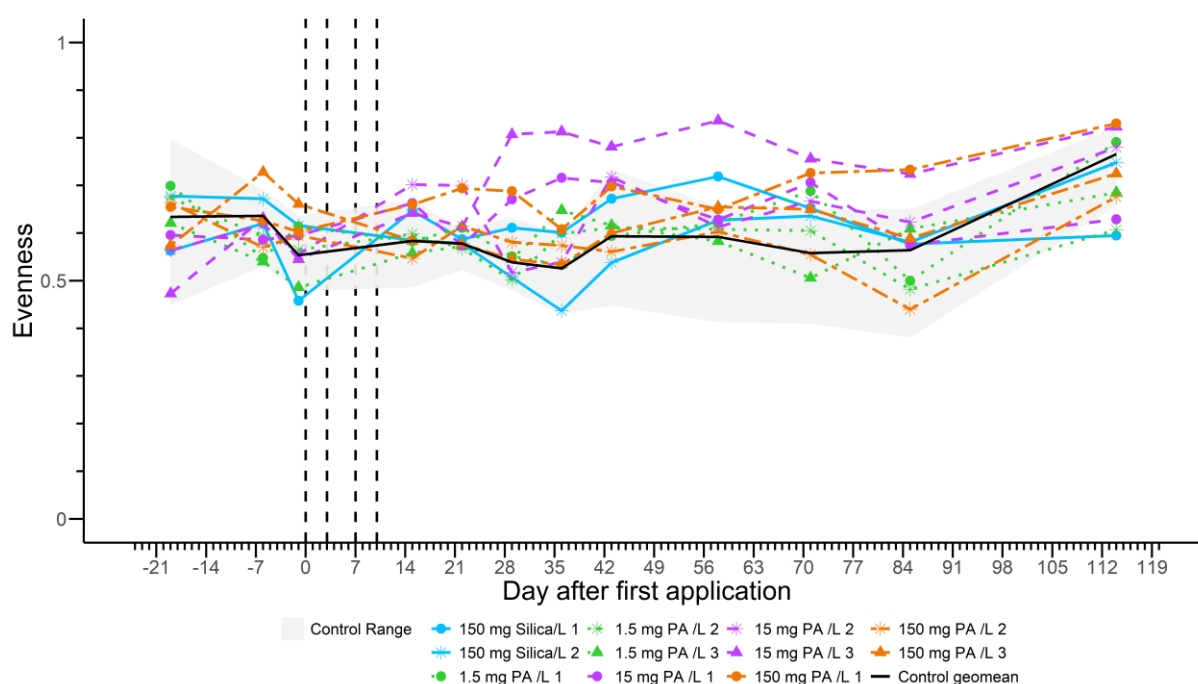
**Figure 54** Number of macroinvertebrate taxa

The mean Shannon index (Figure 55) ranged from  $1.3 \times / \div 1.14$  on day -1 to  $1.9 \times / \div 1.11$  on day 114 in the control. The mean Shannon index ranged from  $1.1 \times / \div 1.29$  on day -1 to  $1.7 \times / \div 1.15$  on day 114 in the Silica treatment. The mean Shannon index ranged from  $1.2 \times / \div 1.09$  on day -1 to  $1.7 \times / \div 1.11$  on day 114 in the LowPA treatment. The mean Shannon index ranged from  $1.1 \times / \div 1.13$  on day -19 to  $1.8 \times / \div 1.12$  on day 114 in the MedPA treatment. The mean Shannon index ranged from  $1.3 \times / \div 1.23$  on day 29 to  $1.8 \times / \div 1.11$  on day 114 in the HighPA treatment. The Shannon index, like the number of taxa rose throughout the study however, with greater dispersion. There were no significant differences compared to the control group throughout the study regarding the Shannon index.



**Figure 55** Shannon index of macroinvertebrate community

The mean evenness (Figure 56) ranged from  $0.53 \times/\div 1.16$  on day 36 to  $0.77 \times/\div 1.09$  on day 114 in the control. The mean evenness ranged from  $0.51 \times/\div 1.25$  on day 36 to  $0.67 \times/\div 1.10$  on day 58 in the Silica treatment. The mean evenness ranged from  $0.52 \times/\div 1.13$  on day 85 to  $0.69 \times/\div 1.14$  on day 114 in the LowPA treatment. The mean evenness ranged from  $0.54 \times/\div 1.13$  on day -19 to  $0.74 \times/\div 1.15$  on day 114 in the MedPA treatment. The mean evenness ranged from  $0.57 \times/\div 1.07$  on day 36 to  $0.74 \times/\div 1.11$  on day 114 in the HighPA treatment. The evenness fluctuated in all mesocosm throughout the study but remained on a similar level. There were no significant differences compared to the control group throughout the study regarding the evenness.



**Figure 56** Evenness of macroinvertebrate community

#### 4.3.6 Statistical evaluation

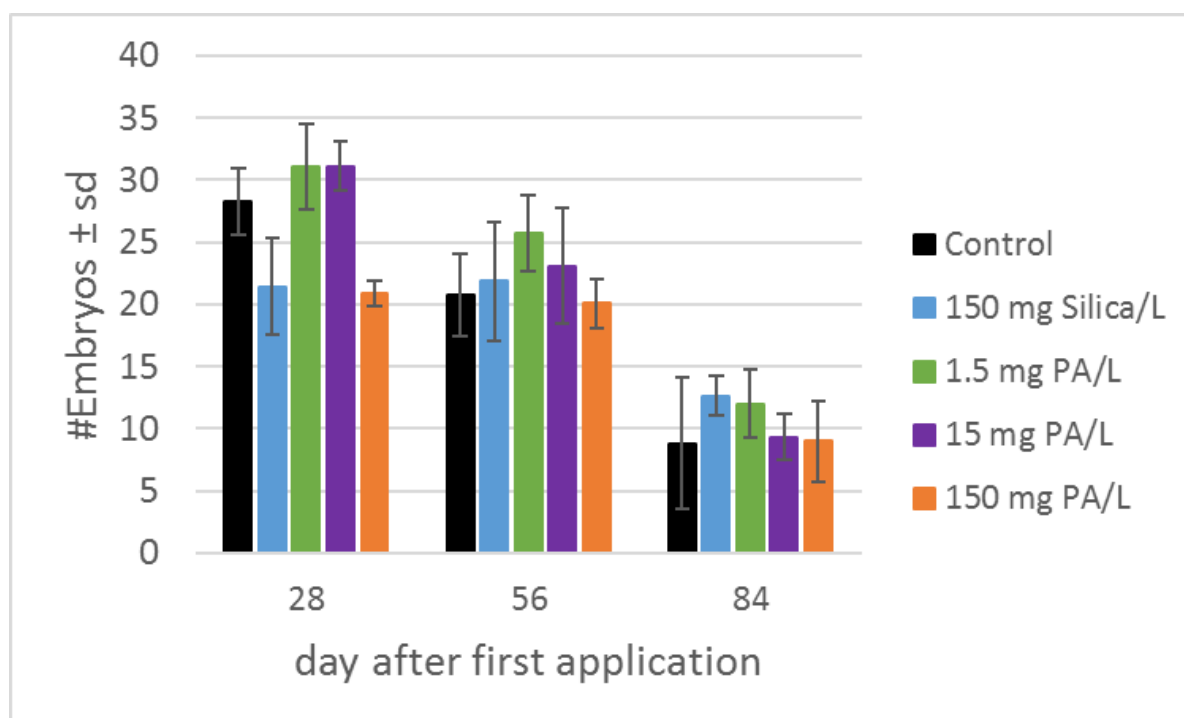
Table 11 shows the effects on the zooplankton taxa. The effects were classified according to EFSA (see Table 4). Neither in the Silica treatment nor in the LowPA or HighPA treatment any taxa were assigned to another class than class 1 (no effects). In the MedPA treatment the larvae of the genus *Chaoborus* were assigned to effect class 3A- due to pronounced direct effects. The sum of Diptera and the total sum were assigned to the same class. All other taxa and sums of taxa were assigned effect class 1 due to not showing effects compared to the control. Overall 15 taxa, including sums are classified as MDD Category 1 and three as MDD Category 2.

**Table 11** Classification of the effect on macroinvertebrates over the entire duration of the study according to EFSA (2013)

MDD Category	Taxon	Silica	LowPA	MedPA	HighPA
1	<i>Asellus aquaticus</i>	1	1	1	1
1	<i>Chaoborus</i> sp.	1	1	3A-	1
1	Chironomidae	1	1	1	1
1	<i>Cloeon dipterum</i>	1	1	1	1
1	Coleoptera larvae	1	1	1	1
1	<i>Lymnaea stagnalis</i>	1	1	1	1
1	Lymnaeidae <0 5 cm	1	1	1	1
1	Naididae	1	1	1	1
1	Planorbidae	1	1	1	1
1	<i>Radix</i> sp.	1	1	1	1
1	Sum Chironomidae	1	1	1	1
1	Sum Diptera	1	1	3A-	1
1	Sum Gastropoda	1	1	1	1
1	Sum Odonata	1	1	1	1
1	Total sum	1	1	3A-	1
2	Acari	1	1	1	1
2	<i>Musculium lacustre</i>	1	1	1	1
2	Trichoptera	1	1	1	1
3	Anisoptera	1	1	1	1
3	<i>Chaoborus</i> sp. pupa	1	1	1	1
3	Chironomidae pupa	1	1	1	1
3	Coleoptera adult	1	1	1	1
3	Corixidae	1	1	1	1
3	<i>Dugesia</i> sp.	1	1	1	1
3	Dytiscidae	1	1	1	1
3	<i>Notonecta glauca</i>	1	1	1	1
3	Sum Coleoptera	1	1	1	1
3	Sum Heteroptera	1	1	1	1
3	Tanypodinae	1	1	1	1
3	Tanypodinae pupa	1	1	1	1
3	Zygoptera	1	1	1	1

#### 4.3.7 *Potamopyrgus antipodarum* bio-assay

The number of embryos was assessed on three samplings (Figure 57). Each ten snails per treatment were sampled, measured and their embryos were counted. On day 28 most embryos were found per snail compared to the other sampling. In the control 28.2  $\times/\div$  2.68 embryos were found. The Silica and HighPA treatment had lower numbers with 21.4  $\times/\div$  3.90 and 20.9  $\times/\div$  1.04 embryos respectively. The LowPA and MedPA treatment had higher numbers compared to the control with 31.1 ( $\times/\div$ 3.47;  $\times/\div$ 1.99) each.



**Figure 57** Mean number of embryos in *Potamopyrgus* in all treatments and the control after 28, 56 and 84 days in bio assays

According to CPCAT calculations no difference was significant compared to the control. Since data is normally distributed (Shapiro-Wilks test  $p=0.8365$ ) and variances are homogenous (Levene Test  $p=0.3376$ ) an ANOVA was performed as well. A  $p$ -value of 0.0058 (Table 13) indicates that variances between the groups are significantly greater than the variances within the groups. To see which treatment differentiates significantly to the control a Dunnett's post-hoc test was performed. The Silica treatment ( $p=0.049$ ) and the HighPA treatment ( $p=0.018$ ) both have significantly lower number of embryos compared to the control.

On day 56 the control had a mean number of embryos of 20.7  $\times/\div$  3.34. The HighPA treatment had the lowest number of embryos with 20.1  $\times/\div$  1.98 and the LowPA treatment the highest with 25.7  $\times/\div$  3.03. No differences compared to the control were significant, with neither the CPCAT approach nor after performing an ANOVA (Table 12, Table 13).

**Table 12** Statistical evaluation of reproduction data using the CPCAT approach. All hypotheses were tested with Monte-Carlo simulations (n= 10000)

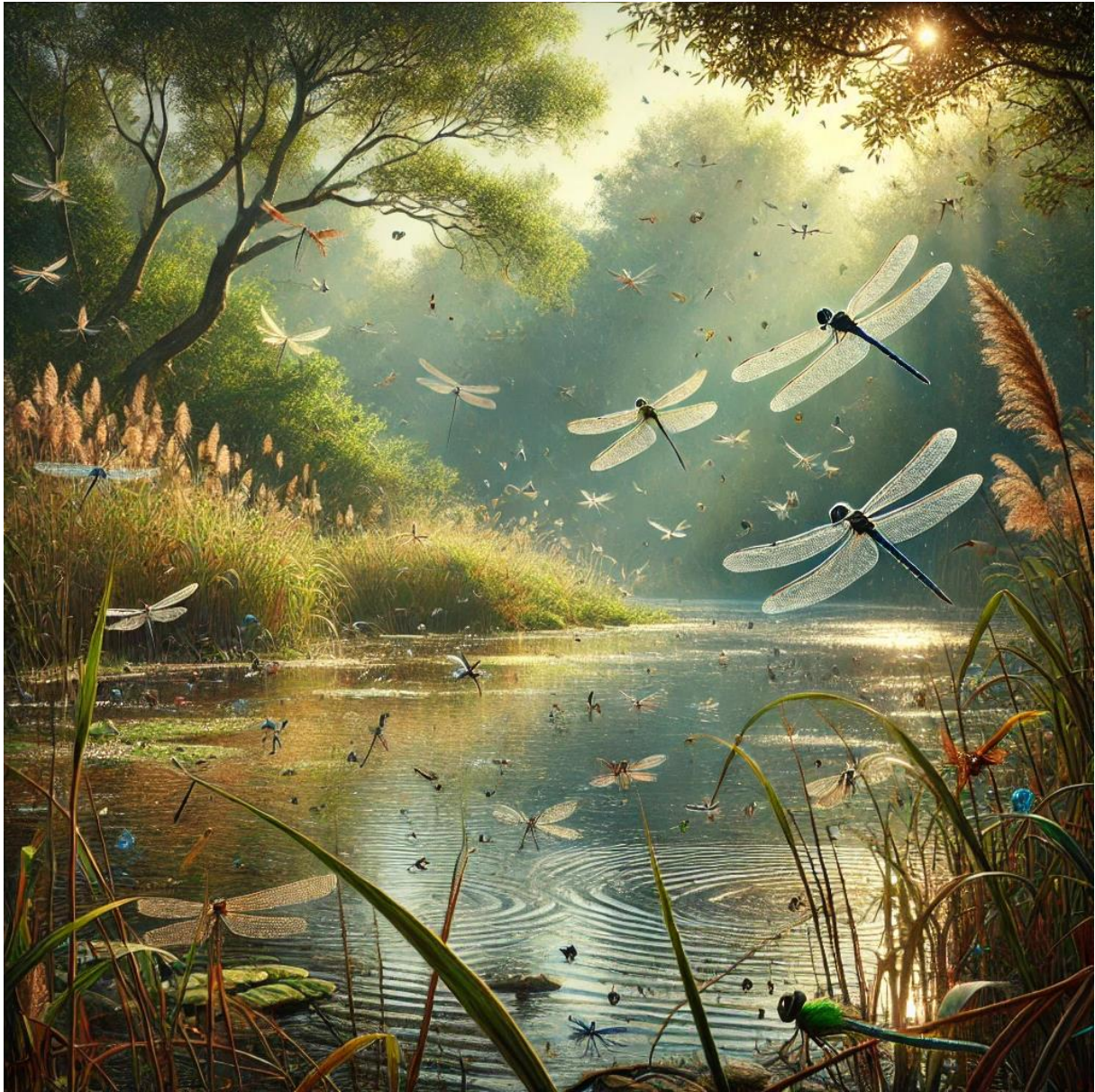
max p-values	Day28	Day56	Day84
Silica	0.2385	0.9304	0.466
LowPA	0.5742	0.6212	0.5657
MedPA	0.5772	0.8892	0.9679
HighPA	0.1613	0.9309	0.9691

The mean number of embryos was the lowest for all treatments on day 84 with  $8.8 \times / \div 5.31$  in the control,  $12.7 \times / \div 1.55$  in the Silica treatment,  $12.0 \times / \div 2.71$  in the LowPA,  $9.3 \times / \div 1.86$  in the MedPA and  $9.0 \times / \div 3.25$  in the HighPA treatment. There was no significant difference compared to the control (Table 12, Table 13).

**Table 13** ANOVA results of embryo count data of *Potamopyrgus* assay

Day	Variance between groups	Variance within groups	Total variance	p-value	F	F*
28	282.79	117.63	400.42	0.0058	6.61	3.36
56	63.67	206.35	270.02	0.5234	0.85	3.36
84	37.63	209.82	247.46	0.7412	0.49	3.36

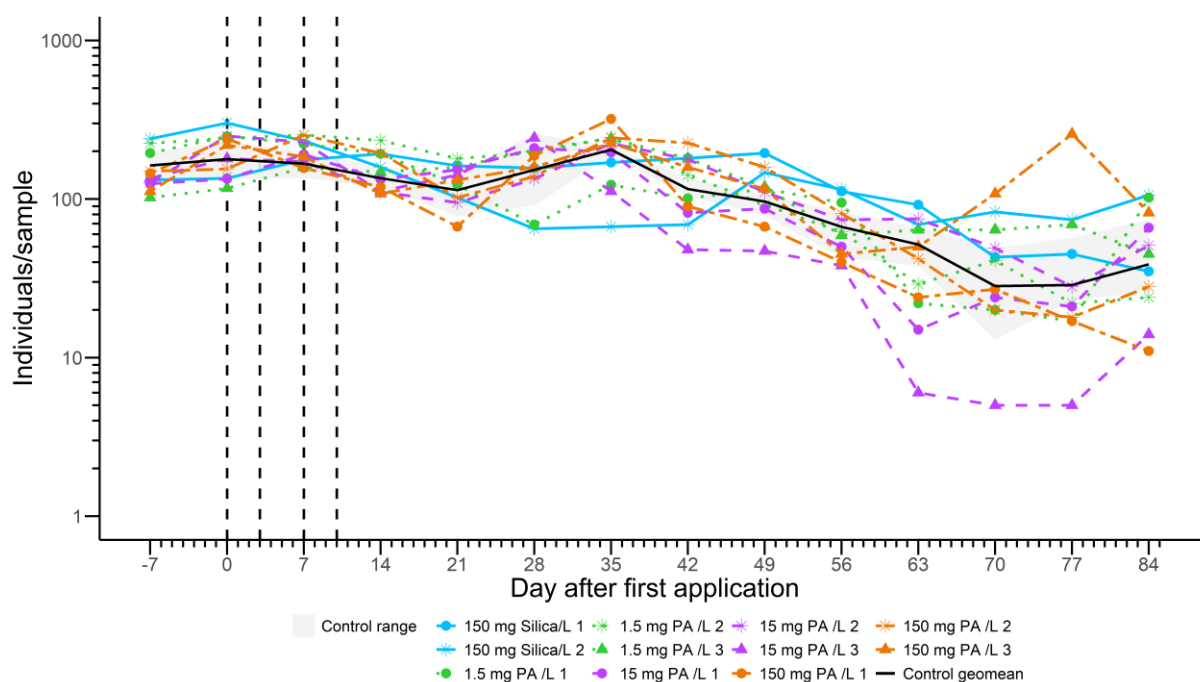
## 4.4 Emerging insects



(Picture generated with OpenAI DALL·E)

#### 4.4.1 Abundance data

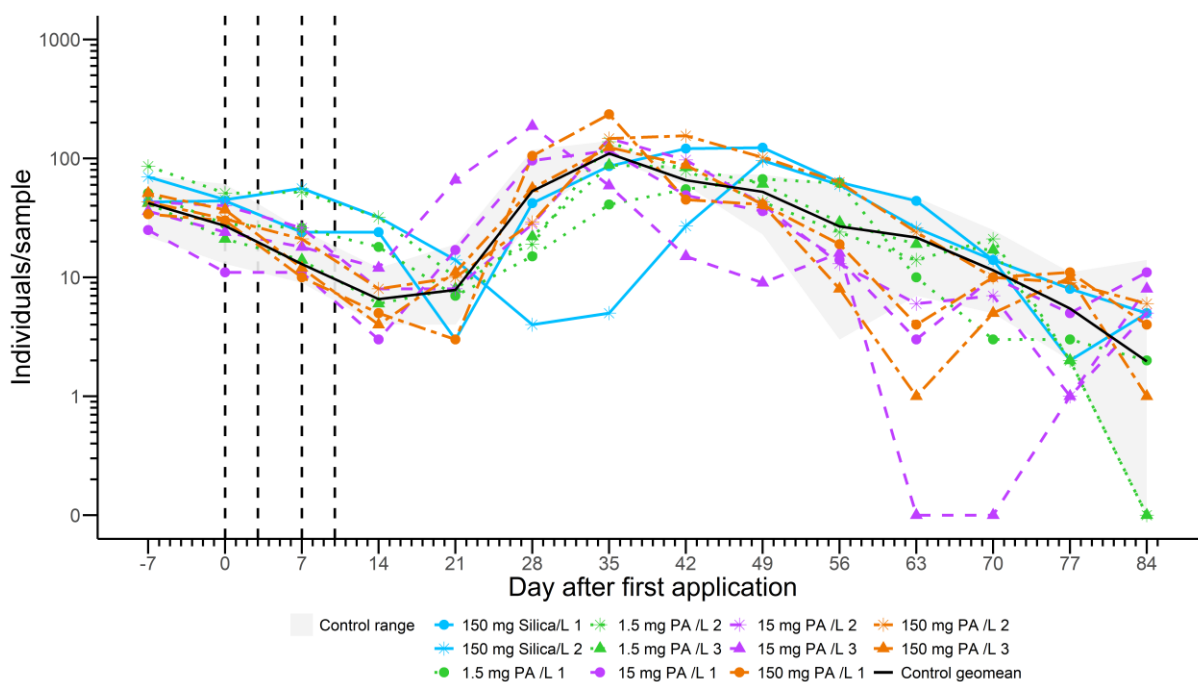
The mean abundance of all emerging insects (Figure 58) ranged from 28.3 Ind. sample<sup>-1</sup>  $\times/\div$  1.66 on day 70 to 205.0  $\times/\div$  1.05 Ind. sample<sup>-1</sup> on day 35 in the control. The mean abundance of all emerging insects ranged from 57.7  $\times/\div$  1.42 Ind. sample<sup>-1</sup> on day 77 to 203.1  $\times/\div$  1.21 Ind. sample<sup>-1</sup> on day 7 in the Silica treatment. The mean abundance of all emerging insects ranged from 29.6  $\times/\div$  2.11 Ind. sample<sup>-1</sup> on day 77 to 207.6  $\times/\div$  1.28 Ind. sample<sup>-1</sup> on day 7 in the LowPA treatment. The mean abundance of all emerging insects ranged from 14.3  $\times/\div$  2.52 Ind. sample<sup>-1</sup> on day 77 to 195.9  $\times/\div$  1.16 Ind. sample<sup>-1</sup> on day 7 in the MedPA treatment.



**Figure 58** Abundance of all emerging insects [Ind. sample<sup>-1</sup>]

The mean abundance of all emerging insects ranged from 29.3  $\times/\div$  2.73 Ind. sample<sup>-1</sup> on day 84 to 260.0  $\times/\div$  1.2 Ind. sample<sup>-1</sup> on day 35 in the HighPA treatment. The total abundance of emerged insects stayed constant during the first six weeks after application and decreased afterwards. With lower abundances the dispersion in the data increased. The Silica treatment had significantly higher abundances of emerging insects on days 49 and 56 compared to the control. Neither polyamide treatment had significant different abundances compared to the control. One replicate of the MedPA treatment had lower abundances than all controls starting day 35 until the end of the study.

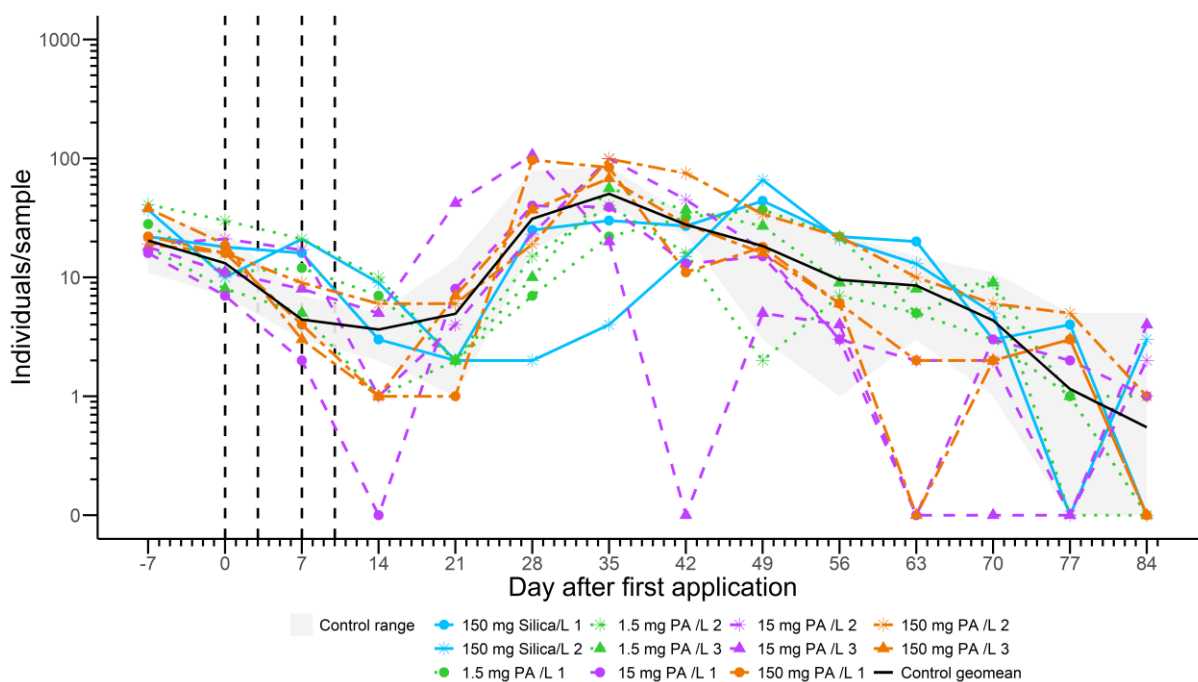
The mean abundance of *Chaoborus* sp. (Figure 59) ranged from  $2.0 \times / \div 6.92$  Ind. sample<sup>-1</sup> on day 84 to  $110.1 \times / \div 1.15$  Ind. sample<sup>-1</sup> on day 35 in the control. The mean abundance of *Chaoborus* sp. ranged from  $4.0 \times / \div 2.67$  Ind. sample<sup>-1</sup> on day 77 to  $108.7 \times / \div 1.19$  Ind. sample<sup>-1</sup> on day 49 in the Silica treatment.



**Figure 59** Abundance of all emerged *Chaoborus* sp. [Ind. sample<sup>-1</sup>]

The mean abundance of *Chaoborus* sp. ranged from  $0.3 \times / \div 5.64$  Ind. sample<sup>-1</sup> on day 84 to  $79.3 \times / \div 1.85$  Ind. sample<sup>-1</sup> on day 35 in the LowPA treatment. The mean abundance of *Chaoborus* sp. ranged from  $1.2 \times / \div 8.95$  Ind. sample<sup>-1</sup> on day 63 to  $100.2 \times / \div 1.61$  Ind. sample<sup>-1</sup> on day 35 in the MedPA treatment. The mean abundance of *Chaoborus* sp. ranged from  $2.9 \times / \div 2.56$  Ind. sample<sup>-1</sup> on day 84 to  $162.4 \times / \div 1.39$  Ind. sample<sup>-1</sup> on day 35 in the HighPA treatment. The abundance of *Chaoborus* sp. was significantly higher compared to the control on day 14 in the Silica treatment. On the last sampling day one replicate of the LowPA treatment had 0 organisms. One replicate of the MedPA treatment had no *Chaoborus* sp. on two consecutive sampling days (63,70). No significant differences were observed in either polyamide treatment. A dip in overall abundance was found in all mesocosms after the applications and a maximum two to three weeks after that. Same applies for male (Figure 60) and female (Figure 61) chaoborids.

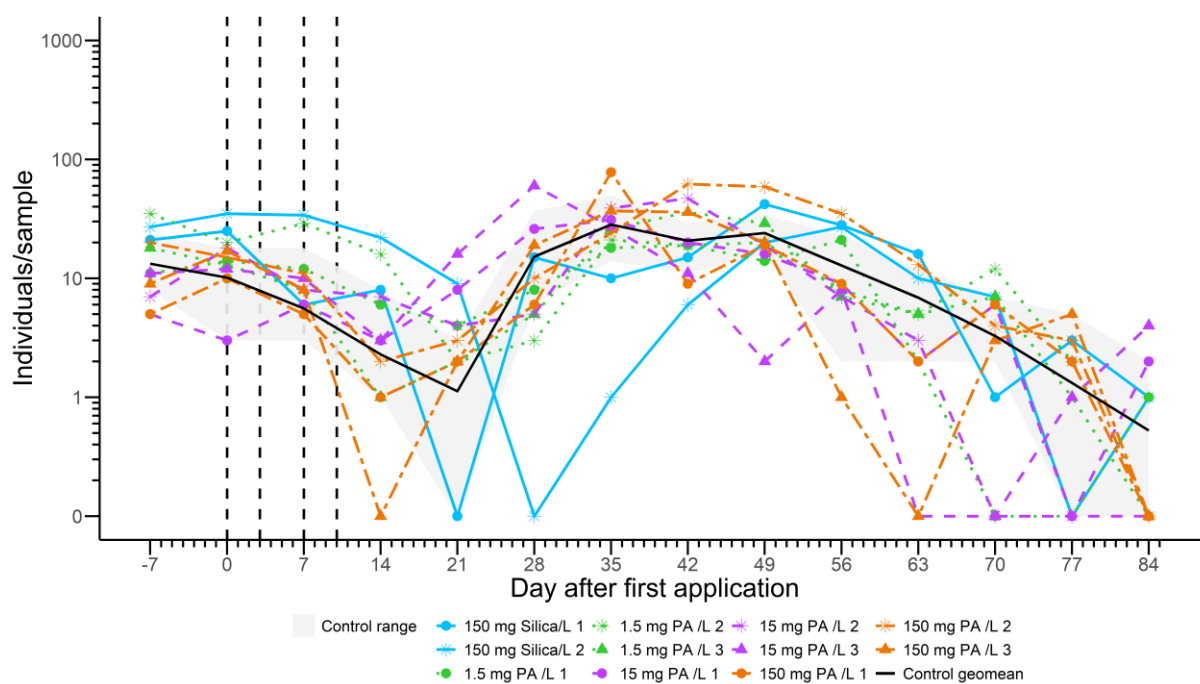
The mean abundance of male *Chaoborus* sp. (Figure 60) ranged from  $0.5 \times/\div 5.41$  Ind. sample<sup>-1</sup> on day 84 to  $50.5 \times/\div 1.38$  Ind. sample<sup>-1</sup> on day 35 in the control. The mean abundance of male *Chaoborus* sp. ranged from  $0.5 \times/\div 11.08$  Ind. sample<sup>-1</sup> on day 84 to  $53.9 \times/\div 1.33$  Ind. sample<sup>-1</sup> on day 49 in the Silica treatment.



**Figure 60** Abundance of male emerged *Chaoborus* sp. [Ind. sample<sup>-1</sup>]

The mean abundance of male *Chaoborus* sp. ranged from  $0.2 \times/\div 3.78$  Ind. sample<sup>-1</sup> on day 84 to  $36.7 \times/\div 1.61$  Ind. sample<sup>-1</sup> on day 35 in the LowPA treatment. The mean abundance of male *Chaoborus* sp. ranged from  $0.3 \times/\div 5.64$  Ind. sample<sup>-1</sup> on days 63 and 77 to  $46.2 \times/\div 2.18$  Ind. sample<sup>-1</sup> on day 28 in the MedPA treatment. The mean abundance of male *Chaoborus* sp. ranged from  $0.2 \times/\div 3.78$  Ind. sample<sup>-1</sup> on day 63 to  $82.7 \times/\div 1.21$  Ind. sample<sup>-1</sup> on day 35 in the HighPA treatment. There were significantly higher abundances on day 56 and on day 84 of male *Chaoborus* sp. In the Silica treatment. In every treatment there were no organisms detected, on individual samplings.

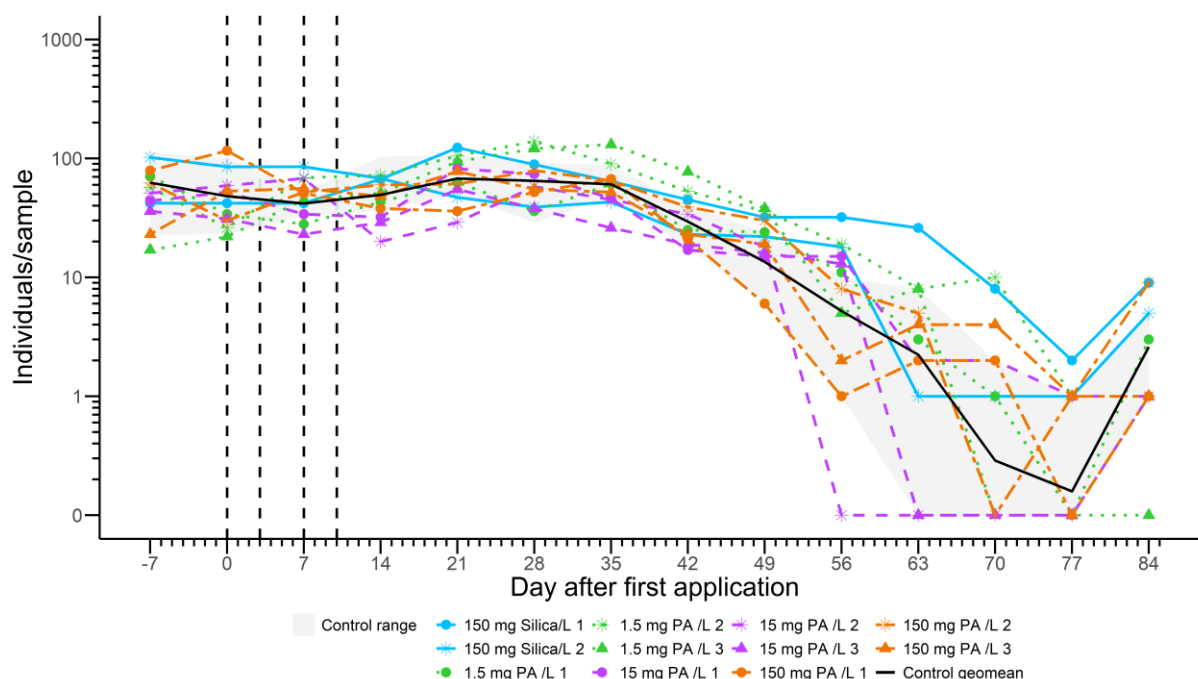
The mean abundance of female *Chaoborus* sp. (Figure 61) ranged from  $0.5 \times/\div 4.67$  Ind. sample<sup>-1</sup> on day 84 to  $28.4 \times/\div 1.68$  Ind. sample<sup>-1</sup> on day 35 in the control. The mean abundance of female *Chaoborus* sp. ranged from  $0.5 \times/\div 11.08$  Ind. sample<sup>-1</sup> on day 77 to  $29.6 \times/\div 1.27$  Ind. sample<sup>-1</sup> on day 0 in the Silica treatment. The mean abundance of female *Chaoborus* sp. ranged from  $0.2 \times/\div 3.78$  Ind. sample<sup>-1</sup> on day 84 to  $23.5 \times/\div 1.45$  Ind. sample<sup>-1</sup> on day 42 in the LowPA treatment. The mean abundance of female *Chaoborus* sp. ranged from  $0.2 \times/\div 3.78$  Ind. sample<sup>-1</sup> on day 63 to  $32.0 \times/\div 1.2$  Ind. sample<sup>-1</sup> on day 35 in the MedPA treatment.



**Figure 61** Abundance of female emerged *Chaoborus* sp. [Ind. sample<sup>-1</sup>]

The mean abundance of female *Chaoborus* sp. ranged from 0 Ind. sample<sup>-1</sup> on day 84 to  $41.1 \times/\div 1.82$  Ind. sample<sup>-1</sup> on day 35 in the HighPA treatment. On day 56, compared to the control, there were significantly more female *Chaoborus* sp. identified in the Silica treatment. In every treatment and the control there were no female individuals found, on individual samplings.

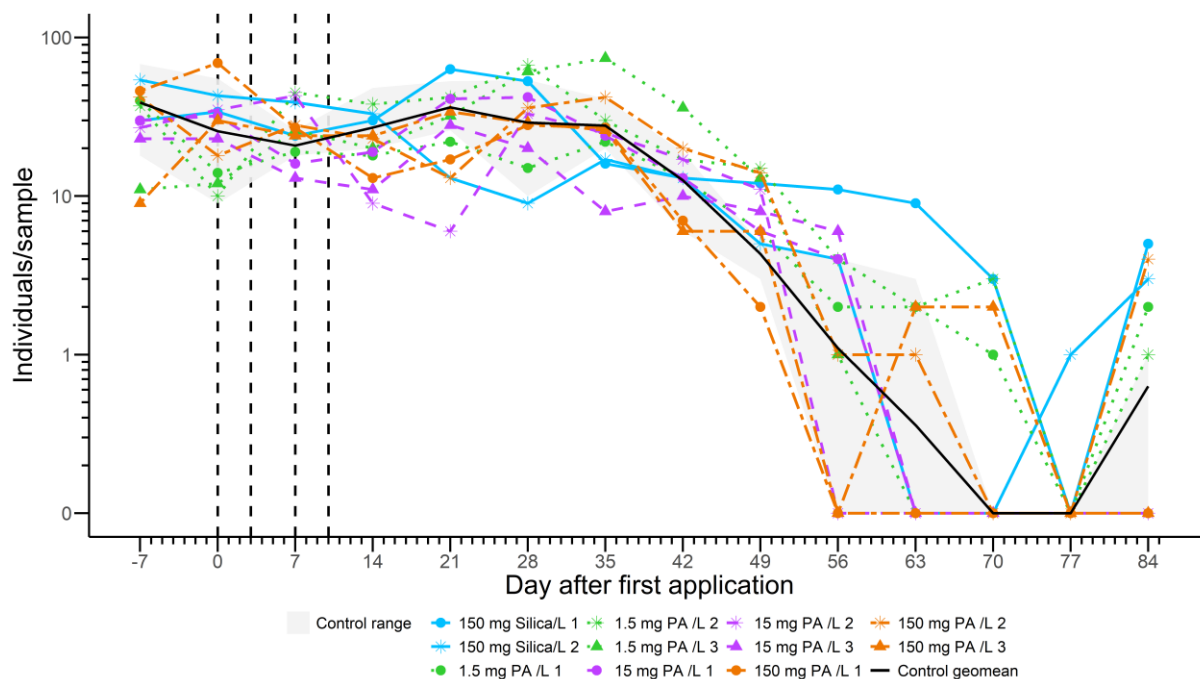
The mean abundance of *Cloeon dipterum* (Figure 62) ranged from  $0.2 \times/\div 2.8$  Ind.sample<sup>-1</sup> on day 77 to  $67.7 \times/\div 1.43$  Ind. sample<sup>-1</sup> on day 21 in the control. The mean abundance of *C. dipterum* ranged from  $1.4 \times/\div 1.63$  Ind. sample<sup>-1</sup> on day 77 to  $76.0 \times/\div 1.97$  Ind. sample<sup>-1</sup> on day 21 in the Silica treatment. The mean abundance of *C. dipterum* ranged from 0 Ind. sample<sup>-1</sup> on day 70 to  $89.6 \times/\div 1.47$  Ind. sample<sup>-1</sup> on day 35 in the LowPA treatment. The mean abundance of *C. dipterum* ranged from  $0.2 \times/\div 3.78$  Ind. sample<sup>-1</sup> on day 77 to  $54.6 \times/\div 1.4$  Ind. sample<sup>-1</sup> on day 28 in the MedPA treatment.



**Figure 62** Abundance of all emerged *Cloeon dipterum* [Ind. sample<sup>-1</sup>]

The mean abundance of *C. dipterum* ranged from  $0.5 \times/\div 3.78$  Ind. sample<sup>-1</sup> on day 77 to  $61.0 \times/\div 1.15$  Ind. sample<sup>-1</sup> on day 35 in the HighPA treatment. On days 56 and 77 the Silica treatment had significantly higher abundances compared to the control. The LowPA treatment had significantly more *Cloeon dipterum* compared to the control on day 49. The MedPA and HighPA treatment showed no significant differences compared to the control, but each two replicates of these treatments had no organisms on one sampling day at latter stages of the study. Starting day 63 the MDD for the Silica treatment and the polyamide treatments exceeded 100, except on the last sampling day (95 and 89 respectively).

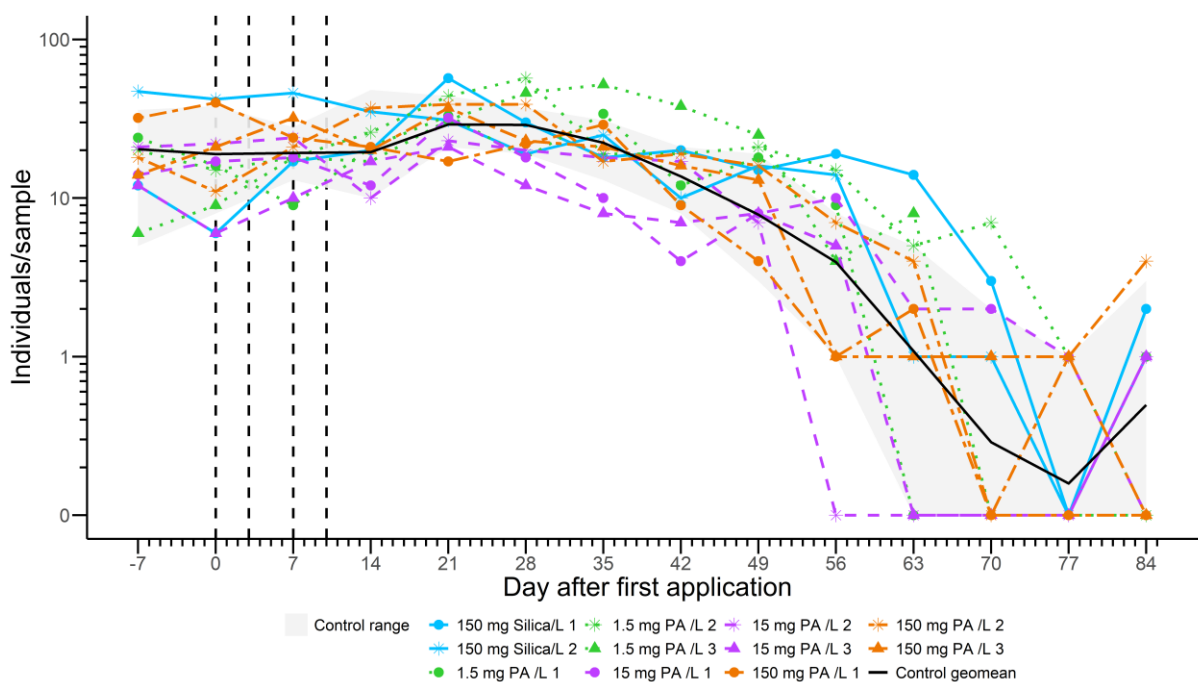
The mean abundance of male *Cloeon dipterum* (Figure 63) ranged from 0 Ind. sample<sup>-1</sup> on days 70 and 77 to 38.9  $\times/\div$  1.69 Ind. sample<sup>-1</sup> on day -7 in the control. The mean abundance of male *C. dipterum* ranged from 0.3  $\times/\div$  5.09 Ind. sample<sup>-1</sup> on day 63 to 40.2  $\times/\div$  1.52 Ind. sample<sup>-1</sup> on day -7 in the Silica treatment. The mean abundance of male *C. dipterum* ranged from 0 Ind. sample<sup>-1</sup> on day 77 to 39.4  $\times/\div$  2.31 Ind. sample<sup>-1</sup> on day 28 in the LowPA treatment. The mean abundance of male *C. dipterum* ranged from 0 Ind. sample<sup>-1</sup> on days 63 through 84 to 30.3  $\times/\div$  1.46 Ind. sample<sup>-1</sup> on day 28 in the MedPA treatment.



**Figure 63** Abundance of male emerged *Cloeon dipterum* [Ind. sample<sup>-1</sup>]

The mean abundance of male *C. dipterum* ranged from 0 Ind. sample<sup>-1</sup> on day 77 to 33.4  $\times/\div$  1.97 Ind. sample<sup>-1</sup> on day 0 in the HighPA treatment. The abundance of male *Cloeon dipterum* was significantly higher compared to the control on days 56 and 84 in the Silica treatment. There were no significant differences in the LowPA, MedPA and HighPA treatments compared to the control.

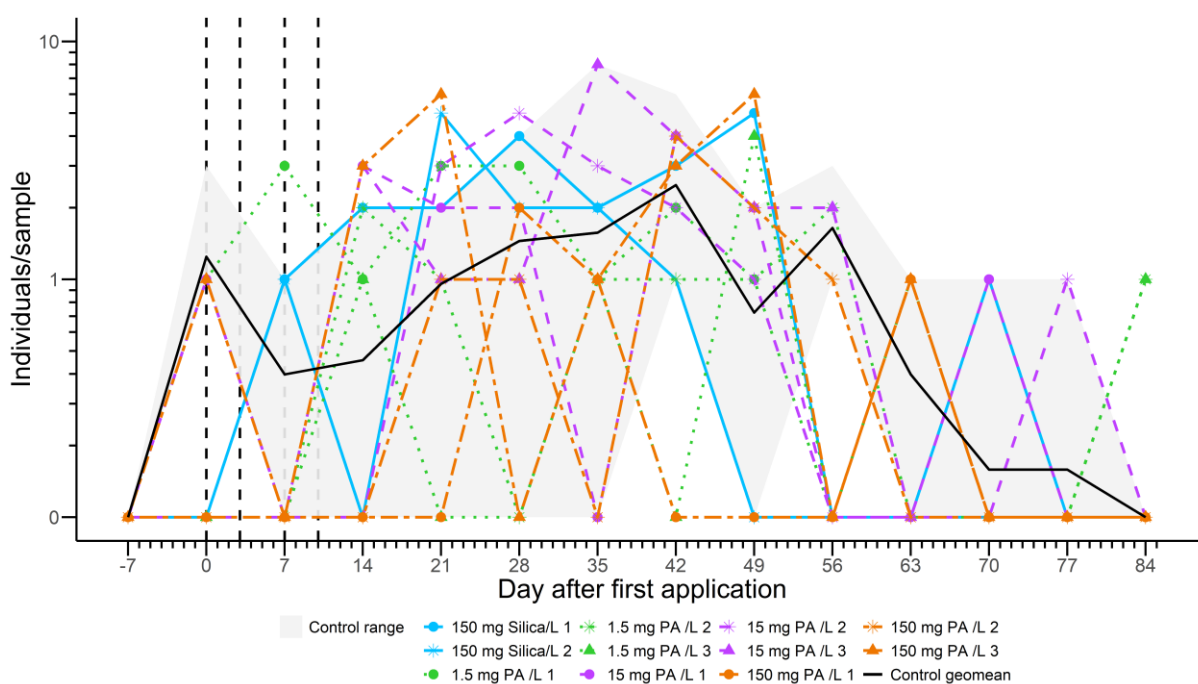
The mean abundance of female *Cloeon dipterum* (Figure 64) ranged from  $0.2 \times/\div 2.8$  Ind. sample<sup>-1</sup> on day 77 to  $29.1 \times/\div 1.44$  Ind. sample<sup>-1</sup> on day 21 in the control. The mean abundance of female *C. dipterum* ranged from 0 Ind. sample<sup>-1</sup> on day 77 to  $42.0 \times/\div 1.54$  Ind. sample<sup>-1</sup> on day 21 in the Silica treatment.



**Figure 64** Abundance of female emerged *Cloeon dipterum* [Ind. sample<sup>-1</sup>]

The mean abundance of female *C. dipterum* ranged from  $0.2 \times/\div 3.78$  Ind. sample<sup>-1</sup> on days 77 and 84 to  $36.1 \times/\div 1.85$  Ind. sample<sup>-1</sup> on day 28 in the LowPA treatment. The mean abundance of female *C. dipterum* ranged from  $0.3 \times/\div 3.78$  Ind. sample<sup>-1</sup> on days 63 through 77 to  $24.9 \times/\div 1.25$  Ind. sample<sup>-1</sup> on day 21 in the MedPA treatment. The mean abundance of female *C. dipterum* ranged from  $0.3 \times/\div 8.41$  Ind. sample<sup>-1</sup> on day 70 to  $29.1 \times/\div 1.59$  Ind. sample<sup>-1</sup> on day 21 in the HighPA treatment. The abundance of male *Cloeon dipterum* was significantly higher compared to the control on days 56 in the Silica treatment. There were no significant differences in the LowPA, MedPA and HighPA treatments compared to the control.

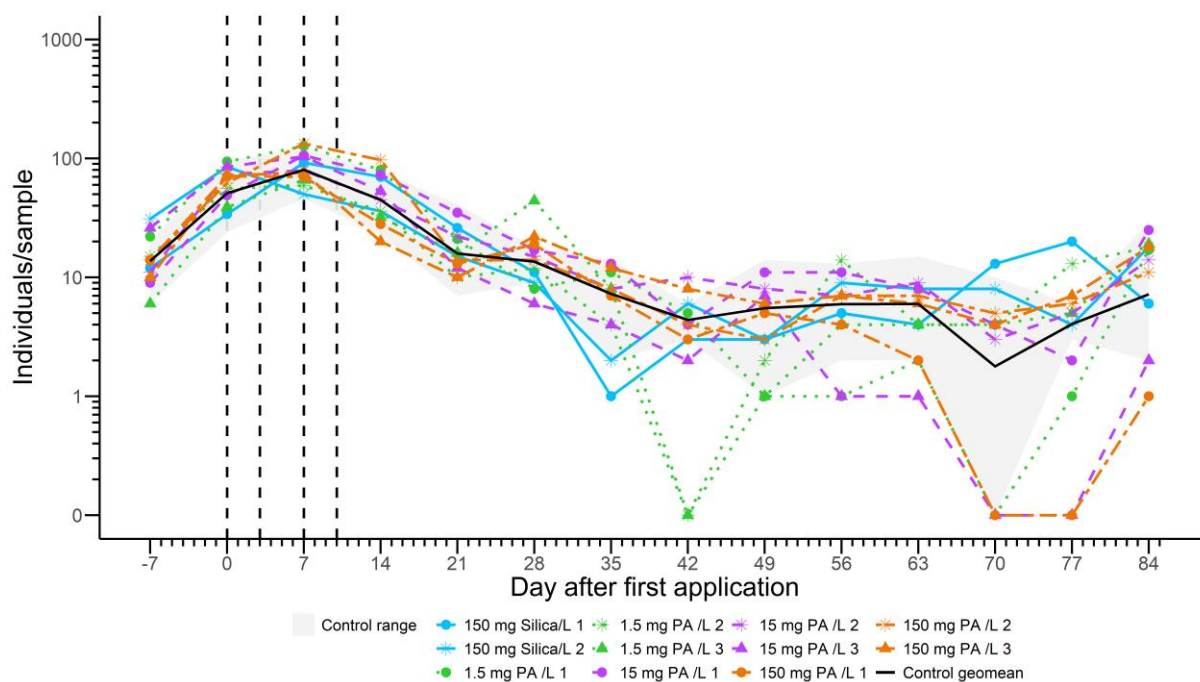
The mean abundance of Coenagrionidae (Figure 65) ranged from 0 Ind. sample<sup>-1</sup> on day -7 and 84 to 2.5 ×/÷ 2.0 Ind. sample<sup>-1</sup> on day 42 in the control. The mean abundance of Coenagrionidae ranged from 0 Ind. sample<sup>-1</sup> on days -7, 0, 56, 63, 77 and 84 to 3.2 ×/÷ 1.91 Ind. sample<sup>-1</sup> on day 21 in the Silica treatment. The mean abundance of Coenagrionidae ranged from 0 Ind. sample<sup>-1</sup> on days -7, 70 and 77 to 1.6 ×/÷ 2.23 Ind. sample<sup>-1</sup> on day 49 in the LowPA treatment.



**Figure 65** Abundance of all emerged Coenagrionidae [Ind. sample<sup>-1</sup>]

The mean abundance of Coenagrionidae ranged from 0 Ind. sample<sup>-1</sup> on days -7, 7, 63 and 84 to 3.2 ×/÷ 1.49 Ind. sample<sup>-1</sup> on day 42 in the MedPA treatment. The mean abundance of Coenagrionidae ranged from 0 Ind. sample<sup>-1</sup> on days -7, 7 and 70 through 84 to 1.1 ×/÷ 7.78 on day 42 in the HighPA treatment. In the LowPA treatment there were significantly more organisms compared to the control on day 84. The abundance of Coenagrionidae fluctuated in all treatments over the whole study. MDD's were over 100 on all sampling days except three (0, 42 and 56), so on all other days only indirect (positive) effects could be statistically confirmed.

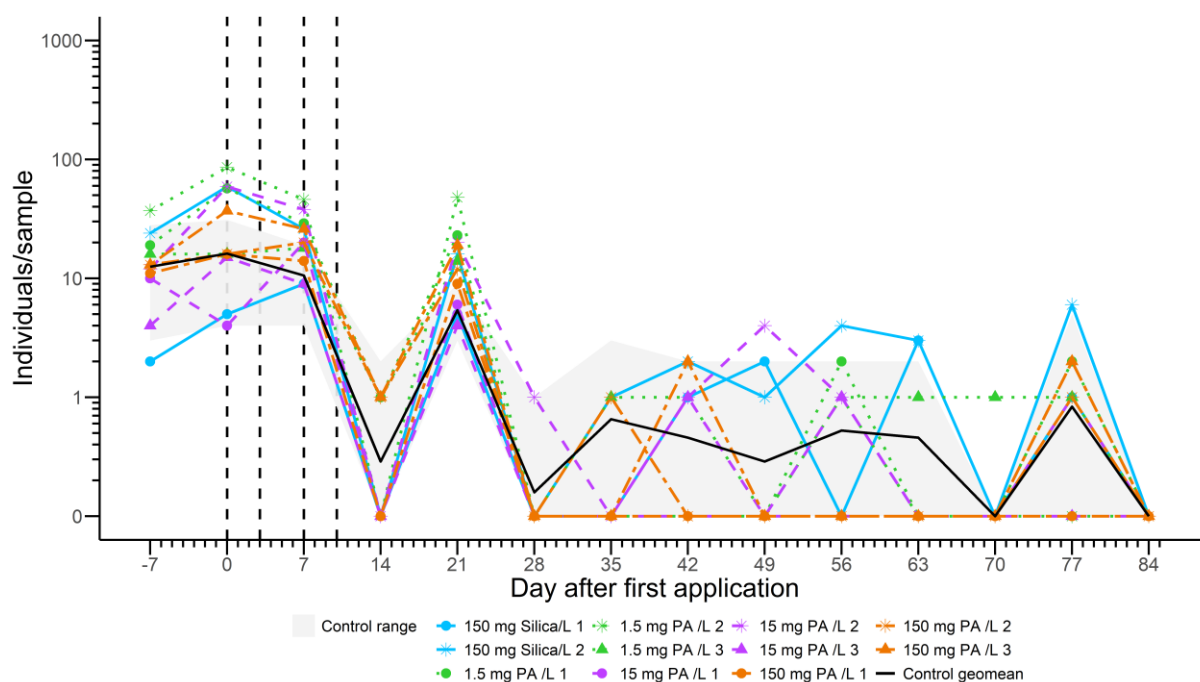
The mean abundance of Tanypodinae (Figure 66) ranged from  $1.8 \times / \div 6.21$  Ind. sample<sup>-1</sup> on day 70 to  $80.4 \times / \div 1.58$  Ind. sample<sup>-1</sup> on day 7 in the control. The mean abundance of Tanypodinae ranged from  $1.4 \times / \div 1.63$  Ind. sample<sup>-1</sup> on day 35 to  $67.8 \times / \div 1.54$  Ind. sample<sup>-1</sup> on day 7 in the Silica treatment. The mean abundance of Tanypodinae ranged from  $0.4 \times / \div 9.57$  Ind. sample<sup>-1</sup> on day 42 to  $79.1 \times / \div 1.51$  Ind. sample<sup>-1</sup> on day 7 in the LowPA treatment.



**Figure 66** Abundance of all emerged Tanypodinae [Ind. sample<sup>-1</sup>]

The mean abundance of Tanypodinae ranged from  $1.0 \times / \div 7.74$  Ind. sample<sup>-1</sup> on day 77 to  $95.5 \times / \div 1.18$  Ind. sample<sup>-1</sup> on day 7 in the MedPA treatment. The mean abundance of Tanypodinae ranged from  $1.3 \times / \div 9.00$  Ind. sample<sup>-1</sup> on day 70 to  $89.1 \times / \div 1.42$  Ind. sample<sup>-1</sup> on day 7 in the HighPA treatment. The abundance of Tanypodinae rose during the application phase to its maximum in all mesocosms and declined afterwards. The dispersion increased in the second half of the experiment. There were no significant differences compared to the control group throughout the study and thus the NOEC is  $> 150$  mg L<sup>-1</sup>.

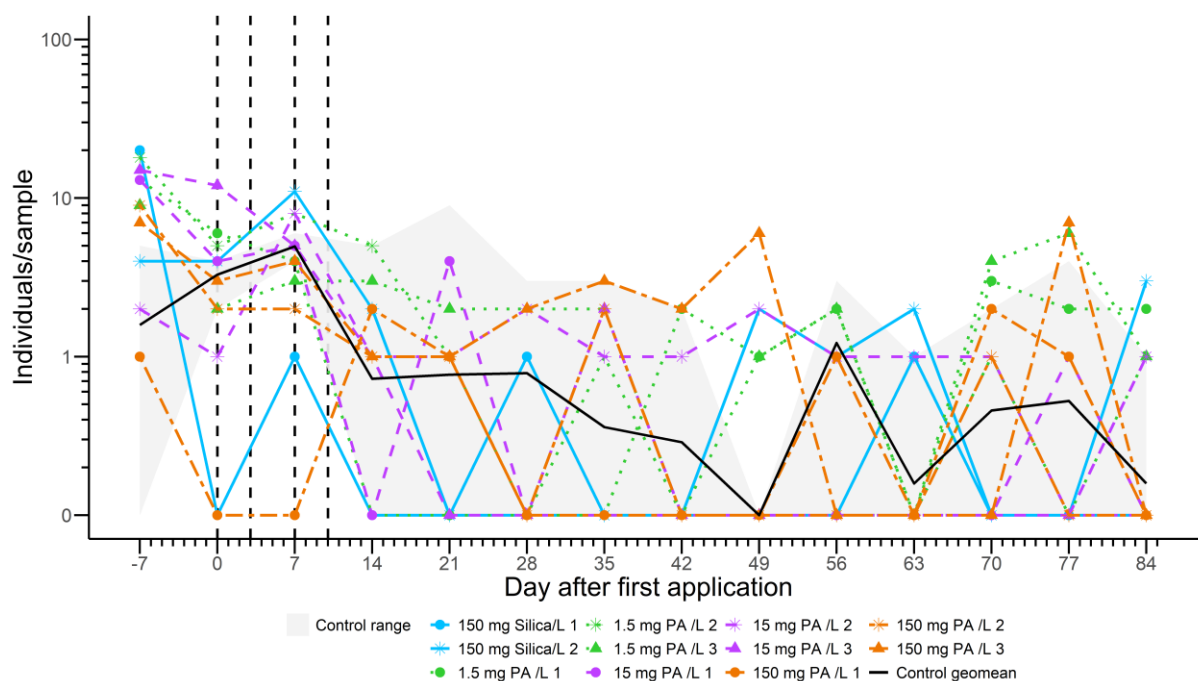
The mean abundance of Orthoclaadiinae (Figure 67) ranged from 0 Ind. sample<sup>-1</sup> on days 70 and 84 to 16.2  $\times/\div$  2.44 Ind. sample<sup>-1</sup> on day 0 in the control. The mean abundance of Orthoclaadiinae ranged from 0 Ind. sample<sup>-1</sup> on days 14, 28, 70 and 84 to 17.2  $\times/\div$  5.73 Ind. sample<sup>-1</sup> on day 0 in the Silica treatment. The mean abundance of Orthoclaadiinae ranged from 0 Ind. sample<sup>-1</sup> on days 28, 49 and 84 to 42.8  $\times/\div$  2.40 Ind. sample<sup>-1</sup> on day 0 in the LowPA treatment. The mean abundance of Orthoclaadiinae ranged from 0 Ind. sample<sup>-1</sup> on days 14, 35, 3, 70 and 84 to 19.0  $\times/\div$  2.06 Ind. sample<sup>-1</sup> on day 7 in the MedPA treatment.



**Figure 67** Abundance of all emerged Orthoclaadiinae [Ind. sample<sup>-1</sup>]

The mean abundance of Orthoclaadiinae ranged from 0 Ind. sample<sup>-1</sup> on days 28, 49 through 70 and 84 to 21.2  $\times/\div$  1.62 Ind. sample<sup>-1</sup> on day 0 in the HighPA treatment. In the LowPA treatment there were significantly more organisms compared to the control on day 21. The Silica treatment had significantly higher abundances on day 63 compared to the control. The MedPA and HighPA treatments showed no significant differences compared to the control. MDD's were over 100 on all sampling days after the first application except two (7 and 21), so on all other days only indirect (positive) effects could be statistically confirmed.

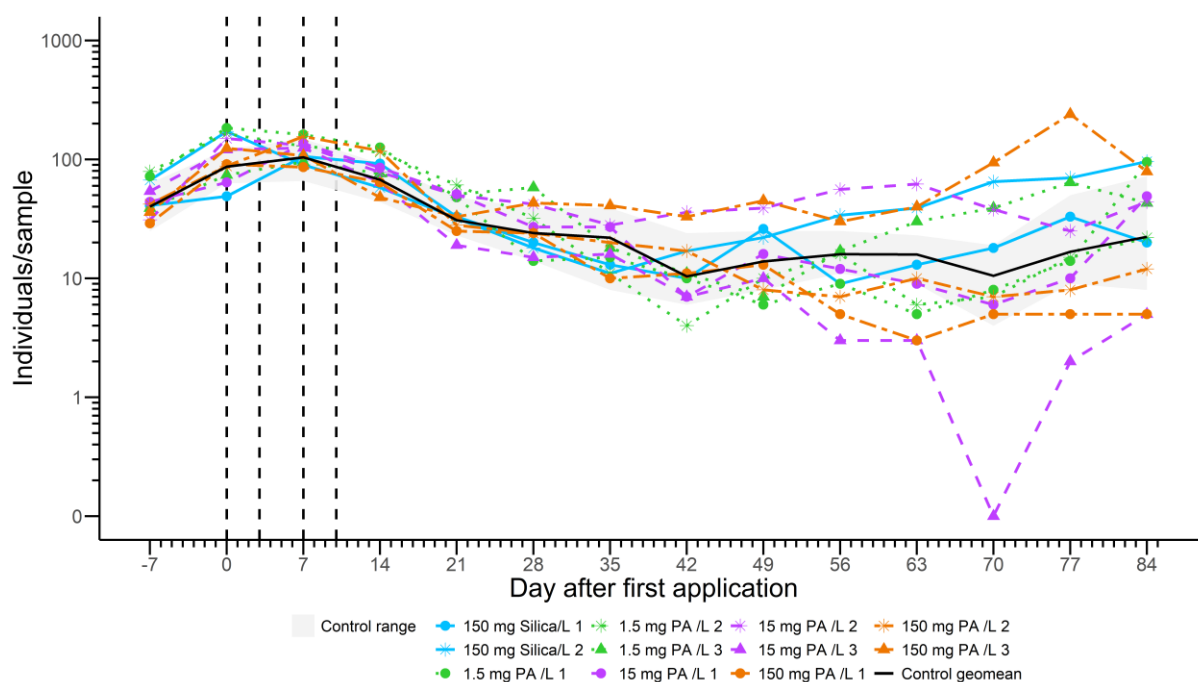
The mean abundance of Chironominae (Figure 68) ranged from 0 Ind. sample<sup>-1</sup> on day 49 to 5.0  $\times/\div$  1.15 Ind. sample<sup>-1</sup> on day 7 in the control. The mean abundance of Chironominae ranged from 0 Ind. sample<sup>-1</sup> on days 21, 35, 42, 70 and 77 to 8.9  $\times/\div$  3.12 Ind. sample<sup>-1</sup> on day -7 in the Silica treatment. The mean abundance of Chironominae ranged from 0 Ind. sample<sup>-1</sup> on day 63 to 12.8  $\times/\div$  1.41 Ind. sample<sup>-1</sup> on day -7 in the LowPA treatment. The mean abundance of Chironominae ranged from 0.2  $\times/\div$  3.78 Ind. sample<sup>-1</sup> on days 42 and 56 through 84 to 7.3  $\times/\div$  3.08 Ind. sample<sup>-1</sup> on day -7 in the MedPA treatment.



**Figure 68** Abundance of all emerged Chironominae [Ind. sample<sup>-1</sup>]

The mean abundance of Chironominae ranged from 0 Ind. sample<sup>-1</sup> on days 63 and 84 to 4.0  $\times/\div$  3.33 Ind. sample<sup>-1</sup> on day -7 in the HighPA treatment. The abundance of Chironominae fluctuated strongly throughout the study in all treatments and the control. On day 63 there were significantly more Chironominae in the Silica treatment compared to the control. Neither polyamide treatment had significant differences compared to the control over the study.

The mean abundance of all Chironomidae (Figure 69) ranged from  $10.4 \times / \div 1.79$  Ind. sample<sup>-1</sup> on day 42 to  $104.1 \times / \div 1.42$  Ind. sample<sup>-1</sup> on day 7 in the control. The mean abundance of all Chironomidae ranged from  $12.0 \times / \div 1.13$  Ind. sample<sup>-1</sup> on day 35 to  $97.7 \times / \div 1.12$  Ind. sample<sup>-1</sup> on day 7 in the Silica treatment. The mean abundance of all Chironomidae ranged from  $7.5 \times / \div 1.3$  Ind. sample<sup>-1</sup> on day 49 to  $131.0 \times / \div 1.64$  Ind. sample<sup>-1</sup> on day 0 in the LowPA treatment. The mean abundance of all Chironomidae ranged from  $2.8 \times / \div 20.91$  Ind. sample<sup>-1</sup> on day 70 to  $130.6 \times / \div 1.04$  Ind. sample<sup>-1</sup> on day 7 in the MedPA treatment.



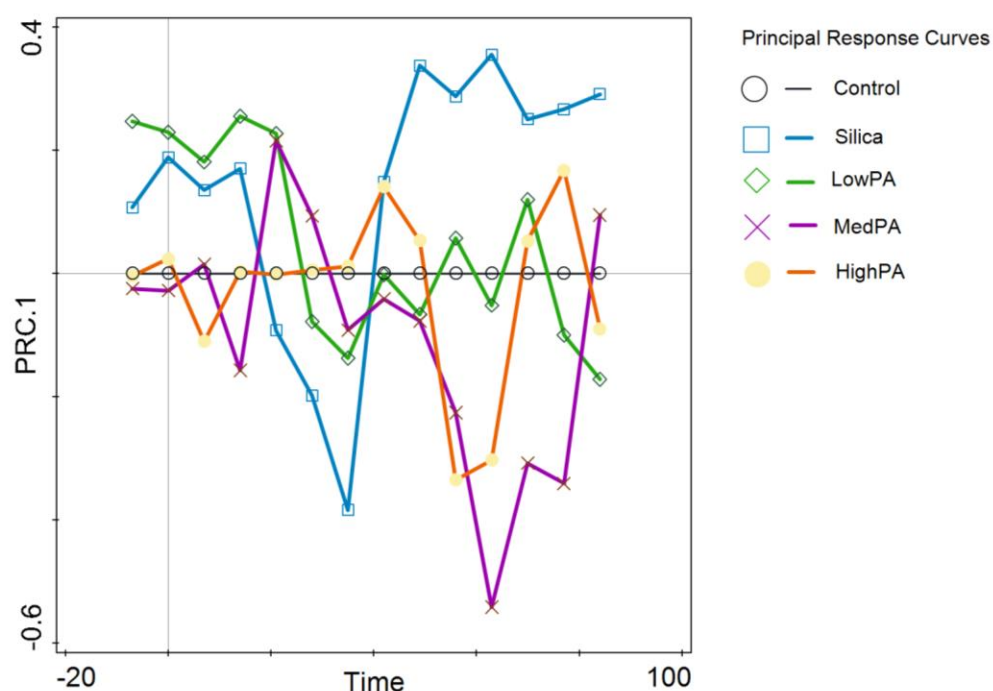
**Figure 69** Abundance of all emerged Chironomidae [Ind. sample<sup>-1</sup>]

The mean abundance of all Chironomidae ranged from  $10.2 \times / \div 2.59$  Ind. sample<sup>-1</sup> on day 56 to  $113.2 \times / \div 1.35$  Ind. sample<sup>-1</sup> on day 7 in the HighPA treatment. The abundance of all Chironomidae declined after the application phase and the dispersion rose after the first half of the study. There were no significant differences compared to the control group throughout the study and thus the NOEC is  $> 150$  mg L<sup>-1</sup>. One replicate of the MedPA treatment had no organisms on day 70. One replicate of each treatment exceeded the control range at the latter stages of the study.

#### 4.4.2 PRC of emerging insect community

The PRC of the emerging insect community (Figure 70) depicts the effect of the treatments on community level. The Silica treatments started with positive effect-like values before application. After the applications the effect decreased and peaked in negative value. After that the effect turned around and the positive effect was stronger than in all polyamide

treatments. The LowPA treatment has moderate effects with a similar starting point as the Silica treatment. After application the values approached the control and remained within the control range until the end of the study. The MedPA treatment, after a positive peak after the last application, shows a negative effect until the end of the study. HighPA treatment, like the MedPA treatment, shows a positive effect of the particles on the community, but with a later peak. After that, at the same time as the MedPA treatment, the HighPA treatment showed a strong negative effect, but recovered two weeks after. Before the first application (vertical line; day 0) there are no differences compared to the control line.



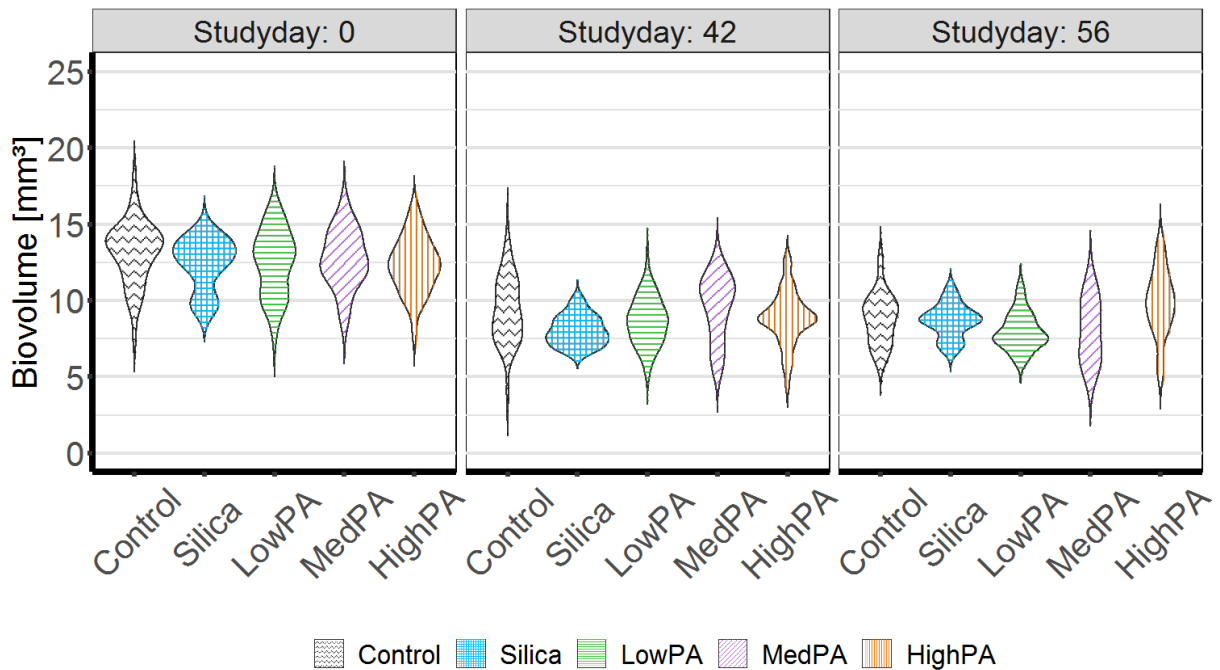
**Figure 70** Principal response curve of emerging insect community

The changes in emerging insect community were not significant ( $p$ -value = 0.258). 7 out of the 19 taxa had species scores with an absolute value over 0.5. Out of those only Thysanoptera had a negative sign with a species score of -0.91. *Chaoborus* sp., with species score of 2.85 followed by Chironomidae with a score of 2.00 had the biggest species scores. The subfamily Orthocladiinae and *Cloeon dipterum* had species scores around 1.5 with 1.55 and 1.44 respectively. Two more subfamilies of Chironomidae had species scores greater than 0.5. Chironominae with 0.90 and Tanypodinae with 0.61 had the fifth and sixth greatest positive species scores.

#### 4.4.3 Biovolume

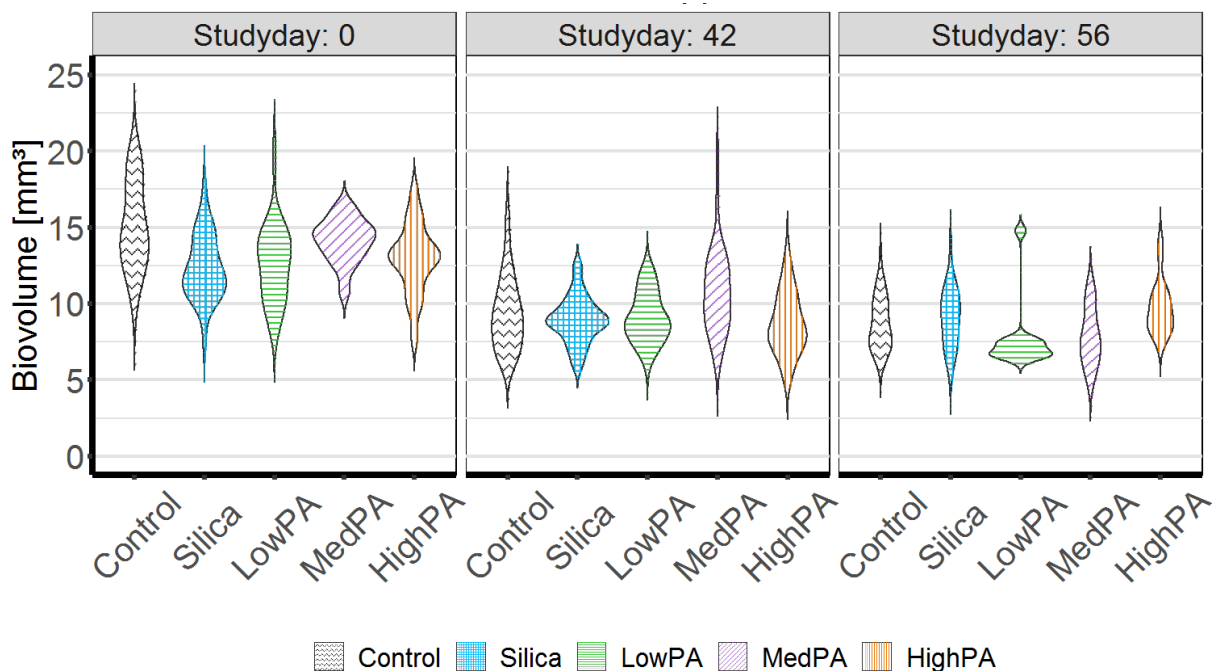
The biovolume of the male *Chaoborus* sp. (Figure 71) showed no significant differences within a sampling date. The frequently occurring biovolumes ranged between 10 and 15 mm<sup>3</sup>

in all treatments and the control on day 0. Study days 42 and 56 showed lower values in biovolumes but no significant differences between the treatments and control.



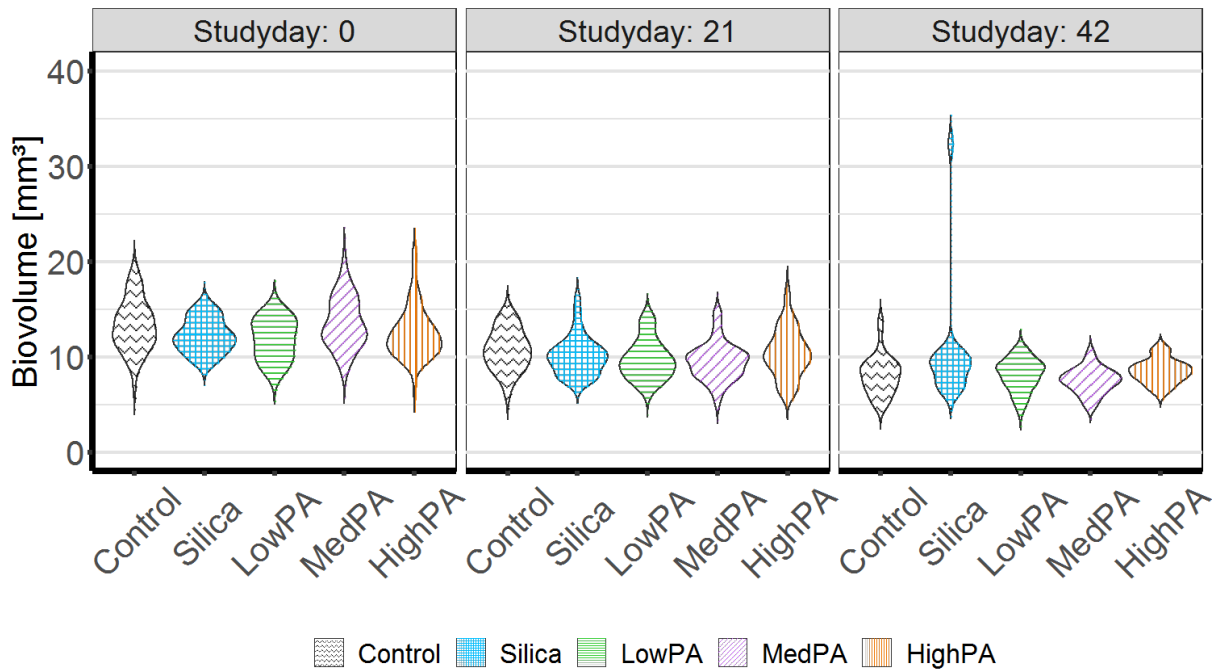
**Figure 71** Biovolume of male emerged *Chaoborus* sp. on days 0, 42 and 56

The female *Chaoborus* sp. (Figure 72) were slightly bigger compared to the male organisms in average on day 0. There were no significant differences between the control and the treatments. The 15 mg PA L<sup>-1</sup> lacked smaller organisms between 5 and 10 mm<sup>3</sup>. On study days 42 and 56 the organisms were smaller than on day 0 in general. There were no significant differences between the control and the treatments after application.



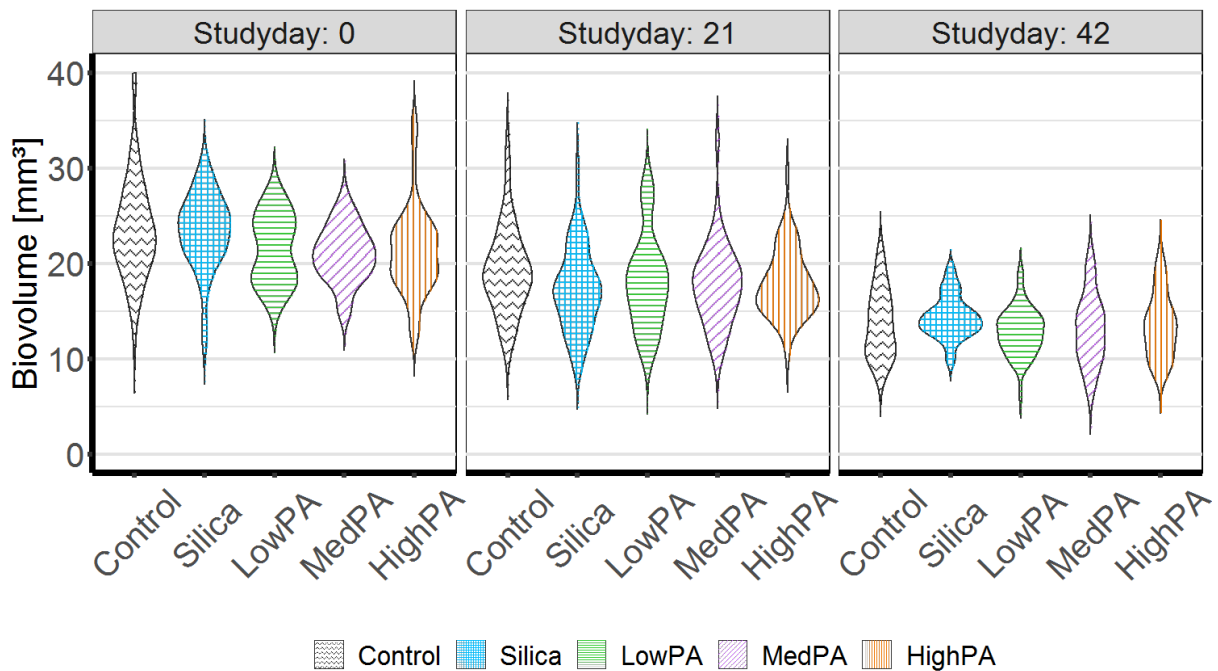
**Figure 72** Biovolume of female emerged *Chaoborus* sp. on days 0, 42 and 56

The biovolume of *Cloeon dipterum* males (Figure 73) ranged between 5 and 20 mm<sup>3</sup> in the control and all treatments at study days 0 and 21. There were no significant differences between the treatments and the control. The organisms were slightly smaller on average on day 21 and even smaller on day 42.



**Figure 73** Biovolume of male emerged *Cloeon dipterum* on days 0, 21 and 42

The biovolume of *Cloeon dipterum* females (Figure 74) had the highest frequencies from 15 to 30 mm<sup>3</sup> before application and after application on day 21. On study day 42 the mean biovolumes of organisms in the treatments and the control were lower. No significant differences were detected.



**Figure 74** Biovolume of female emerged *Cloeon dipterum* on days 0, 21 and 42

#### 4.4.4 Sex ratio

The sex ratio of  $n_{\text{males}}/n_{\text{total}}$  of *Chaoborus* sp. (Table 14) showed significant differences compared to the control on three samplings in the Silica treatment. On days 0 and 14 the proportion of males was significantly lower compared to the control. On day 21 the proportion was less than half compared to the control, but no significance could be found due to insufficient sample size. On day 49 the proportion of males was significantly higher in the Silica treatment compared to the control. The LowPA and MedPa showed no significant differences compared to the control throughout the study. The proportion of males in the HighPA treatment was significantly higher on day 28. After day 56 not all treatments and after day 70 neither treatment had sufficient numbers for statistical evaluation.

**Table 14** Sex ratio of *Chaoborus* sp. Proportion calculated as  $n_{\text{males}}/n_{\text{total}}$ . Bold green cells show significant differences to control. Italic orange cells indicate low abundances ( $n < 30$ ) and unreliable statistical analysis.

Day	Control ratio ( $n_{\text{males}}/n_{\text{total}}$ )	Silica	LowPA	MedPA	HighPA
-7	60.8	55.1	57.6	69.7	69.9
0	55.3	<b>31.8</b>	51.0	54.2	54.8
7	39.7	48.1	43.7	52.9	40.0
14	55.9	<b>28.6</b>	43.9	<i>31.6</i>	<i>72.7</i>
21	78.6	<i>30.8</i>	<i>42.9</i>	65.9	<i>66.7</i>
28	66.7	64.3	66.7	65.1	<b>81.4</b>
35	62.7	75.6	64.5	62.1	64.4
42	56.2	66.7	53.5	42.6	51.6
49	49.8	<b>64.0</b>	51.2	50.0	41.0
56	45.5	43.9	51.4	29.4	43.0
63	55.1	55.9	<i>60.0</i>	<i>28.6</i>	<i>44.4</i>
70	60.9	<i>50.0</i>	52.5	<i>45.5</i>	<i>43.5</i>
77	45.5	<i>57.1</i>	<i>40.0</i>	<i>66.7</i>	<i>52.4</i>
84	58.3	<i>60.0</i>	<i>50.0</i>	<i>53.8</i>	<i>100.0</i>

The proportion of  $n_{\text{males}}/n_{\text{total}}$  of *Cloeon dipterum* (Table 15) showed no significant differences compared to the control in neither treatment. Starting day 63 no significant differences were observable due to insufficient numbers of individuals. The proportion of males in the control stayed around 50 to 60% through the first 8 samplings (day -7 to day 42). The proportion sunk after.

**Table 15** Sex ratio of *Cloeon dipterum*. Proportion calculated as  $n_{\text{males}}/n_{\text{total}}$ . Italic orange cells indicate low abundances ( $n < 30$ ) and unreliable statistical analysis.

Day	Control ratio ( $n_{\text{males}}/n_{\text{total}}$ )	Silica	LowPA	MedPA	HighPA
-7	63.6	58.7	63.8	63.0	60.2
0	58.9	61.6	47.4	66.7	61.9
7	51.0	50.0	66.7	58.1	50.6
14	55.0	53.4	54.3	50.0	43.5
21	55.0	46.3	47.1	49.7	40.8
28	53.1	55.9	54.2	65.5	52.5
35	55.3	43.4	54.5	61.3	58.6
42	48.6	46.4	48.9	58.8	42.9
49	29.7	35.4	34.7	52.1	40.0
56	27.3	31.3	20.0	40.0	10.0
63	33.3	37.5	23.5	0.0	30.0
70	0.0	42.9	36.4	0.0	66.7
77	0.0	100.0	0.0	0.0	0.0
84	44.4	72.7	75.0	0.0	50.0

#### 4.4.5 Dominance

In the control (Table 16), the mayflies *Cloeon dipterum* were initially the most dominant taxon with 43.4% (eudominant). At the end of the study, the taxon was less represented with 7.2% and classified as dominant. On day 21, the mayflies showed a peak with over 60% relative dominance. *Chaoborus* sp. peaked at 58.2% (eudominant) on day 49, with the lowest proportions of less than 10% (dominant) recorded in the first weeks after first application. The taxon Chironomidae increased continuously towards the end of the study, while the subfamilies Orthocladiinae, Tanypodinae and Chironominae decreased steadily. The Silica treatment showed a similar trend to the control, whereby the dominance of the Chironomidae was even higher at 63.1% at the last sampling. The LowPA treatment confirms this trend. However, there was a very high dominance of Tanypodinae on day 7 with almost 50%. *Chaoborus* sp. was also eudominant on day 35 with 60.3%. As in the other treatments, *Cloeon dipterum* was not recedent to subdominant at the end of the study. In the HighPA treatment, *Chaoborus* sp. on day 35 with 64.3% and Chironomidae with 78.1% on day 77 achieved the highest relative dominances across all treatments. *Cloeon dipterum* started high at 40.3% (day -7), then dropped dramatically to 9.1% (dominant) on day 84 with an interim peak on day 21 at just below 60%.

**Table 16** Relative densities [%] of most abundant taxa of emerging insects

<b>Control</b>														
<b>Day</b>	<b>-7</b>	<b>0</b>	<b>7</b>	<b>14</b>	<b>21</b>	<b>28</b>	<b>35</b>	<b>42</b>	<b>49</b>	<b>56</b>	<b>63</b>	<b>70</b>	<b>77</b>	<b>84</b>
Anisoptera	0.0	0.0	0.0	0.6	0.0	0.4	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
<i>Chaoborus</i> sp.	28.2	17.2	8.3	5.2	7.7	36.8	54.1	56.6	58.2	55.8	48.9	44.5	20.8	12.0
Chironomidae	4.1	5.2	3.0	13.1	3.6	5.4	4.2	3.3	6.8	8.8	15.5	21.9	40.9	43.3
Chironominae	1.8	1.9	3.0	1.5	2.2	0.7	0.5	0.5	0.0	2.9	0.4	2.6	3.9	0.5
<i>Cloeon dipterum</i>	43.4	31.3	25.2	39.8	60.9	43.0	30.5	26.2	15.6	9.6	8.3	1.9	0.6	7.2
Coenagrionidae	0.0	0.8	0.4	0.6	1.2	1.5	1.5	2.6	1.0	2.6	1.1	0.6	0.6	0.0
Orthoclaadiinae	9.9	11.3	7.2	0.4	4.9	0.1	0.7	0.7	0.6	1.5	1.5	0.0	7.1	0.0
Tanypodinae	9.0	30.6	51.3	35.9	16.4	8.7	4.2	4.0	7.6	10.8	14.0	12.9	13.6	26.4
Thysanoptera	2.2	0.9	0.2	0.4	0.5	2.1	1.2	2.2	5.1	5.3	6.8	10.3	2.6	1.4
<b>Silica</b>														
<b>Day</b>	<b>-7</b>	<b>0</b>	<b>7</b>	<b>14</b>	<b>21</b>	<b>28</b>	<b>35</b>	<b>42</b>	<b>49</b>	<b>56</b>	<b>63</b>	<b>70</b>	<b>77</b>	<b>84</b>
Anisoptera	0.0	0.0	0.5	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Chaoborus</i> sp.	30.4	20.4	19.5	16.0	6.4	20.7	38.6	59.2	64.0	54.2	43.8	22.2	8.4	7.1
Chironomidae	3.5	7.8	1.7	11.1	1.1	7.2	7.6	6.0	10.8	10.6	18.8	48.4	60.5	63.1
Chironominae	6.5	0.9	2.9	0.6	0.0	0.5	0.0	0.0	0.6	0.4	1.9	0.0	0.0	2.1
<i>Cloeon dipterum</i>	38.7	29.1	31.0	38.6	64.4	57.7	45.3	27.2	15.8	22.0	16.9	7.1	2.5	9.9
Coenagrionidae	0.0	0.0	0.5	0.6	2.7	2.7	1.7	1.6	1.5	0.0	0.0	0.8	0.0	0.0
Orthoclaadiinae	7.0	14.6	8.5	0.0	8.0	0.0	0.4	1.2	0.9	1.8	3.8	0.0	5.9	0.0
Tanypodinae	11.6	27.2	34.6	30.3	15.5	9.0	1.3	3.6	1.8	6.2	7.5	16.7	20.2	17.0
Thysanoptera	1.6	0.0	0.5	0.3	0.4	0.9	3.4	0.4	3.5	2.6	3.1	3.2	0.0	0.0

**Table 16** (continued) Relative densities [%] of most abundant taxa of emerging insects

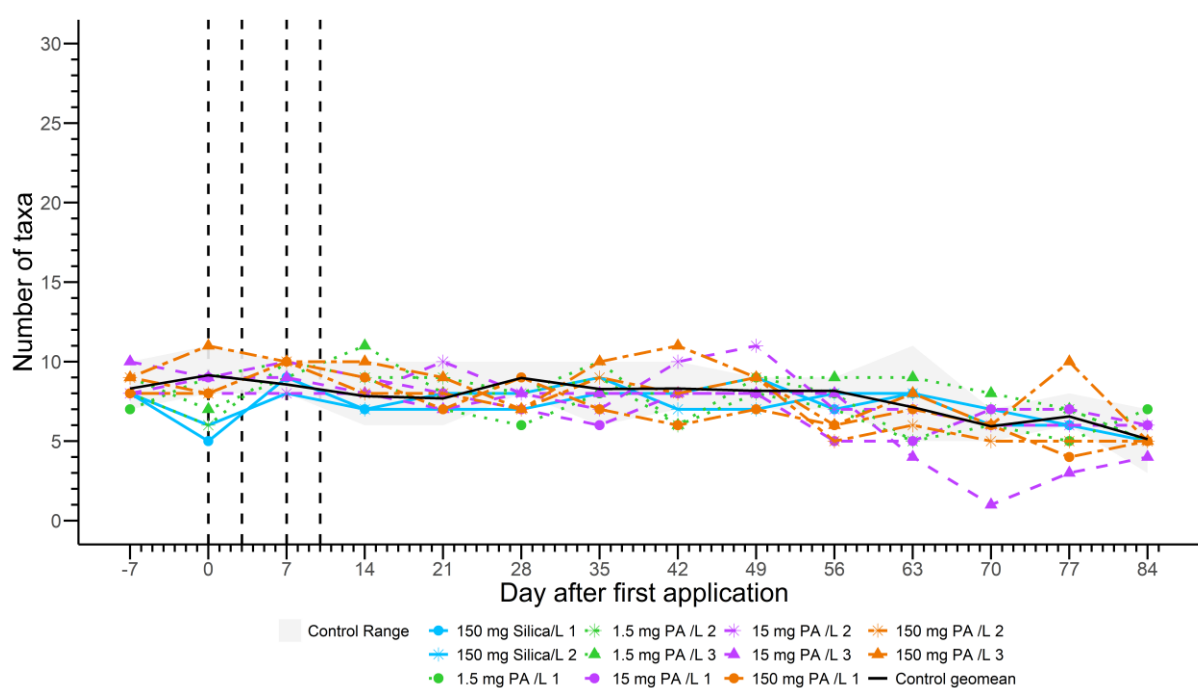
<b>1.5 mg/L PA</b>														
<b>Day</b>	<b>-7</b>	<b>0</b>	<b>7</b>	<b>14</b>	<b>21</b>	<b>28</b>	<b>35</b>	<b>42</b>	<b>49</b>	<b>56</b>	<b>63</b>	<b>70</b>	<b>77</b>	<b>84</b>
Anisoptera	0.0	0.0	0.0	1.4	0.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Chaoborus</i> sp.	34.3	16.4	14.5	9.8	5.4	11.9	43.9	51.5	52.3	53.2	37.4	32.8	6.5	1.2
Chironomidae	6.5	10.0	3.6	27.6	5.4	7.2	1.8	4.0	2.4	6.9	26.1	29.6	57.4	59.6
Chironominae	7.7	2.1	2.4	1.4	0.6	0.4	0.5	0.5	0.6	1.9	0.0	6.4	7.4	2.3
<i>Cloeon dipterum</i>	27.8	13.5	22.4	29.0	56.7	63.1	46.4	36.6	30.0	16.2	16.5	8.8	0.9	2.3
Coenagrionidae	0.0	0.2	0.5	0.7	0.9	0.6	0.5	0.7	1.8	0.9	0.9	0.0	0.0	1.2
Orthoclaadiinae	13.8	26.2	14.6	0.3	18.4	0.0	0.2	0.2	0.0	1.9	0.9	0.8	2.8	0.0
Tanypodinae	8.2	31.1	39.7	25.8	10.2	13.6	3.8	1.2	1.2	8.8	8.7	6.4	17.6	31.6
Thysanoptera	0.4	0.0	0.5	0.5	0.0	0.6	1.2	3.5	7.6	8.3	6.1	12.0	2.8	0.6
<b>15 mg/L PA</b>														
<b>Day</b>	<b>-7</b>	<b>0</b>	<b>7</b>	<b>14</b>	<b>21</b>	<b>28</b>	<b>35</b>	<b>42</b>	<b>49</b>	<b>56</b>	<b>63</b>	<b>70</b>	<b>77</b>	<b>84</b>
Anisoptera	0.0	0.0	0.0	1.1	0.5	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
<i>Chaoborus</i> sp.	26.9	13.3	9.3	6.5	23.3	53.1	60.3	52.3	35.4	26.5	9.4	21.8	13.0	18.3
Chironomidae	6.7	5.1	2.9	15.2	4.1	6.8	7.7	10.1	11.9	30.2	56.3	46.2	48.1	42.7
Chironominae	7.8	3.0	3.0	0.6	1.3	0.3	0.6	0.3	0.8	0.6	1.0	1.3	1.9	0.8
<i>Cloeon dipterum</i>	33.9	25.2	21.1	22.8	42.6	29.0	21.9	22.7	20.2	17.3	2.1	2.6	1.9	2.3
Coenagrionidae	0.0	0.4	0.0	1.7	1.5	1.4	2.1	3.2	2.1	1.2	0.0	1.3	1.9	0.0
Orthoclaadiinae	6.7	13.8	11.3	0.0	7.7	0.2	0.0	0.6	1.6	1.2	0.0	0.0	1.9	0.0
Tanypodinae	11.9	36.9	48.8	48.0	17.7	6.5	4.7	5.2	10.7	11.7	18.8	9.0	13.0	31.3
Thysanoptera	3.9	1.1	1.7	1.4	0.3	1.4	2.2	3.6	11.5	9.9	8.3	15.4	5.6	0.0

**Table 16** (continued) Relative densities [%] of most abundant taxa of emerging insects

<b>150 mg/L PA</b>														
<b>Day</b>	<b>-7</b>	<b>0</b>	<b>7</b>	<b>14</b>	<b>21</b>	<b>28</b>	<b>35</b>	<b>42</b>	<b>49</b>	<b>56</b>	<b>63</b>	<b>70</b>	<b>77</b>	<b>84</b>
Anisoptera	0.0	0.0	0.5	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>Chaoborus</i> sp.	31.4	16.0	7.2	4.0	8.0	39.1	64.3	60.6	53.8	54.2	25.0	16.1	10.3	9.1
Chironomidae	2.9	3.6	0.7	17.3	2.0	5.8	4.2	8.8	13.2	13.9	32.8	60.6	78.1	54.5
Chironominae	4.2	0.8	1.0	0.9	1.0	0.4	0.6	0.4	1.8	0.6	0.0	1.9	2.7	0.0
<i>Cloeon dipterum</i>	40.3	32.4	26.7	34.6	58.1	38.3	23.4	17.5	16.1	6.6	9.5	3.9	0.7	9.1
Coenagrionidae	0.0	0.2	0.0	0.7	2.3	0.6	0.3	1.5	2.3	0.6	1.7	0.0	0.0	0.0
Orthoclaadiinae	9.1	11.2	10.1	0.5	13.3	0.0	0.1	0.4	0.0	0.0	0.0	0.0	1.0	0.0
Tanyptodinae	9.3	33.4	46.9	34.4	12.3	11.3	3.4	3.2	4.1	10.8	12.9	5.8	4.5	24.8
Thysanoptera	0.7	0.7	1.0	0.9	0.3	1.6	1.8	4.2	6.1	10.2	13.8	7.7	0.3	0.8

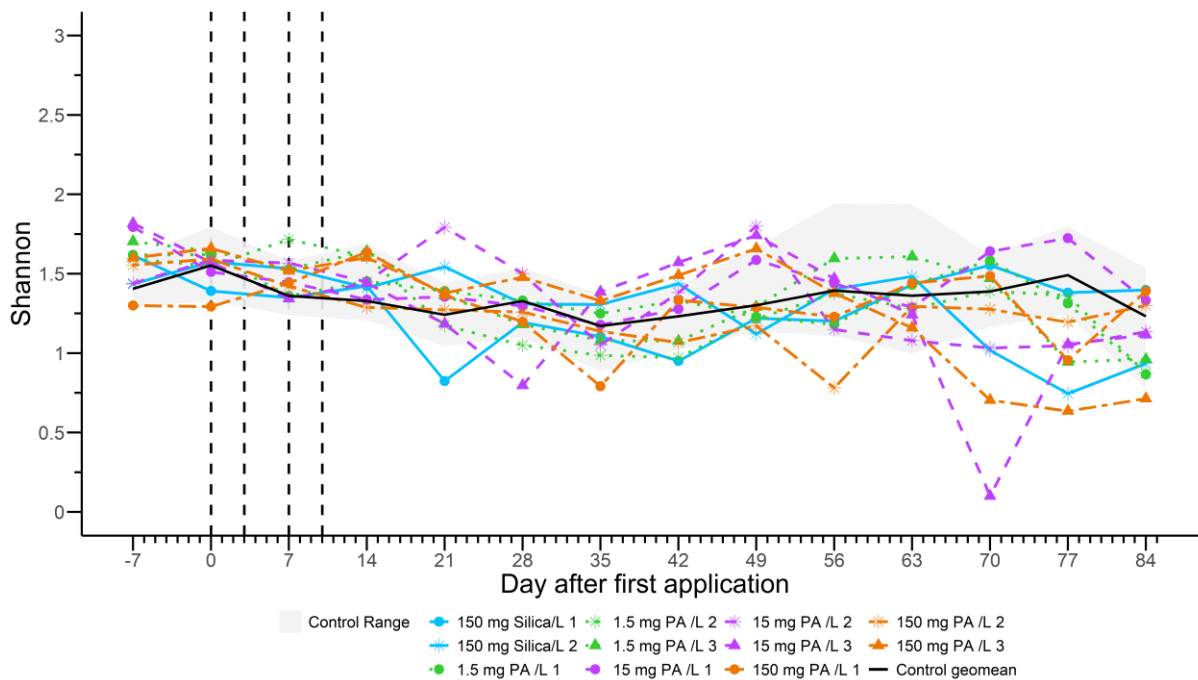
#### 4.4.6 Diversity indices

The mean number of taxa (Figure 75) ranged from  $5.1 \times / \div 1.46$  on day 84 to  $9.2 \times / \div 1.12$  on day 0 in the control. The mean number of taxa ranged from  $5.0 \times / \div 1.00$  on day 84 to  $8.5 \times / \div 1.09$  on day 7 in the Silica treatment. The mean number of taxa ranged from  $5.6 \times / \div 1.21$  on day 84 to  $9.7 \times / \div 1.06$  on day 7 in the LowPA treatment. The mean number of taxa ranged from  $3.5 \times / \div 2.95$  on day 70 to  $9.0 \times / \div 1.12$  on day 7 in the MedPA treatment. The mean number of taxa ranged from  $5.0 \times / \div 1.00$  on day 84 to  $10.0 \times / \div 1.00$  on day 7 in the HighPA treatment. On day 0, before application the number of taxa in the Silica treatment was significantly lower compared to the control. The number of taxa was significantly lower compared to the control on day 56 in the HighPA treatment.



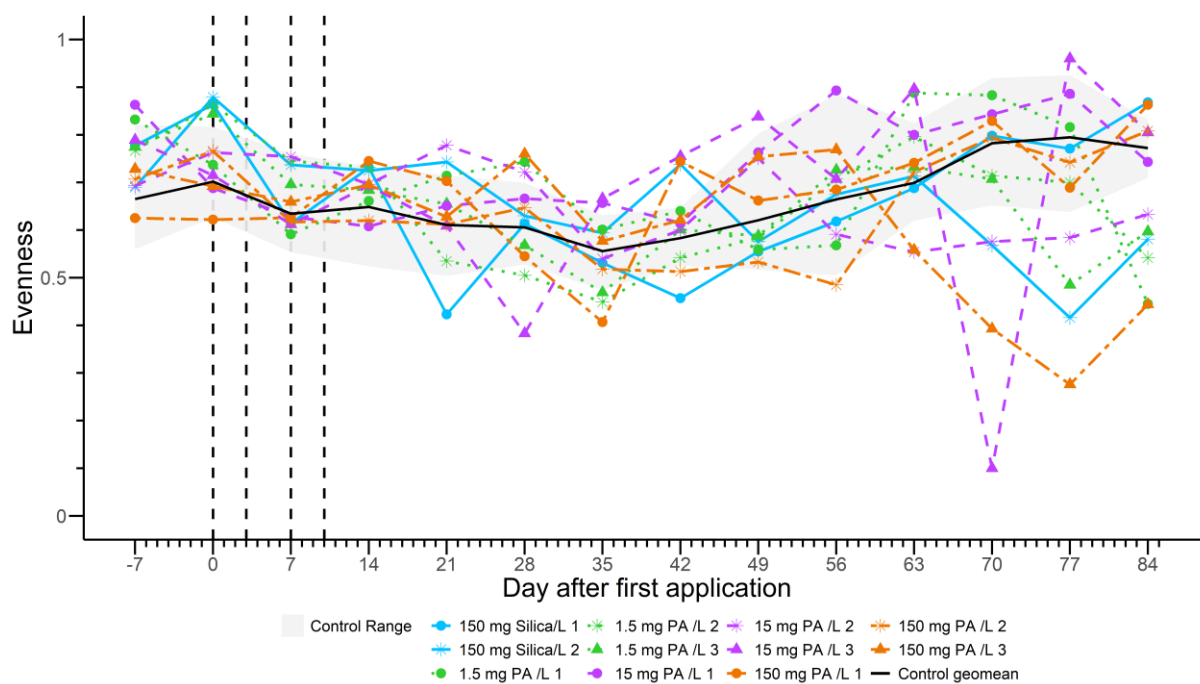
**Figure 75** Number of taxa of emerging insects

The mean Shannon index (Figure 76) ranged from  $1.2 \times / \div 1.21$  on day 35 to  $1.6 \times / \div 1.14$  on day 0 in the control. The mean Shannon index ranged from  $1.0 \times / \div 1.55$  on day 77 to  $1.5 \times / \div 1.09$  on day -7 in the Silica treatment. The mean Shannon index ranged from  $0.9 \times / \div 1.06$  on day 84 to  $1.6 \times / \div 1.03$  on day -7 in the LowPA treatment. The mean Shannon index ranged from  $0.6 \times / \div 4.48$  on day 70 to  $1.7 \times / \div 1.07$  on day 49 in the MedPA treatment. The mean Shannon index ranged from  $0.9 \times / \div 1.38$  on day 77 to  $1.5 \times / \div 1.14$  on day 0 in the HighPA treatment. There were no significant differences compared to the control group throughout the study regarding the Shannon index.



**Figure 76** Shannon index of emerging insect community

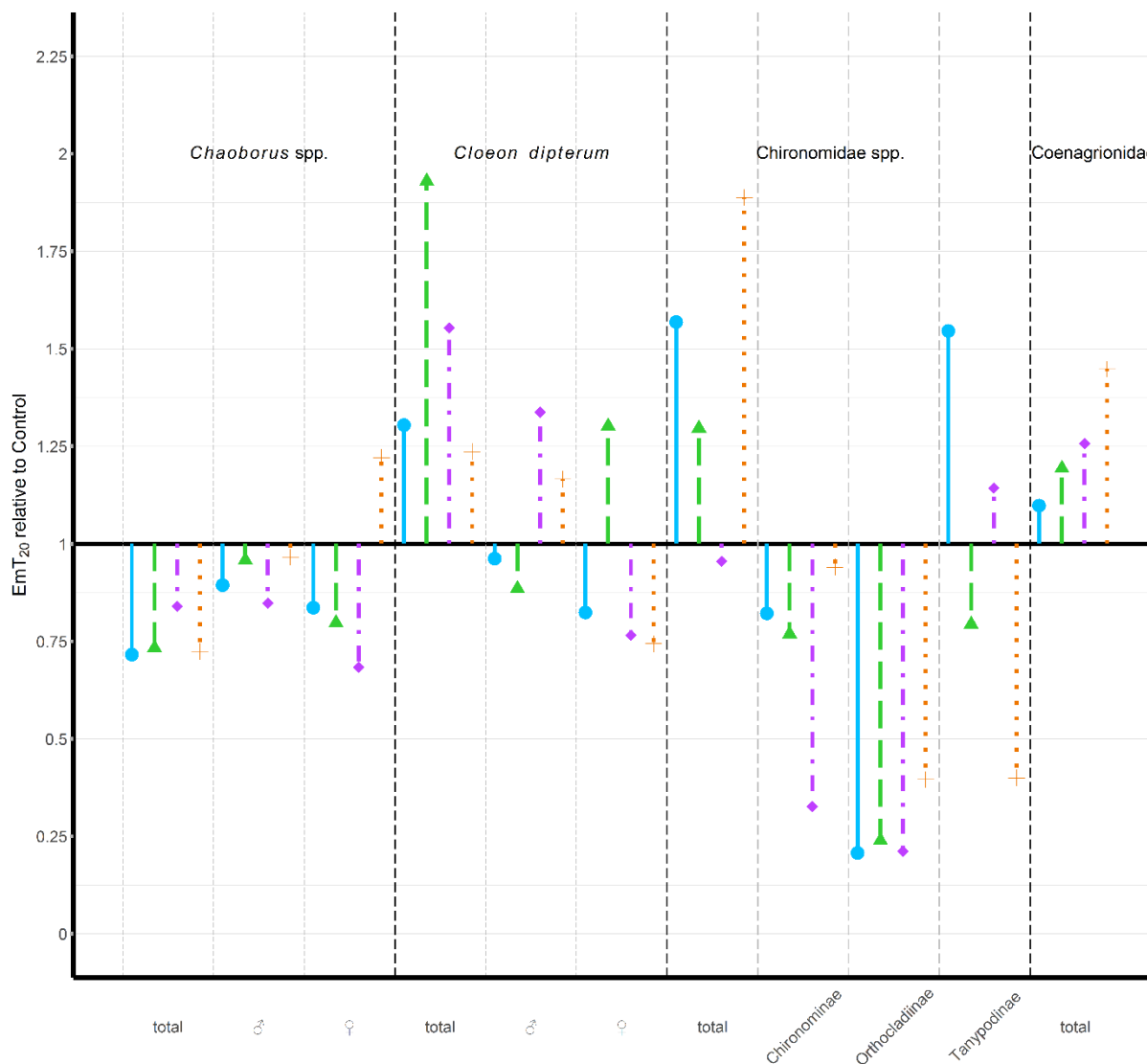
The mean evenness (Figure 77) ranged from 0.56  $\times/\div$  1.14 on day 35 to 0.79  $\times/\div$  1.15 on day 77 in the control. The mean evenness ranged from 0.56  $\times/\div$  1.49 on day 21 to 0.87  $\times/\div$  1.01 on day 0 in the Silica treatment. The mean evenness ranged from 0.50  $\times/\div$  1.17 on day 35 to 0.81  $\times/\div$  1.09 on day 0 in the LowPA treatment. The mean evenness ranged from 0.36  $\times/\div$  3.12 on day 70 to 0.79  $\times/\div$  1.31 on day 77 in the MedPA treatment. The mean evenness ranged from 0.50  $\times/\div$  1.20 on day 35 to 0.69  $\times/\div$  1.11 on day 0 in the HighPA treatment. There were no significant differences compared to the control group throughout the study regarding the evenness.



**Figure 77** Evenness of emerging insect community

#### 4.4.7. Emergence mean Time

The emergence mean Time for a proportion of 20% of the organisms ( $EmT_{20}$ ) was normalized to the control value (Figure 78). The control value is fixed at a value of 1. The total sum of chaoborids, as well as the male and female individuals separately have  $EmT_{20}$  values below 1 in all treatments. The only exception were the female *Chaoborus* sp. in the HighPA treatment, where the organisms emerged around 25% later than in the control. The males have  $EmT_{20}$  values closer to 1 compared to the females and the total sum, which also includes the organisms whose sex was not distinguishable. The total sum of *Cloeon dipterum* had  $EmT_{20}$  values over 1 in all treatments with the organisms in the LowPA treatment emerged the latest followed by the MedPA treatment. 20% of the *Cloeon dipterum* males emerged slightly early in the Silica and LowPA treatment compared to the control. The organisms in the MedPA and HighPA treatment emerged 20% to 40% later than the ones in the control. The female *Cloeon dipterum* emerged early compared to the control in all treatments except the LowPA treatment.

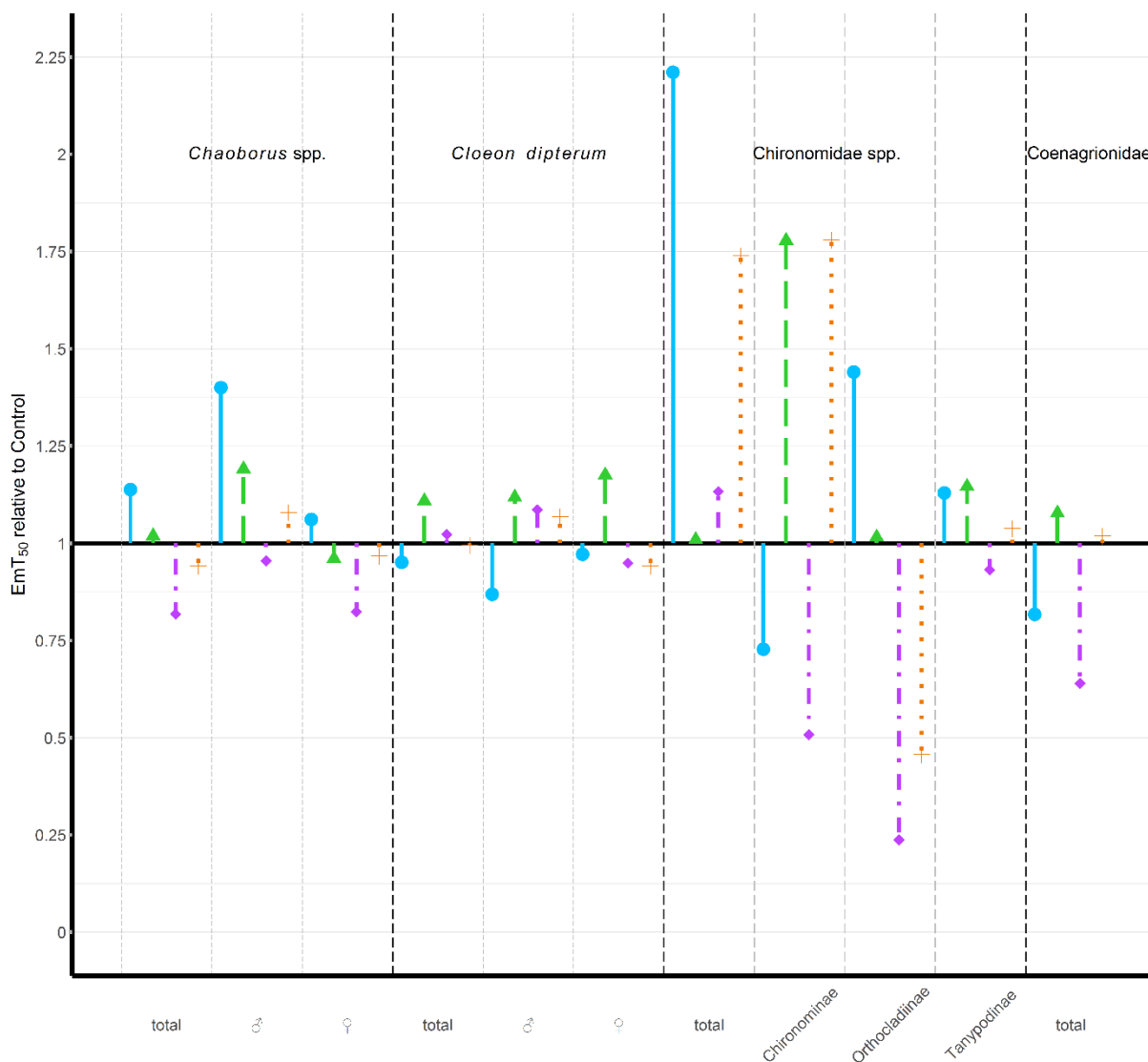


**Figure 78** Emergence mean Time for 20% of the emerging insects normalized to control

The Chironomidae and its subfamilies Tanypodinae, Chironominae and Orthoclaadiinae had the strongest changes in  $EmT_{20}$  compared to the control. Orthoclaadiinae had the lowest  $EmT_{20}$  values in all treatments. The organisms emerged four times earlier compared to the control. The difference was not significant with MDD values over 120% in all treatments. Chironominae emerged earlier in all treatments as well, with the closest  $EmT_{20}$  value to the control in the HighPA treatment followed by the Silica treatment. The organisms in the MedPA treatment emerged three times earlier than the individuals in the control. The MDD, however, was over 100% in all treatments. The Tanypodinae show no clear pattern. The individuals emerged 60% later than in the control. The organisms in The MedPA treatment had slightly delayed emergence. The individuals in the LowPA and HighPA treatments emerged early compared to the control. The Tanypodinae met the MDD criteria, with MDD values of 100% in the Silica treatment and 98% in the polyamide treatments however. The total sum of Chironomidae, which also includes organisms of other subfamilies or not distinguishable organisms showed delayed emergence compared to the control in all treatments except the MedPA treatment. The organisms emerged nearly 90% later in the HighPA treatment followed

by the Silica treatment and the LowPA treatment. The Coenagrionidae emerged later compared to the control in all treatments. The organisms in the HighPA treatment had the most delayed emergence followed by the MedPA, LowPA and Silica treatments. There were no significant differences in the 20% Emergence mean Time compared to the control.

The EmT<sub>50</sub> (Figure 79) shows a different pattern than the EmT<sub>20</sub>. *Chaoborus* sp. total, males and females emerged later in the Silica treatment. Males also emerged earlier in the LowPA and HighPA treatments. The females emerged slightly earlier in all polyamide treatments. The EmT<sub>50</sub> of the total sum of *Cloeon dipterum* and both sexes ranged around control value of 1.

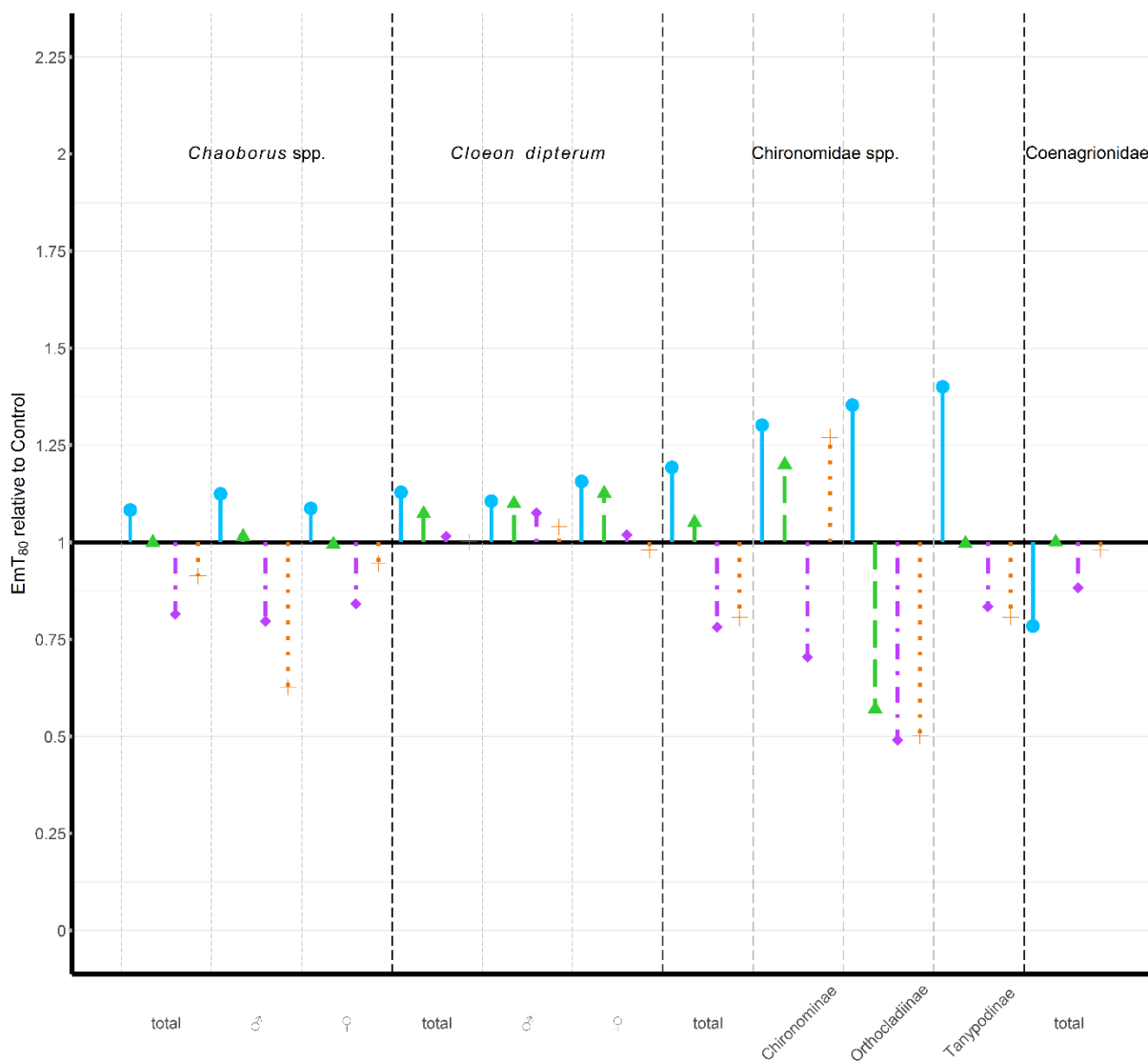


**Figure 79** Emergence mean Time for 50% of the emerging insects normalized to control

The total number of Chironomidae and the Orthoclaadiinae in the Silica treatment emerged later than in the control. The total sum emerged nearly 125% later in the Silica treatment than in the control. Chironominae in the LowPA and HighPA treatments and the total sum in the HighPA treatment emerged around 75% later than in the control.

Chironominae in the MedPA treatment and Orthoclaadiinae in the HighPA treatment emerged around 100% earlier than the individuals in the controls.  $EmT_{50}$  of Tanypodinae ranged around the control value. The  $EmT_{50}$  of Coenagrionidae was lower in the Silica and MedPA treatments. The LowPA and HighPA values ranged around the control. There were no significant differences between any treatment and the control.

The Emergence mean Time of 80% of total emergence (Figure 80) normalized to control was delayed in the Silica treatment for all taxa, except Coenagrionidae. *Chaoborus* had an early emergence in both sexes and the total sum in the MedPA and HighPA treatments. While the males and females had a similar  $EmT_{80}$  relative to the control in the MedPA treatment, the  $EmT_{80}$  relative to the control of the males was much lower than of the females.



**Figure 80** Emergence mean Time for 80% of the emerging insects normalized to control

The EmT<sub>80</sub> relative to the control of *Cloeon dipterum* was delayed or on control level in total sum, males and females in all treatments but less than 20%. The changes in EmT<sub>80</sub> relative to the control in the Silica treatment were the strongest in the subfamily Tanypodinae, followed by Orthocladiinae and Chironominae. The MedPA treatment caused an early EmT<sub>80</sub> relative to the control in all taxa belonging to the Chironomidae. The strongest effect can be found in the subfamily Orthocladiinae in the MedPA and HighPA treatments. These effects are significant compared to the control. The early emergence of Orthocladiinae in the LowPA treatment is not significant compared to the control. The changes in EmT<sub>80</sub> relative to the control for Coenagrionidae were the highest in the Silica treatment. These changes were not significant.

#### 4.4.8. Statistical evaluation

Table 17 shows the effects on the zooplankton taxa. The effects were classified according to EFSA (see Table 4). In the Silica treatment there were some taxa that were assigned to effect class 2+. Even though the male and the sum of all *Chaoborus* showed slight indirect effects the female individuals were assigned to effect class 1 “no effects”. The opposite is true for *Cloeon dipterum*, where females and the total sum showed slight indirect effects (class 2+), but males showed no effects (class 1). Out of the subfamilies of the family of Chironomidae only the Chironominae and Orthocladiinae showed slight indirect effects (class 2+). The total sum of emerging insects was also assigned class “+ as there were slight effects on the abundance. In the LowPA treatment the sum of all Chironomidae and the total sum of *Cloeon dipterum* as well as the Orthocladiinae showed slight indirect effects (class 2+). *Chaoborus* sp. males and Thysanoptera showed slight indirect effects in the MedPA treatment. All taxa were assigned class 1 “no effects” in the HighPA treatment. Overall 13 taxa, including sums and sexes are classified as MDD Category 1 and four as MDD Category 2.

**Table 17** Classification of the effect on emerging insects over the entire duration of the study according to EFSA (2013)

MDD Category	Taxon	Silica	LowPA	MedPA	HighPA
1	<i>Chaoborus</i> sp.	2+	1	1	1
1	<i>Chaoborus</i> sp. female	1	1	1	1
1	<i>Chaoborus</i> sp. male	2+	1	2+	1
1	Chironomidae	1	2+	1	1
1	<i>Chironomus</i> sp.	1	1	1	1
1	<i>Cloeon dipterum</i>	2+	2+	1	1
1	<i>Cloeon dipterum</i> female	2+	1	1	1
1	<i>Cloeon dipterum</i> male	1	1	1	1
1	Orthoclaadiinae	2+	2+	1	1
1	Sum Chironomidae	1	1	1	1
1	Tanypodinae	1	1	1	1
1	Thysanoptera	1	1	2+	1
1	Total sum	2+	1	1	1
2	Chironominae	2+	1	1	1
2	Coenagrionidae	1	1	1	1
2	Hydrophilidae	1	1	1	1
2	Sum Odonata	1	1	1	1
3	Anisoptera	1	1	1	1
3	Ceratopogonidae	1	1	1	1
3	Chironomidae <2mm	1	1	1	1
3	Coleoptera	1	1	1	1
3	Culicidae	1	1	1	1
3	Curculionidae	1	1	1	1
3	<i>Dixidae</i> sp.	1	1	1	1
3	Dytiscidae	1	1	1	1
3	<i>Helophorus</i> sp.	1	1	1	1
3	Mymaridae	1	1	1	1
3	Nematocera	1	1	1	1
3	Podonominae	1	1	1	1
3	Psychodidae	1	1	1	1
3	Trichoptera	1	1	1	1



## 5. Discussion

The mesocosm study was conducted in a big pond which was set up 18 months before the start of the pre application phase. This allowed the formation of a stable, a near-natural bioscenoecosis. Six weeks before the first application individual mesocosms were created by introducing stainless steel enclosures. 16 of these 1000 L systems were used in a design with five controls, two particulate controls and three triplicated microplastic treatments. Polyamide and silica were applied each four times to reach nominal concentrations of 1.5 mg L<sup>-1</sup> (LowPA), 15 mg L<sup>-1</sup> (MedPA) and 150 mg L<sup>-1</sup> (HighPA and Silica) respectively. Over a span of over 100 days on 158 individual samplings and 15 endpoints a total of over 2528 samples were taken. Some of these samples were not analyzed as they were only for optional analysis only. In total 67 taxa were taxonomically differentiated. The fate of the particles was calculated mathematically and confirmed by turbidity measurements in situ. The effects on individual endpoints and taxa are discussed below and then put within a larger context at the ecosystem level.

### 5.1 General study design

This design, together with the balanced biocoenosis and extensive monitoring of various endpoints ensured a statistically sufficient power to show effects in eight potentially endangered species at least. Since the mode of action of the polyamide particles was not clear the aim was to have a sound biodiversity in the mesocosm test systems to demonstrate any impact. Minimum Detectable Difference (MDD) values over 100% signify that it is impossible to show significant toxic effects in given test design. Since a change of more than 100% in abundance is needed to show significant effects compared to the control and an abundance value is not able to fall below a limit of zero, no relative change of abundance can surpass 100%. The number of replicates for the polyamide treatments was sufficient to not be influenced by outliers in general. The triplicates ensured a statistically powerful design. One replicate of the MedPA treatment had positive and negative outlier values for some endpoints and taxa. This however had no influence on the statistical evaluation since geometric means were used and the other two replicates had stable values. The Silica treatment had two replicates. This was sufficient to show whether a particle effect occurred. There were some significant differences between the Silica treatment and the controls. These are discussed in the respective subchapters.

To provide a better understanding of the test system's sensitivity, it is helpful to consider the MDD values of the primary producers and consumers in more detail. The primary producers, especially the phytoplankton color classes had MDD values below 100% for the total chlorophyll content, the diatoms and the blue algae throughout the study. The green algae showed MDD values over 100% on day 21 and all other samplings after day 31. After the first application, the cryptomonads had MDD values below 100% starting study day 6, except the Silica treatment on day 13. In general, the MDD values of the cryptomonads were relatively high compared to the other color classes. The sum of all zooplankton organisms met the MDD criteria on all samplings in all treatments after the first application. So did the taxa *Alonella* sp., *Cephalodella* sp., *Chaoborus* sp., Chironomidae, *Chydorus* sp., Cyclopidae,

*Daphnia longispina*, *Euchlanis* sp., *Hexarthra* sp., *Lecane* sp., *Lepadella* sp., *Mytilinia* sp., Nauplia, Ostracoda, *Polyarthra* sp., *Simocephalus* sp. and all sums (Rotaria, Cladocera and Copepoda). All other taxa exceeded a MDD value of 100% on at least one sampling and in at least one treatment. Bdelloidae on day 56 and *Keratella quadrata* on day 84 only surpassed a MDD value of 100% once and in all treatments. Out of the macroinvertebrate taxa, that were sampled more often than the zooplankton organisms and counted mostly alive, less taxa fulfilled the MDD criteria throughout the whole study. These taxa were *Chaoborus* sp., *Cloeon dipterum*, Naididae, Planorbidae and the sums of all organisms and Diptera. The sum of all Chironomidae had MDD values below 100% after application in the polyamide treatments. The Chironomidae had an MDD value of 101% on days 71 and 85 in the Silica treatment. The sum of all emerged insects and the sum of all chironomids were the only two taxa of the emerged organisms that met the MDD criteria throughout the study. The sum of all *Chaoborus* sp. met the criteria until day 77, while the male organisms met the criteria until day 70 and the females until day 63. The sum of *Cloeon dipterum* met the criteria until day 56 in the polyamide treatments and until day 49 in the Silica treatment. The female organisms had a MDD value below 100% until day 56 and the males until day 49 in all treatments. Tanypodinae met the MDD criteria until day 63 and on day 84 in all treatments and additionally on day 77 in the polyamide treatments. Over all endpoints 49 taxa, life stages and sexes and 10 sums met the respective criteria and were classified in MDD class 1, 8 taxa and life stages were MDD class 2 and the remaining 32 taxa and life stages were categorized as MDD class 3. This meets the requirements of the EFSA, which requires 8 potentially sensitive taxa in MDD class 1 for aquatic mesocosms (EFSA, 2013).

Beyond biological sensitivity, the spatial allocation of the mesocosms also played a crucial role in ensuring experimental reliability. The position of the mesocosms were assigned to the respective treatments based on the data of macroinvertebrates and physicochemical parameters prior to the first application. The assignment was done to prevent false effects due to varied solar radiation at different spots of the big outer pond. Additionally, organism blooms at certain spots before placement of the enclosures could not be excluded. By assigning the mesocosms with educated randomness with reference to pre-sampling results, false effects before application could be prevented with a few exceptions of non-relevant taxa. Before first application there were significant differences to the control for *Daphnia pulex*, Coleoptera, Acari and the emerged Chironomidae in the LowPA treatment, and for female emerged *Chaoborus* sp. in the Silica treatment. Neither difference continued to the next sampling and thus had no influence in the assessment of the direct and indirect effects of the silica and polyamide microparticles. In addition to biological factors, physical and chemical water parameters provide further insights into the system's comparability between treatments. Temperature, oxygen content and conductivity, with one exception on day 86 in the MedPA treatment, showed no significant differences compared to the control. This again is an indicator, that the allocation of the mesocosm was done evenly. The pH in the Silica treatment was significantly lower on days 57 to 86, except on day 78, with a p-value of 0.067. This may be explained by the different algal compositions in the mesocosms. There were more

diatoms and less blue algae in the Silica treatment. Zepernick et al. (2021) stated that in waters with higher pH, due to cyanobacterial blooms, diatoms have a competitive disadvantage, because of the pH-dependency of bio silicification. The total chlorophyll content was not different between controls and Silica treatment.

While physicochemical differences were minimal, sedimentation dynamics and turbidity patterns offer additional relevant context for the exposure scenario. Since there were no reliable analytics of water and sediment samples regarding microplastic concentration in the respective environmental medium, the turbidity measurements give an important insight into the distribution over time. Additionally, the turbidity can also describe the sinking dynamics for the silica particles, which would not have been possible with analytical methods, since silica itself is a component of the natural sediment. Due to the higher density of the silica particles, they sediment faster than the polyamide particles (Figure 10). The silica particles, however, were slightly smaller on average, which leads to the assumption that the mixture of different particle sizes sedimented relatively even. The calculated sedimentation time of the lower limit of the mean particle size of the polyamide particle and the upper limit of the mean particle size of the silica particle differed by only 0.1 hours. The particles were stirred up again, especially during the application phase, as the system was affected by wind and sampling.

It has been shown that hydrodynamic processes and climatological conditions affect microplastic sinking and resuspension into and from sediment (Castro-Castellon et al., 2022; Hurley et al., 2017; Rodrigues et al., 2018; Dahms et al., 2020; Zhang and Chen, 2020). For this reason, macroinvertebrates were not sampled while the applications were ongoing, since this sampling disturbs the mesocosms strongly. By splitting the total quantity to be applied at four points in time, particles were constantly present in the water phase over a period of around 14 days. This made it possible to simulate the continuous input that also occurs in nature more precisely.

The calculated sinking velocity of the particles and the resulting sedimentation time for 100 cm (the initial water level minus the depth of application) and the measured turbidity match. Both methods suggest that most of the particles arrived in the lower water layers or on the sediment after about 24 hours. Only the highest polyamide treatment was selected for the graph, as measurement with a higher particle count is more reliable. With the mean particle size, given by the distributor (see Appendix A),  $5 \cdot 10^{10}$  particles were applied in the HighPA treatment. A tenth of that in the MedPA and a hundredth in the LowPA treatment respectively. With a diameter of the mesocosms of 1.10 m and an area of 9500 cm<sup>2</sup> there were around one million particles per cm<sup>3</sup> sediment, respecting the bioactive and bioturbated upper 5 cm horizon. That is approximately 2.4 mg PA g<sup>-1</sup> wet sediment in the HighPA treatment. Arshad et al. (2023) found 0.67 mg g<sup>-1</sup> wet sediment in samples from the present study. They analysed sediment samples with fluorescence microscopy and discussed in the master thesis that their analyses might certainly under represent the microplastic particles. This corresponds well with the values of a maximum of 4000 items kg<sup>-1</sup> (or 1 g kg<sup>-1</sup>) sediment

found in beach sediment of the Rhine-Main area in Germany (Klein et al., 2015). Assuming that no particles above 50  $\mu\text{m}$  were used in this study and that the LowPA and MedPA treatment had lower amounts of plastic, one can assume environmentally relevant concentrations. Stankovic et al. (2020) calculated  $8 \text{ g cm}^{-2}$  as environmentally relevant. With a surface of approximately  $1 \text{ m}^2$  and a Volume of around  $1 \text{ m}^3$  the concentrations used in this study were  $1.5 \text{ g m}^{-2}$  in the LowPA treatment,  $15 \text{ g m}^{-2}$  in the MedPA treatment and  $150 \text{ g m}^{-2}$  in the HighPA and Silica treatment respectively. The HighPA concentration however exceeds the “worst-case” stated by Stankovic et al. (2020) of  $80 \text{ g m}^{-2}$ . Furthermore, it can be assumed that in the environment the reservoirs, which are supposed to be represented by the mesocosms, are more likely to be regarded as sinks for the particles than the Rhine or the Main. In the surface water of the Danube River, a maximum of only  $150 \text{ items m}^{-3}$  were found (Lechner et al., 2014). This clearly shows that microplastic particles, depending on their density and biochemical processes sediment over time and are washed away in lotic waters. In the project “MikroPlaTaS” to which this dissertation belongs, the highest polyamide concentrations were found at the Lippe (site Lippe 1 before the weir) with  $16.5 \mu\text{g m}^{-3}$  and in Quitzdorf with  $36.1 \mu\text{g m}^{-3}$ . The most frequently found polymer was PS. PE was found in the highest concentrations of up to  $928.9 \mu\text{g m}^{-3}$ . However, these data refer to fractions of 100-500  $\mu\text{m}$  (Wendt-Potthoff et al., 2022).

In this study spherical virgin primary polyamide microplastic particles were used. In other studies, weathered particles, other shapes and sizes and polymers are used, which can complicate comparability of the results from different studies. Various approaches to improving comparability are conceivable. In the future, a standard mixture of different particles could be used (de Ruijter et al., 2023). The analysis of environmental samples can be standardized in order to achieve a better comparison of the different loads of the habitats. Otherwise, consideration must be given to comparing results of similar particle sizes and the same polymers separately, as both represent different potential mechanisms of action.

In the present mesocosm study, little significant effects of the applied microplastic particles on the investigated components of the aquatic biocenosis compared to the control systems could be detected. This outcome is particularly noteworthy in light of ongoing debates regarding the potential ecological risks of microplastics, as it suggests a low or even negligible ecological relevance of the specific particles used under near-natural conditions. A key element in interpreting these findings lies in the use of environmentally relevant concentrations and particle sizes, as typically detected in freshwater habitats. Unlike many laboratory studies that rely on artificially high concentrations, the experimental design in this study aimed to simulate microplastic exposure under realistic environmental conditions. The absence of significant effects under these circumstances thus supports the conclusion that the specific microplastic particles used at the tested concentration do not pose an acute threat to the investigated aquatic community. It is also important to highlight that mesocosm approaches bridge the gap between laboratory and field studies by combining controlled conditions with ecological complexity. In this study, a multi-trophic community structure was considered, allowing for a realistic assessment of ecosystem responses. Especially in this

context, the lack of observable changes is highly meaningful, as it points toward a potential resilience of the studied communities to the specific microplastic stressors introduced.

Nevertheless, these findings should not be interpreted as a general dismissal of potential risks associated with microplastics in aquatic environments. Rather, they indicate that not all types of microplastic particles and concentrations necessarily induce harmful effects. The observed ecological robustness may be attributed to the physicochemical properties of the particles used (e.g., polymer type, size, surface characteristics). This highlights the importance of a differentiated assessment of microplastic impacts, which must consider both the material characteristics and the environmental context, including concentration and exposure duration.

## 5.2 Primary producers

The temporal course of abundance, expressed by the chlorophyll a content, is influenced by sunlight, available nutrients and the presence of primary consumers. The chlorophyll a content per liter that can be found in nature varies strongly between different water bodies and also vary seasonally. Pre-alpine lakes are mostly oligotrophic waters with  $> 5 \mu\text{g L}^{-1}$ . Mesotrophic waters, often found in the low mountain regions, have a chlorophyll a content of  $5 - 20 \mu\text{g L}^{-1}$ . Eutrophic waters, such as the Elbe, but also the Bautzen dam, are characterized by a content of  $20 - 75 \mu\text{g chlorophyll L}^{-1}$  (Caspers 1984, LAWA 1998). The Bautzen dam has frequently exhibited blue algae blooms for several years. Levels above  $75 \mu\text{g chlorophyll a L}^{-1}$  indicate a hypertrophic water body (Öffentlichkeitsarbeit RK 2018). These are often found in heavily agricultural used regions. However, it is important to note that the standard method for wet chemical chlorophyll measurement according to DIN 38409-60 is not directly comparable with this measurement method. In the delayed fluorescence measurement, only alive, photosynthetically active cells are measured, which promises better informative value, especially in ecotoxicological studies. The chlorophyll a content in the main growth period, in which the study was performed, suggest that the mesocosms were mesotrophic to moderately eutrophic, with one mesocosm in the MedPA treatment being hypertrophic on occasional samplings, due to visually determinable *Volvox* occurrence.

In this study, there were some effects on the phytoplankton. Most striking was the rapid and significant increase in chlorophyll content in diatoms compared to the control after the first application and up to three days after the last application in the Silica treatment. This can be explained by the fact that the limiting factor for the growth of diatoms in the mesocosms was silica, which they build their scaffold with (Gilpin et al., 2004, McNair et al., 2018, Gibson et al., 2000). This effected the total chlorophyll a content as well for the same period of time. After this period, there were no significant effects anymore, indicating that the excess silica has been metabolized or the particles have at least left the water phase, and were not bioavailable any more for the plankton, which is consistent with the calculated sinking velocity. There were no significant effects compared to the control in the LowPA treatment over the whole study, while the effects in the MedPA treatment can be

assigned to the one mesocosm with the spherical algae bloom. All significant effects in this treatment were in the green algae and thus affected the total chlorophyll a content, except one. The pattern is a different one in the HighPA treatment, where the only consecutive significant changes can be found in the blue algae. There the chlorophyll content increased compared to the control during the four-application process. During this time, all treated mesocosms, except the Silica treatment with strong competition by diatoms, were slightly higher in chlorophyll-a content than the control. The community response to the particulate stressors expressed by the PRC was significant compared to the control. RDA was performed to determine where the significance occurred. Significant differences compared to the control were observed on days 6, 9, and 31. The treatment explained 20%, 13%, and 28% of the changes on these days, respectively. It is noticeable that two of these days are during the application process. It cannot be ruled out that these changes were caused by the turbidity caused by the particles.

In addition to overall community responses, size-specific fractions provide further insights into potential mechanisms of action. The 30  $\mu\text{m}$  fraction that was measured occasionally as well showed less significant differences between the control and the treatments. This can be partly explained by the smaller number of samplings. The total chlorophyll a content of the 30  $\mu\text{m}$  fraction was between 65% to over 100% of the whole sample. The values above 100% can be explained by the fact that the subsamples are slightly different in the composite sample. As the algae are sometimes severely damaged after the measurement, a repeated measurement is difficult to carry out. The 30  $\mu\text{m}$  fraction is exactly in the size range of the silica and polyamide microplastic and an important food source for filter feeders such as *Daphnia*. An experiment in the same mesocosms as this main study, showed that the position of the mesocosm in the big outer pond was more relevant for biofilm structure than the polymer type it grew on (unpublished data; personal communication Prof. Dr. Bodo Philipp). Since there were limited data in literature that the chosen particle size and polymer type has direct toxic effects on phytoplankton and primary producers in general, a more precise taxonomic evaluation was not performed. Likewise, an analysis of periphyton was not carried out, since many of the species overlap. The macrophyte mapping showed no significant differences to the control. The macrophytes grew evenly in all mesocosms and have steadily increased their coverage and thus their biomass. Single ground coverage values exceeding 100% are normal and possible due to the different growth heights of the species and the overlapping coverages in different depths.

Beyond the present study, existing literature offers additional evidence and contrasting findings on the interaction between microplastic and primary producers. A marine study (Kvale et al 2021) has shown that the existence of microplastic in an ecosystem can positively influence the growth of algae. This is caused by the reduced feeding pressure by zooplankton organisms. Microplastic may also act as substrate for biofilms. Since in this study the particles were very small and were spherical even particles, this reason can be denied for this study. Abinandan et al 2023 found that the photosynthetic activity of *Raphidocelis subcapitata* was negatively influenced by PET, PVC and PS microplastic.

Rani-Borges et al. (2020) reviewed studies on the toxicity of microplastic particles on microalgae. They found that the least research was done with PA particles, on particles in a size range of 20-50  $\mu\text{m}$  and on exposure times of over 30 days. All these criteria were met in this study which gives an impression of the importance of this research. Most studies used similar concentration levels than the ones in this study (Rani-Borges et al., 2020). Lagarde et al. (2016) found that *Chlamydomas reinhardtii* overexpressed genes in the biosynthesis pathway after an exposure of over 70 days. Since the generation time of algae is very short, this is an indication that the effects of the microplastic continuum are constantly changing and that long-term experiments also offer important added value. Wu et al. (2019) were able to determine an inhibitory effect on photosynthetic activity for PVC and PP particles. This effect was up to over 50% at concentrations of 250  $\text{mg L}^{-1}$ . Cao et al. (2022) found similar effects, but in their experiment smaller particles (1  $\mu\text{m}$ ) were more toxic than bigger particles (5  $\mu\text{m}$ ). Since particles of 5  $\mu\text{m}$  were the smallest used in this study, this could explain the lack of direct toxic effects. Like Cao et al. (2022) PS microplastic were also used by Natarajan et al. (2022). They also found a higher increase of oxidative stress in smaller (1  $\mu\text{m}$ ) than bigger (12.5  $\mu\text{m}$ ) particles.

The results indicate that the impact of microplastic particles on phytoplankton varies depending on particle type, size, and concentration, with silica availability notably enhancing diatom growth. These findings underscore the necessity for long-term studies to fully elucidate the dynamic and complex effects of microplastics on primary producers.

### 5.3 Zooplankton

The zooplankton organisms are an important link in the food chain and are involved both as primary and secondary consumers, transferring carbon compounds produced by the photoautotrophic organisms to higher trophic levels. Since the particles used in the presented study fall within the size spectrum of food that is ingested by many of the animal planktonic organisms (Scherer et al., 2018), it can be assumed that the microplastic particles are also ingested. In the laboratory, with a mixture of feed algae and dyed particles of the same batch as in the mesocosm experiment, it was observed that *Daphnia magna* ingested the particles as well as the algae (Appendix F). No significant effects were detected at the population level using the PRC. Nevertheless, several effects were detected on different taxa in the mesocosm experiment. The most sensitive taxon was the class Ostracoda. The seed shrimps showed significant differences compared to the control in all polyamide treatments on day 70. No significant differences were observed in the previous weeks, but p-values below 0.2 from day 41 in the MedPA and HighPA treatments and from day 56 in the LowPA treatment. On day 84, the differences compared to the control were significant in the HighPA treatment and showed a clear trend in the MedPA treatment with a p-value of 0.0502. Professor Dr. Burkhard Scharf (personal communication) has identified the ostracods present in the samples as *Cyprina ophthalmica*, *Cypridopsis vidua* und *Notodromas monacha*. All three species belong to the order Podocopida. Ostracoda begin their development as nauplii larvae. Only at a later stage could they be clearly identified as ostracods. Due to the partly grazing, partly predatory food intake, both silica and polyamide particles can be ingested and have a negative influence on

development (Scherer et al 2018). Particle ingestion may also have led to death due to subsequent adhesion in the intestine (Cole et al., 2013). Furthermore, it is striking that the Ostracoda reacted to the Silica treatment with a decrease, which was not significant. The species all live at least temporarily on the sediment surface and are therefore zooplankton representatives that are in direct contact with the sedimented plastic particles. The oral cavities and chewing organs of the Ostracoda are located on the underside of their bodies. Intestinal analysis confirmed that they had induced ingestion. Various quantities and particle sizes from 5  $\mu\text{m}$  to 25  $\mu\text{m}$  were regularly found. The particles appeared to be ingested more or less non-selectively. It is possible that the particles were masked or enveloped by bio fueling of phytoplankton or other organics prior to ingestion (Long et al., 2017). The particles may also have become entangled in the leg hairs of the organisms and also been sucked in. It may also have clung to the outside of the food and also been ingested. Otherwise, it could be proven that the Ostracoda had a lot of pollen in them and few microplastic particles in their gastrointestinal tract. It is assumed that it was the pollen of apple tree blossoms from the surrounding area. As the pollen is comparable in size to the particles, the particles may also - were picked up non-selectively by the ostracods (Appendix F), which was already shown some taxa of the class in other studies (Scherer et al. 2017). To quantify this more precisely, a feeding experiment in the laboratory with pollen and polyamide particles could provide clarity.

In addition to Ostracoda, the Rotifera exhibited abundance patterns that may also reflect interactions with the microplastic particles used. On day 56 of the study in the two highest microplastic treatments (MedPA and HighPA) had significantly lower abundances compared to the control, while there were no effects, but tendencies, in the LowPA treatment. The phylum Rotifera, which includes the genera *Keratella*, *Synchaeta* and *Hexarthra*, was very dominant in terms of abundance throughout the course of the study. The abundance development of Rotifera in the control can be attributed to the natural influences of the physical parameters. According to Voigt and Koste (1978) and Donner (1973), Rotifera can occur in water bodies under a wide range of different conditions. The decrease in the first half of the study may be due to rising temperatures and the increasing abundance of secondary consumers. In the alkaline range, it can be assumed that abundances are steadily increasing (Donner 1973). Although the pH value did not fall below 7 in this study, this could not be observed in this experiment. This can also be attributed to the increasing numbers of predators. The courses of the treatments are similar to those of the control, but with a somewhat greater decline. The drop in abundance in the MedPA treatment may be due to the high concentrations of spherical algae, which, unlike cryptomonads, are not a food source for Rotifera and compete with the other algae (Voigt and Koste 1978). This decline in abundance may also be linked to the ingestion of microplastic particles, as demonstrated in previous studies.

Since Rotifera can ingest microplastic particles (Setälä et al 2014), these effects may also be due to the PA particles. At 5 to 40  $\mu\text{m}$ , the algae that are ingested by Rotifera by swirling correspond exactly to the plastic particles used (Campbell et al 2009). As these were pure particles, it is likely that the animals did recognize them as dangerous via their chemoreceptors. If the particles are ingested in the gastrointestinal tract, it cannot be ruled out that the particles may hinder the digestive process and cause death. Cole et al. (2013) found that the plastic can stick together in the intestine. Although Voigt and Koste (1978) state that Rotifera can excrete undigested food via the mouth or intestine. In the class of Bdelloidea, for example, the food is first made slimy in the stomach and formed into pills. It is unknown what effect this process can have on plastic. However, it should also be noted that most of the particles sank after day 14 and were only partially resuspended by sampling. Thus, after about two weeks following the first application, the particles were less bioavailable to the Rotifera. The silica used in Silica treatment promoted the growth of diatoms in these mesocosms. Various Rotifera species feed on these diatoms (Voigt and Koste, 1978). This is also accompanied by the development of the genera *Synchaeta* and *Hexarthra*. With regard to the abundance trends of Rotifera, it can be stated that minor effects of PA on the genus of *Rotaria* cannot be ruled out, as the stress levels occasionally show lower numbers of individuals than the average value of the control. However, for the total abundance of Rotifera, only isolated significant differences to the control can be identified, so that it can be assumed that the total population is not endangered by the polyamide. In addition, the abundance recovered from the minima on day 56 within four weeks. The significance of the individual taxa showed no clear correlation.

Unlike Rotifera, Cladocera and Copepoda showed more resilience, though indirect effects cannot be excluded. Cladocera, to which the genera *Daphnia* and *Simocephalus* belong, show natural fluctuations due to temperature, generational changes and changes in the biocenosis over time. According to research by Jaikumar et al. (2018), *Daphnia* were able to ingest particles with a size of 1-5  $\mu\text{m}$ , with temperature-dependent toxicities of  $10^5$  particles  $\text{ml}^{-1}$  at 18° C. Khosrovyan and Kahru (2022) found no effects of neither virgin nor weathered microplastic on *Daphnia magna* reproduction. No significant differences between the treatments and the control were observed for the total Cladocera. Especially because the abundances in the treatments increase again after day 41 or 56, the theory suggests that the cladocerans are able to avoid the polyamide particles or that even if they are ingested, they can be released harmlessly. The taxa, which belong to the Cladocera, also showed no effects.

A closer look at copepod abundance and developmental patterns further illustrates the complex interplay between biological and environmental variables. Developmental processes of nauplii and copepodite stages are temperature-dependent; 2 °C less can slow down development by two days (Einsle, 1993). Generation changes in the sense of newly hatched nauplii and naturally dead adult copepods that have already reached the end of their lifespan in spring can also have an influence on the fluctuations. However, these changes cannot be discussed further, as these cycles and the lifespan depends on the species. The taxonomic determination was only carried out up to the family level. It can be assumed that

different species with specific generation changes occur in the enclosures. Likewise, no copepodite or nauplius stages could be subdivided, so that no further assumptions can be made. The most dominant taxon of the Copepoda were the nauplii larvae, which are the first larval stage of many crustaceans, especially barnacles, ostracods, copepods and some higher crustaceans. The nauplii larvae were assigned to copepods for the summation, as the ostracods showed only low abundances. These feed filter-feeding on algae colonies. At certain stages the base food source of Cyclopidae switches to animal food. For example, the ingestion of microplastic pellets with diameters of 7.3  $\mu\text{m}$  into the stomach was observed in adult filter-feeding calanoids in a laboratory experiment (Cole et al., 2013). Rodríguez-Torres et al. (2020) also showed comparable results but found that the predominant phytoplankton content determines ingestion. Studies by Cole et al. (2013) have shown that plastic particles can stick together in the intestine after ingestion. Fagiano et al. (2022) also demonstrated a negative correlation between microplastic in the sea and copepod abundance. Thus, the lack of abundance peaks of copepods in the treatments can be seen as a decline in reproductive performance due to the altered nutritional basis caused by the polyamide. The ingestion of at least 5  $\mu\text{m}$  small particles by the nauplii is possible from the first stage onwards (Henriksen et al., 2007). The ingestion of carnivorous Cyclopidae may have been secondary to the consumption of filter-feeding Rotifera, calanoids or cannibalism. The decreases in the MedPA treatment can be assumed to be outliers caused by *Volvox* mass occurrence. This form of green algae is not a food source for Copepoda or the prey of Cyclopidae but instead competes with their food algae for nutrients. Furthermore, Cyclopidae and their associated nauplii follow specific sinking and movement behaviors to capture their prey. This process may have been further disadvantaged by the spherical algae abundance (Henriksen et al., 2007). The earlier recovery of the population at the LowPA treatment is due to the lower concentration. The general declines after day 70 can be superimposed on the onset of the dormancy phase or diapause, so that egg laying or further development ceases at the transition to fall/winter or due to the declining food supply (Einsle, 1993). In summary, the copepod population is not affected over the entire experimental period.

The study demonstrates that microplastic particles within the size range of natural food sources are ingested by various zooplankton taxa, with Ostracoda showing the highest sensitivity to polyamide exposure. While some taxa, such as the rotifers, exhibited minor fluctuations possibly linked to microplastic presence, overall zooplankton populations including Cladocera and Copepoda showed resilience over the experimental period. In order to assess the impact on the very small rotifers more precisely, a denser sampling plan would be necessary. These findings highlight the complex interactions between microplastics and zooplankton communities, emphasizing the need for further research to clarify long-term ecological consequences and species-specific responses.

#### 5.4 Macroinvertebrates

With the three macroinvertebrate sampling techniques 37 distinct taxa were detected on twelve sampling occasions. Nine of those samplings took place after the application phase and three before the first application. The last sampling of the macroinvertebrates was on day 114 after the first application. This biological endpoint was the least sensitive in the study. Here, only three taxa effects were assigned according to EFSA in the MedPA treatment. Two of these taxa are sums that depend on the one taxon with an effect. The glass midges *Chaoborus* showed a strong effect in the MedPA treatment. However, this was mainly due to a replicate that already had the lowest abundance of this genus before application (Figure 39). Two species were identified, *Chaoborus crystallinus* and *Chaoborus obscuripes*, which according to Cockroft et al. (2022) are frequently found in small, fishless and man-made ponds in Europe. The distribution of biovolume of these larvae shows that there is a shift to higher (L3/L4) larval stages over the course of the year, particularly on study day 114 (mid-September). This is related to the generally univoltine life cycle of the two *Chaoborus* species, in which only one generation change occurs per year and the larvae of the second generation overwinter in the fourth larval stage (L4). Like the phantom midges, Chironomidae have a total of four larval stages (Vallenduuk and Moller 2007). The number of generations per year is not uniform due to the high diversity of the family. Many species have an univoltine, others a bivoltine or trivoltine life cycle, always depending on the living conditions in relation to physical and chemical parameters in the water (Vallenduuk and Moller 2007).

Since *Cloeon dipterum* usually has a bivoltine generation cycle, with a generation change from the first to the second generation from May to June (Lichtenberg, 1973), the individuals found after the application phase can be assigned to the rapidly developing second generation. This is also confirmed by the emergence data for *Cloeon dipterum* in this study, which showed the highest emergence in mid-June. The increase in the availability of diatoms for the second generation of the year in the Silica treatment could have led to an increase in the abundance of the slowly developing and later overwintering subsequent generation, which was still in a younger, smaller larval stage on day 85 (mid-August) and still not as big on day 114 as on day 36.

Aside from temporal dynamics, the potential for recolonization is a key factor when assessing the recovery potential of macroinvertebrate populations. The macroinvertebrates are the only group assessed in this experiment, where extrinsic recovery can be an option. As many of the taxa have an emergent adult phase the organisms from the control and treatments, other outer pond on the test facility or nearby ponds and streams can recolonize potentially harmed mesocosms. Some other taxa like *Asellus aquaticus* or *Lymnaea stagnalis* are not able to recover extrinsic. A potential effect followed by recovery has to be intrinsic. That means that surviving individuals reach the age of reproduction and recolonize the system from within the system. An example of extrinsic recovery in this study are the *Chaoborus* larvae, where significant reductions in the MedPA treatment compared to the control can be seen in the first third to half of the experiment. As the study progresses, extrinsic recolonization takes place. For ecotoxicological recovery and effect classification it is

important to ensure that the stressed systems abundance approaches the control values and not the other way around. As there were no other pronounced direct effects followed by recovery, no example of intrinsic recovery within this group.

Despite the potential for recovery, methodological limitations must also be considered when interpreting low abundances and high variability in the macroinvertebrate data. High MDD values of many individual taxa may be a reason for the absence of significant direct differences compared to the control. These high MDD values are partly due to high variances and partly due to low abundances of individual taxa. As no distinction was made between the three sampling techniques, it can only be assumed that, due to the relatively small sized mesocosms, the netting sampled too small of a water column. The other two methods should not suggest any difference to the larger mesocosms, while each of the three netting hauls was about 30 cm shorter than in the systems that are normally used. It is therefore possible that the relatively low abundances of individual taxa can be attributed to this methodological adjustment and that in future at least four or five net pulls should be carried out with these systems. The reason for the smaller systems is related to the availability of the outer pond and the number of replicates, that could no longer have been selected with the larger mesocosm systems in the available outer pond (see Figure 5, A).

To further strengthen the validity of the findings, a complementary bio-assay was conducted using *Potamopyrgus antipodarum* as a model organism. Two different statistical methods were used in the bio-assays with *Potamopyrgus antipodarum*. The method specified in the guideline (OECD 242), which suggests an ANOVA and a suitable pos-hoc test (e.g., according to Dunnett) and the method according to Lehmann et al. (2018), which works with a closure principle computational approach test (CPCAT). Both methods were used to handle the analysis as protectively as possible. With CPCAT, no significant differences were found between the treatments and the control. An ANOVA of the data showed that both treatments with the highest particle load differed significantly from the control in embryo number on day 28. That contrasts with the findings of Lehmann et al. (2018) which found no real differences in the capability of detecting significant differences between multiple t-test, such as Dunnett's and CPCAT. The mudsnails are a proxy organism for endocrine effects (Duft et al., 2003, Duft et al., 2007, Schmitt et al., 2010) and have been invasive in Europe for decades (Ponder 1988, Kinzelbach 1995, Alonso and Castro-Diez 2008). It has already been shown that *P. antipodarum* ingests microplastic (Imhof et al., 2013). Imhof and Laforsch (2016) found no effects of microplastic on this species. Romero-Blanco et al. (2021) also found no effects on reproduction or survival but were able to demonstrate a significant effect on the reaction time of the snails. Since the effects only occurred on the first sampling (day 28) and only in the treatments with a particle load of  $150 \text{ mg L}^{-1}$ , it is reasonable to assume that the effect is due to the greatly increased turbidity, which was present in these two treatments for about half of the time up to the first sampling. Even if there were only indirect effects due to the turbidity, a certain risk can be deduced from the significantly reduction in the number of embryos. Nevertheless, it is important to ecotoxicologically investigate the potential endocrine effects of plastic materials, as many of them release estrogenic chemicals (Yang et al., 2011).

Macroinvertebrate communities showed limited direct sensitivity to polyamide microplastics, with observed effects largely influenced by natural variability and extrinsic recolonization, while subtle reproductive impacts in *Potamopyrgus antipodarum* were likely driven by increased turbidity rather than direct toxicity.

### 5.5 Emerging insects

The analysis of emerging insects makes it possible to gain insights into both direct effects and effects that delay development. By implementing the EmT<sub>x</sub>, it is possible for the first time to establish this standard laboratory endpoint (e.g., OECD 218) for semi-outdoor studies for mesocosms where there are no precisely defined starting conditions. A more defined discussion for the non-standard analysis like sex ratio, biovolume and the EmT<sub>x</sub> can be found in the following subsection 5.7. The emerging insects are the most regularly analysed endpoint. It is noticeable that there is a negative correlation between the polyamide concentration and the number of significant effects compared to the control. The LowPA treatment shows nine significant differences compared to the control, the MedPA treatment a third of them and the HighPA treatment again a third of them. The Silica treatment had the most significant effects with a total of 16 significant deviations from the control. All of these significant differences, except for one, indicated indirect effects, meaning higher abundances in the Silica treatment compared to the control. The only case, for which the Silica treatment had significant direct effects compared to the control was the Shannon index before application on day 0 and thus not relevant for effect analysis.

To better understand these findings, it is essential to consider the ecological niches and exposure pathways of the different insect taxa. Since the different taxa of emergent insects colonize the entire water phase and the sediment, different effects were to be expected. The Chironomidae larvae, which mainly reside in the sediment, only came into closer contact with the particles after they had sunk. For some aquatic invertebrates, it has already been shown that abiotic (e.g., sedimentation rate) and biotic (e.g., morphology, diet) factors play a major role in the uptake of microplastic (Scherer et al., 2017). No significant effects can be identified for the abundance development of Chironomidae from the subfamilies Orthoclaadiinae, Podonominae, Chironominae and Tanypodinae, which spend their different larval stages benthically. For the chironomid *Chironomus riparius*, it has already been shown that soft PE microplastic cause negative effects at the metabolic and cellular level (Muñiz-González et al., 2021). In addition, for *C. riparius* in connection with PVC microplastic a negative effect on emergence, development and weight in relation to the concentration of microplastic concentration has been detected (Scherer et al., 2020).

While Chironomidae showed limited effects in this study, other insect taxa revealed clearer patterns of sensitivity to the treatments applied. The most sensitive taxon was *Cloeon dipterum* with a total of three significant differences after application compared to the control. Mayflies are considered important bioindicators for the pollution of ecosystems (Carde and Resh 2009). Filter feeders and detritus eaters are more likely to ingest microplastic due to their low selectivity in food choice (Scherer et al., 2018). The larvae of *Cloeon dipterum*

feed according to this pattern. In addition, the larvae are organisms that live in the pelagic zone of stagnant water (Eastham 1958). It is therefore possible that the larvae ingested microplastic during the application period, but that this had no direct effect on the abundance of adult individuals. The limited time that the microplastic is in the water column could be a limiting factor here. The second generation of *Cloeon dipterum*, which probably emerges from study day 21 onwards, may not have come into sufficient contact with the microplastic, as most of the particles should already be near the sediment at this time. Whether microplastic are ingested by the larvae and subsequently spread by the adult individuals out of the test system further into the terrestrial environment is the subject of further studies. However, it has already been shown that aquatic caddisfly larvae can take up nanoparticles and spread them terrestrially as adults (Bundschuh et al., 2019). The same has been shown in this study for chaoborids (Michler-Kozma et al., 2022). Although the number of effects on individual taxa was limited, total community metrics still offer important complementary insights. Only the total sum had two consecutive significant differences compared to the control (in the Silica treatment). Both were indirect effects with increasing abundance. The standardized abundance-based evaluation suggests that there was no influence of the polyamide or silica particles on the emerging insects. Only a few individual taxa met the MDD criteria throughout the study. It was therefore important to also consider the totals of the order Odonata, the family Diptera and the total sum of all organisms, even if this makes it difficult to assign effects to individual species or genera. But even the sums have shown little to no effect.

Beyond abundance, sublethal parameters such as biovolume and emergence time may reveal delayed or masked responses in the insect community. The biovolume of male and female *Chaoborus* sp. and *Cloeon dipterum* was analyzed on three samplings each. The limiting factor for the selection of these days was the condition of the organisms. Since each sample was a bulk sample of the emergence per week some organisms were no longer intact and could therefore not be measured. On the respective sampling days, each one before application and two after application the most intact male and female organisms could be measured. Organisms do not have to be intact to be taxonomically interpreted, but for exact measurements. Fewer fluctuations were detected than in the larvae, as the adult insects no longer grow. However, both *Chaoborus* sp. and *Cloeon dipterum*, especially the females, are smaller later in the year. This indicates that these organisms belong to the faster growing but smaller summer generation. Emerging insect taxa exhibited limited direct adverse effects from polyamide microplastic exposure, with most significant differences observed at lower concentrations and primarily reflecting indirect or environmental influences. The silica treatment elicited more pronounced indirect effects, often increasing abundances. Sensitive taxa like *Cloeon dipterum* showed some significant responses, likely related to feeding behavior and habitat use, but overall abundance and community-level metrics remained largely unaffected by polyamide particles. Sublethal parameters such as biovolume and emergence timing revealed subtle seasonal or generational shifts rather than clear toxicological impacts, indicating that polyamide microplastics may have minimal immediate effects on emerging insect communities within the study's timeframe.

## 5.6 Trophic and ontogenetic transfer

An evaluation by project partners at the WWU Münster has shown that polyamide particles were found in larvae of the genus *Chaoborus*. The particles were found in the gastrointestinal tract of the individuals. (Michler-Kozma et al., 2022). Since the particles have a comparable size to the algae on which daphnids feed non-selectively and filter-feeding (Scherer et al., 2018), it is reasonable to assume that the predatory *Chaoborus* sp. larvae have ingested the particles via the daphnids or other prey, as described by Cuthbert et al. (2019). This would be a sign of bioaccumulation of microplastic particles. Particles were also detected in the adult, emerged individuals. However, these were individual findings. Al-Jaibachi et al. (2018) also found a similar picture in *Culex*, where the number of microplastic particles found in the organisms decreased steadily from the larvae to the pupae to the adult insects. The transition of microplastic particles from the larval stage to the adult organism has also been observed in another mosquito in laboratory tests (Simakova et al. 2022). These observations highlight the relevance of ontogenetic transfer as a mechanism for trophic and environmental redistribution of microplastic. Yan et al. (2022) provide an overview of the potential impact of microplastic on trophic chains. Some taxa, such as various Diptera and Ephemeroptera, are an important food source for organisms such as birds which influences their abundance and population size (Lewis-Phillips et al., 2020). This ontogenetic transfer opens up the possibility of aquatic microplastic accumulating in terrestrial biocenoses. Dipterans do not tend to avoid microplastic contaminated ponds when laying eggs (Cuthbert et al., 2019), which makes the cycle start all over again. In a mesocosm experiment, Yildiz et al. (2022) showed that microplastic particles were transferred trophically from daphnids to odonate larvae, and that chironomids retained these particles in their bodies during ontogenesis. Castro-Castellon et al. (2021) provide an overview that there is a transfer of microplastic in freshwater food webs. Despite uncertainties in quantification, the ecotoxicological relevance of such transfer pathways becomes evident when regional exposure levels are considered. In general, the ecotoxicological risk that microplastic has on the ecosystem in Europe and North America, should be acceptable, compared to Asia (PEC/PNEC > 1) (Adam et al., 2019). But non-direct toxic effects must also be considered when it comes to risks to ecosystems. The presence of microplastic can lead to macroinvertebrates adapting their feeding habits, as in some cases over 20% of the food they eat is composed of plastic (Martinez Rodriguez et al., 2024). Also, microplastic presence can lead to behavioral changes and influence the drift frequency of riverine invertebrates (Mora-Teddy et al., 2024).

While concentrations used in experimental studies often exceed environmental levels, the relevance of realistic dosages is increasingly emphasized. Although 100 microplastic L<sup>-1</sup> can be considered a high concentration in water (Cunningham and Sigwart 2019), they found higher impacts with lower dosages on benthic organisms. Lenz et al. (2016) propose a maximum concentration of 1 mg L<sup>-1</sup> as environmentally relevant. The concentration of 1.5 mg L<sup>-1</sup> in the LowPA treatment falls exactly within this range. Bucci et al. (2019) found that most of the particles used in laboratory studies are below the sizes found in the environment. However, it should be noted that detecting small particles analytically is still a challenge and

it is likely that the amount of plastic in the environment is underestimated (Lindeque et al., 2020). Finally, the experimental design of the present study allows for important conclusions on particulate vs. plastic-specific effects. Cunningham and Sigwart (2019) propose the idea of including a non-plastic particulate control, which was not done at the time their study was published. In this study the Silica treatment serves as such. Finding effects only in the HighPA and Silica treatment is a strong hint, that effects are more particulate-related than plastic-related. In their review Weis and Palmquist (2021) state that microplastic research lacks long-term exposure studies. Spivak et al. (2011) stated in a study with phytoplankton that the results of longer term mesocosm experiments generally give good insights on ecosystemic reactions. In a field study Stankovic et al (2021) found no microplastic related effects on macroinvertebrate community with a concentration of 80 g m<sup>-2</sup>.

Polyamide microplastic particles were detected in *Chaoborus* sp. larvae and adults, indicating trophic transfer and potential bioaccumulation within aquatic food webs. This ontogenetic transfer raises concerns about microplastic movement from aquatic to terrestrial ecosystems, with possible ecological impacts including altered feeding behavior and population dynamics. Although experimental concentrations often exceed environmental levels, the study's inclusion of a non-plastic particulate control (silica) suggests observed effects are primarily related to particle presence rather than plastic-specific toxicity. Long-term and realistic exposure studies remain crucial to fully understand the ecological risks of microplastic pollution.

### **5.7 New endpoints and ecosystemic effects**

To ensure the highest possible level of protection, it is also important to record and evaluate non-lethal endpoints, as the lowest effect level often does not come from a lethal endpoint. The emergence time is a measure of the time the organisms need to fully develop. In laboratory studies, with synchronized individuals at the start of the experiment, the exact time that the larvae need to pass through metamorphosis from the youngest larval stage to hatching can be determined. A change in this emergence time can be induced by a change in temperature, different food availability, increased predation pressure or a chemical stressor. The emergence time is constant and typical for a given species (Corbet 1964). In higher-tier studies, organisms can also be specifically added to record this endpoint. However, this is only possible to a limited extent and with only a few species at the same time.

Given the logistical limitations in semi-field experiments, a novel calculation method was applied to estimate emergence time retrospectively. In order to be able to use a defined number of age-synchronized organisms, for other species and studies in which a defined number of organisms are not used at the application point, a method was developed and tested in this work to calculate the emergence time based on the total emergence per taxon and replicate. With this method, the emergence time can be calculated for each replicate based on the available organisms represented by the total emergence. For this purpose, only data after the first application is used and to achieve good accuracy, the emergent organisms should be sampled and analyzed continuously. This is ensured by weekly sampling of a pooling emergence trap. The ecological relevance of emergence timing becomes particularly evident

when considering implications for population continuity and predator-prey interactions. A change in emergence time can have numerous negative effects on the biocoenosis. On the one hand, for many organisms it is necessary that enough organisms hatch at the same time to create a new generation. Delayed hatching of one sex can also prevent sufficient organisms capable of mating from being available at the same time. Also, insects are an important food source for higher order aquatic and terrestrial consumers and delay in emergence can potentially delay the development of these consumers.

Mayflies for example sometimes emerge highly synchronized in large masses (Bauernfeind and Soldán, 2012). Due to the short lifespan, a sex-dependent shift can therefore have far-reaching consequences for the further development and survival of a mayfly population. The percentage of males was lower on day 14 and 21, especially in the HighPA treatment. However, the shift observed here does not necessarily have to be induced by microplastic, since the differences were not significant compared to the control. Besides chemical stress, other environmental drivers such as temperature and predation must be considered as alternative or interacting factors. For other mayfly species, it has been shown that increased water temperature and predators can influence development (Peckarsky et al., 2001; Harper and Peckarsky, 2006). For *Cloeon dipterum*, the combination of food availability and predation risk appears to play an important role in relation to development (Šupina et al., 2016). It is unlikely that water temperature has an influence on hatching time in this case, as no deviations in the temperature curve can be detected. Predators and food availability could have had an influence on the emergence time, but should also not deviate between controls and treatments.

In parallel, the subfamily Chironominae showed trends in emergence dynamics, but detection of effects was limited by sampling constraints. The subfamily of Chironominae showed no direct or indirect effect by the silica nor the polyamide. Due to the low and fluctuating abundances and the MDD value below 100% only once post first application no direct effects could be observed. Khosrovyan and Kahru (2021), showed a delayed emergence of *Chironomus riparius*, which belongs to Chironominae in their study. However, no effect on the total emergence was observed in the treated mesocosms. Something comparable, with similar concentrations, was also found in the present higher tier study. The  $EmT_{50}$  and  $EmT_{80}$  of the Chironominae was clearly, although not significantly, delayed in the HighPA and LowPA treatment. This leads to the assumption that here too, with slowly ageing particles, there was a tendency towards a sublethal effect. A deeper look at sublethal parameters across treatments highlights the sensitivity of such endpoints even in the absence of significant abundance changes. The fact that this trend is also not evident in the  $EmT_{20}$  could be due to the particles. Khosrovyan and Kahru (2021) also found no effects with virgin polyamide particles and only found effects with particles that were aged by UV-weathering for 26 days. For the Chironominae in this study, the  $EmT_{20}$  in the control is 7 days, the  $EmT_{50}$  is 21 days and the  $EmT_{80}$  is 45 days. As this was an outdoor trial, it can be assumed that the ageing processes of the particles were similar.

Nevertheless, even in the  $EmT_x$  calculation, the abundance was too low to demonstrate any effects. In the MedPA treatment, there was an even lower and more fluctuating total emergence, which led to the individuals emerge earlier than in the control. Orthocladiinae, in

contrast, revealed more consistent patterns across abundance and emergence time endpoints. A different picture emerged for the Orthoclaadiinae. There, significantly more organisms were found in the LowPA treatment on day 21 and in the Silica treatment on day 63. The  $EmT_{80}$ , on the other hand, shows a significantly earlier emergence for this subfamily compared to the control in the MedPA and HighPA treatment. The LowPA treatment also shows the tendency of an effect with a p-value of 0.06. In contrast to the abundance evaluation, no significant differences were found for the Silica and LowPA treatment in the  $EmT_x$  calculation compared to the control. This shows that an evaluation of the sublethal endpoints is also important in order to obtain a general overview of the effects of a substance. To increase the robustness of future studies, methodological adaptations and improved representativeness are essential.

It has been shown that for a calculation of  $EmT_x$  per treatment it is better to calculate each replicate individually and then calculate mean values. This also takes into account the deviations that naturally occur between individual mesocosms, as the replicates are comparable but never identical. It can be assumed that small effects are masked in abundances, especially in less abundant species. These may then be detectable in the  $EmT_x$ . In addition, size-based metrics such as biovolume offer insights into population structure and timing of generation shifts. By evaluating the size distribution of the organisms, whether by assessing length, calculating length-weight regression or the biovolume, one gets an insight into the age structure of the populations. This can help to find the point in time, when certain taxa are most vulnerable. Younger, smaller organisms are often more likely to be affected by stress than older animals. By comparing the biovolume and abundances of *Cloeon dipterum* larvae and adults it is possible to determine when the generation changes from the winter to the summer generation and back to the next winter generation occurred. For future experimental designs, incorporating long-term data and timing of life stages will be crucial for ecological relevance. Accordingly, both for the planning of future semi-field studies and in the evaluation, the timing when the most vulnerable life stages dominate the population should be considered. For this it is also important, as for biological monitoring, that data has been collected over many years and reused continuously to create the closest possible approximation to reality.

This is further supported by complementary studies using other stressors, such as veterinary pharmaceuticals, which underline the relevance of  $EmT_x$ -based approaches. Another study, with the veterinary drug eprinomectin, showed a strong dose-response relationship for macroinvertebrates. Accordingly, the emerging insects also responded. The calculation of the  $EmT_x$  (Appendix G) confirmed the assumptions from this study, showing that no  $EmT_{50}$  could be calculated at the highest concentration for *Cloeon dipterum*, as no organisms emerged. At the low concentration, very early emergence was observed. Based on the knowledge of the effect, this is a sign of a toxic effect that develops in the long term. This means that the substance in the given concentration is not directly toxic for the larger organisms, but is likely toxic to smaller organisms. In the medium concentration, a delayed emergence is seen for the total amount of *Cloeon dipterum*. The substance was toxic to all life stages, but recovery was possible and organisms were able to emerge towards the end of the study. It should also be noted that in the medium concentration the females emerged early

and the males late. This is an indication of a difference in sensitivity between the two sexes. In contrast, other taxa such as *Chaoborus* sp. and Tanypodinae demonstrated clearer dose-dependent effects on emergence. The strongly differing emergence times can have serious effects on mating and reproductive success of the population. The picture is different for chaoborids. Here, all concentrations were highly toxic to the larvae. All  $EmT_{50}$  values are well below 1, which indicates that the larvae that were about to hatch at the time of application emerged at least partially at the beginning of the study and hardly any organisms emerged later. This can be clearly seen in the highest concentration, where the  $EmT_{50}$  values are almost 0, which means only single findings of individuals. Finally, the Tanypodinae, with their sediment-dwelling larvae, are discussed. A clear concentration-effect relationship can be seen here. While the  $EmT_{50}$  in the low concentration is more or less equal to the control, it decreases further with increasing concentration. This is also an indication of strong toxic effects without recovery.

The further expansion of mesocosm data, by capturing sublethal effects, biovolume and biomass, can provide a basis for ecosystem models to expand risk assessment in the future. For this it is important to find constant patterns and to understand the variability in natural occurring dynamics (Loerracher et al., 2023). Finally, statistical approaches and sampling biases should be revisited to ensure comparability across taxa. Furthermore, the existing methods could also be adapted in the future. Given the example of the PRC for macroinvertebrates, which describes the response of the community to a stressor. The current procedure for mesocosm experiments is that the PRC is calculated from the raw data of organisms that were caught. However, it is doubtful whether the sampling methods for all taxa sample a comparable proportion of organisms. For example, if 20% of all *Chaoborus* sp. larvae in the system are sampled and only 5% of all *Chironomus* sp. larvae, the *Chaoborus* sp. larvae will be overrepresented in the evaluation, as one fifth of a population is compared to one twentieth. The same applies to diversity indices such as Shannon and Evenness. In order to improve this in the future, the representativeness of the different taxa must be examined for all sampling methods.

In conclusion, the absence of significant effects on certain organism groups and endpoints in this mesocosm study should not be seen as a methodological limitation, but rather as a valuable contribution to the environmental risk assessment of microplastics. Since the communities in ecosystems are interconnected in many ways, the loss of one species can have unpredictable consequences. In order to better predict these consequences and potentially endangered groups, species, and life stages, a comprehensive prospective and retrospective risk assessment is necessary. In this experiment, there were only isolated effects on primary producers. Effects were observed on small animal planktonic organisms, whereas larvae and adult winged macroinvertebrates showed exclusively non-lethal effects. However, in order to achieve the protective goal of ecotoxicology, which is to protect populations, these sublethal effects are also relevant, as they too can cause lasting changes to the communities. The results suggest that certain types of microplastic, under environmentally realistic conditions, may pose limited ecotoxicological risk. Thus, this study helps to bring nuance to the broader discussion around microplastics in freshwater ecosystems and emphasizes the importance of controlled, ecologically relevant experiments in evaluating the environmental

safety of emerging contaminants. In this experiment, unaged pure microplastic particles were used. In the environment, polyamide has the potential to absorb other pollutants, such as PFAS or Bisphenol A. In the short term, this can even reduce the toxicity of the chemical substances to organisms, but it also carries the potential for the substances to be transported further or to remain available for longer due to continuous desorption (Mejías et. al, 2023; Rehse et. al, 2018). Other additives, phthalates, or metal pigments can also bind to the organic polymer matrix, which often has hydrophobic and hydrophilic areas. This is different from silica particles, which were used as particulate control. These have a negatively charged surface at alkaline pH and can bind cations, but also certain pharmaceuticals (Guo et. al, 2019). However, in this mesocosm experiment, there was neither a heavy metal contamination nor a measurable concentration of chemical substances (Appendix H). Therefore, no statement can be made about the vector function of the particles in this experiment.

### 5.8 Evaluation of research questions and hypotheses

All shown data and evaluations help answer the research questions and hypotheses defined at the beginning of this thesis. All of these are answered in detail below by classifying the results and highlighting potential further emerging questions.

#### *Research questions*

- A) Does the presence of the added particles have an influence on the physicochemical composition of the model ecosystems and thus on the living conditions of the organisms?*

As described in the results the physicochemical parameters (pH, conductivity, temperature and oxygen content) did not differ significantly compared to the control. Neither did the chemical parameters (hardness, phosphate, nitrate). All parameters were more or less in the control range throughout the study. The turbidity changed, depending on the particle amount and type. After each application the turbidity rose up to tenfold compared to the control in the HighPA treatment. After the particles had settled, which happened after few days, the turbidity values returned to the control level. The sole influence of the particles on the living conditions was posed by the addition of the silicon dioxide particles in the Silica treatment which caused a short-term increase of chlorophyll a originating from diatoms and thus diatom abundance. So, overall, there was no impact on the physicochemical parameters, posed by the particles.

- B) Is there a group in the aquatic invertebrate food web whose abundance is particularly influenced positively or negatively by the particles, i.e. which are the species of risk?*

As described for research question A) the diatoms were positively impacted by the Silica particles short-term. The presence of polyamide microplastic particles did positively affect the blue and green algae, expressed by the chlorophyll a content. Both indirect effects were found in the HighPA treatment. There were no treatment related direct effects for the chlorophyll a content, the macrophyte ground coverage or the abundance of macroinvertebrates or emerging insects. The abundance of some zooplankton organisms however was negatively affected by the polyamide treatment. The abundance of Cyclopidae and the total sum of all

organisms in the MedPA and HighPA treatment and of Ostracoda in all polyamide treatments was lower compared to the controls on various samplings. Cyclopidae and Ostracoda abundance was lower in the latter stages of the study. As many species of the family of Cyclopidae live near detritus and are benthic or semi benthic and all found Ostracoda species are associated with sediment or macrophytes, it can be assumed that effects on small, partly grazing organisms are only to be expected after the particles have completely sunk and accumulated on the sediment, and that these organisms are therefore among the most potentially endangered.

*C) Does a refined evaluation in higher tier studies, especially for stressors that are not typically tested in mesocosms, provide the opportunity to better assess effects and expand a risk analysis?*

The evaluation of biovolume showed no significant differences between the control and the treatments with the high particle load neither for macroinvertebrates nor emerged insects. Nevertheless, this evaluation can help to track the development of larvae and other invertebrates over the season. This, in turn, can help in future studies to identify the points in time when the most sensitive stages of development occur and adapt the study design accordingly. Furthermore, the biovolume of the emerged insects may give hints on the reproductive ability of these individuals. The larger the organisms are, the more food intake during larval development. Since the food intake is driven by availability and ability to feed of the larvae themselves this may give an insight into the functionality of the bioscoenosis.

The evaluation of sexratio of emerged Chaoboridae and Baetidae has revealed some (limited by the abundances) significant differences compared to the control. With a higher catch rate, for example through larger traps and better sample fixation, this endpoint is potentially suitable for indicating a different influence on ontogenetic development in the two sexes.

Calculating the EmT<sub>x</sub> allows to provide an additional laboratory endpoint for more complex field studies. Knowledge of the development time of the allows effects to be better differentiated. This makes it possible to distinguish toxic effects from those that are more likely to be developmental delays. In addition, this study showed that taxa that did not exhibit any direct or indirect toxic effects, such as Orthoclaadiinae, exhibited significantly different development times when particles were added.

### *Hypotheses*

- 1) *As the particles have a higher density than water and are relatively small, they will sediment slowly. They can limit the light intensity available to the algae and higher aquatic plants for a short period of time.*

There were no negative effects on algal or macrophyte growth. The sedimentation time was less than three days and thus, despite multiple applications in quick succession no effects were observed. The hypothesis is rejected.

- 2) *Zooplankton organisms, especially filter feeders, will ingest the particles unselectively as they are similar in size to their preferred food. Once the particles have been ingested, they can have an effect on the small animal organisms in various ways.*

Ostracods and cyclopoids showed significant lower abundances in the microplastic treated systems towards the end of the study. Both groups consist of grazing and predatory taxa which suggests and was shown for Ostracoda that particles were actively picked up or ingested through food. This hypothesis is accepted.

- 3) *Larger aquatic invertebrates, so-called macroinvertebrates, can ingest plastic particles directly as well as through their food and in some cases pass the particles on ontogenetically. This can cause effects on the organisms.*

The food intake of and ontogenetic transfer in macroinvertebrates was shown for *Chaoborus* sp. Since even smaller organisms ingested plastic particles via grazing and filtering it is reasonable that other macroinvertebrates did likely. The in some cases significantly early or delayed emergence speaks for an effect of the particles on larval development. This hypothesis can be accepted.

## 6. Conclusion

An obvious advantage of mesocosm experiments over laboratory experiments is the increased realism. However, this also makes standardization and replicability more difficult. Nevertheless, these higher-tier experiments are also assigned a low safety factor by regulatory authorities, which in some cases can be only 0.1% of that of an acute laboratory experiment. Particularly in the case of stressors that cannot be subordinated to the classic regulatory procedure, mesocosm experiments can give useful hints to ecotoxicological risk potential. These, admittedly very complex, experiments can provide an initial insight into the endangered species, populations, trophic levels or developmental stages. This top-down approach to risk assessment could be particularly useful for stressors such as particles, oil films or temperature changes, as the more natural conditions can reveal a more realistic response of the biocoenosis. In addition to these positive aspects, the mesocosm experiment also revealed some weaknesses in the design. Firstly, the number of concentrations in a (semi-) field study is limited if sufficient replicates are to be used at the same time. Secondly, a stressor for which the effect thresholds cannot be clearly defined requires a very high spacing factor compared to laboratory tests in order to assess the potential effect limits as no range finding experiments can be done easily. It is always important to ensure that one does not end up in a concentration that is far from all naturally expected concentrations, as the greatest benefit of such studies is their closeness to reality. With a duration of over 100 days after the first application this study is one of few to include long exposure, a particulate treated control and at least one environmentally realistic concentration.

All in all, however, it could be shown that no unacceptable risk to aquatic ecosystems is to be expected in the given experiment with non-aged, primary, spherical polyamide microplastic particles in a size of 5 - 50  $\mu\text{m}$  and in concentrations of 1.5, 15 and 150  $\text{mg L}^{-1}$ . This dissertation has laid the foundation of knowledge to gain a basic understanding of the effects of small, untreated microplastic particles on aquatic invertebrate biocoenosis. This opens up the potential to expand the knowledge through further adaptations and questions and to integrate it into a holistic risk analysis. A comprehensive risk assessment must cover the entire life cycle for these particulate stressors. Future studies should include a more diverse range of particles (polymer type, size, shape) and focus on non-lethal endpoints in addition to the positive aspects of mesocosm studies. Additionally, interactions with chemicals, that adhere to the particles during aging or recycling, should be a focal point in the future.



## VII. References

- Abinandan S, Praveen K, Venkateswarlu K, Megharaj M. 2023. Microalgae-microplastic interactions at environmentally relevant concentrations: Implications toward ecology, bioeconomy, and UN SDGs. *Water Res.* 247(120778):120778. doi:10.1016/j.watres.2023.120778. <http://dx.doi.org/10.1016/j.watres.2023.120778>.
- Adam V, Yang T, Nowack B. 2019. Toward an ecotoxicological risk assessment of microplastic: Comparison of available hazard and exposure data in freshwaters: Environmental risk assessment of microplastic. *Environ Toxicol Chem.* 38(2):436–447. doi:10.1002/etc.4323. <http://dx.doi.org/10.1002/etc.4323>.
- Alijagic A, Kotlyar O, Larsson M, Salihovic S, Hedbrant A, Eriksson U, Karlsson P, Persson A, Scherbak N, Färnlund K, u. a. 2024. Immunotoxic, genotoxic, and endocrine disrupting impacts of polyamide microplastic particles and chemicals. *Environ Int.* 183(108412):108412. doi:10.1016/j.envint.2023.108412. <http://dx.doi.org/10.1016/j.envint.2023.108412>.
- Al-Jaibachi R, Cuthbert RN, Callaghan A. 2018. Up and away: ontogenic transference as a pathway for aerial dispersal of microplastic. *Biol Lett.* 14(9):20180479. doi:10.1098/rsbl.2018.0479. <http://dx.doi.org/10.1098/rsbl.2018.0479>.
- Aloisi M, Poma AMG. 2025. Nanoplastics as Gene and Epigenetic Modulators of Endocrine Functions: A Perspective. *Int J Mol Sci.* 26(5):2071.
- Alonso A, Castro-Díez P. 2008. What explains the invading success of the aquatic mud snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca)? *Hydrobiologia.* 614(1):107–116. doi:10.1007/s10750-008-9529-3. <http://dx.doi.org/10.1007/s10750-008-9529-3>.
- Amaral-Zettler LA, Zettler ER, Mincer TJ. 2020. Ecology of the plastisphere. *Nat Rev Microbiol.* 18(3):139–151. doi:10.1038/s41579-019-0308-0. <http://dx.doi.org/10.1038/s41579-019-0308-0>.
- Amato-Lourenço LF, Carvalho-Oliveira R, Júnior GR, Dos Santos Galvão L, Ando RA, Mauad T. 2021. Presence of airborne microplastic in human lung tissue. *J Hazard Mater.* 416(126124):126124. doi:10.1016/j.jhazmat.2021.126124. <http://dx.doi.org/10.1016/j.jhazmat.2021.126124>.
- Andrades R, Dos Santos RA, Martins AS, Teles D, Santos RG. 2019. Scavenging as a pathway for plastic ingestion by marine animals. *Environ Pollut.* 248:159–165. doi:10.1016/j.envpol.2019.02.010. <http://dx.doi.org/10.1016/j.envpol.2019.02.010>.
- Andrady AL, Neal MA. 2009. Applications and societal benefits of plastics. *Philos Trans R Soc Lond B Biol Sci.* 364(1526):1977–1984. doi:10.1098/rstb.2008.0304. <http://dx.doi.org/10.1098/rstb.2008.0304>.
- Arshad S, Sommer M, Wendt-Potthoff K, Dunker S. 2023. Analysis of Polyamide Microplastics using Multispectral Imaging Flow Cytometry Master thesis. Technische Universität Chemnitz (not published)
- Balian EV, Segers H, Lévêque C, Martens K. 2008. The Freshwater Animal Diversity Assessment: an overview of the results. *Hydrobiologia.* 600(1):313–313. doi:10.1007/s10750-008-9302-7. <http://dx.doi.org/10.1007/s10750-008-9302-7>.

- Bänsch-Baltruschat B, Brennholt N, Kochleus C, Reifferscheid G, Koschorreck J. 2017. Conference on Plastics in Freshwater Environments. Umweltbundesamt.
- Barboza LGA, Gimenez BCG. 2015. Microplastic in the marine environment: Current trends and future perspectives. *Mar Pollut Bull.* 97(1–2):5–12. doi:10.1016/j.marpolbul.2015.06.008. <http://dx.doi.org/10.1016/j.marpolbul.2015.06.008>.
- Barrick A, Champeau O, Chatel A, Manier N, Northcott G, Tremblay LA. 2021. Plastic additives: challenges in ecotox hazard assessment. *PeerJ.* 9(e11300):e11300. doi:10.7717/peerj.11300. <http://dx.doi.org/10.7717/peerj.11300>.
- Barros J, Seena S. 2021. Plasticsphere in freshwaters: An emerging concern. *Environ Pollut.* 290(118123):118123. doi:10.1016/j.envpol.2021.118123. <http://dx.doi.org/10.1016/j.envpol.2021.118123>.
- Bauernfeind E, Soldan T. 2012. The mayflies of Europe (Ephemeroptera). Apollo Books. <http://dx.doi.org/10.1163/9789004260887>.
- Baumgartner A, Liebscher HJ. 1996. Lehrbuch der Hydrologie / Allgemeine Hydrologie - quantitative Hydrologie. 2. Aufl. Liebscher HJ, Herausgeber. Stuttgart: Borntraeger.
- Besseling E, Wang B, Lürling M, Koelmans AA. 2014. Nanoplastic affects growth of *S. obliquus* and reproduction of *D. magna*. *Environ Sci Technol.* 48(20):12336–12343. doi:10.1021/es503001d. <http://dx.doi.org/10.1021/es503001d>.
- Beuter L-K, Dören L, Hommen U, Kotthoff M, Schäfers C, Ebke KP. 2019. Testing effects of pesticides on macroinvertebrate communities in outdoor stream mesocosms using carbaryl as example test item. *Environ Sci Eur.* 31(1). doi:10.1186/s12302-019-0185-1. <http://dx.doi.org/10.1186/s12302-019-0185-1>.
- Bhat RAH, Sidiq MJ, Altinok I. 2024. Impact of microplastic and nanoplastics on fish health and reproduction. *Aquaculture.* 590(741037):741037. doi:10.1016/j.aquaculture.2024.741037. <http://dx.doi.org/10.1016/j.aquaculture.2024.741037>.
- Bolívar-Subirats G, Rivetti C, Cortina-Puig M, Barata C, Lacorte S. 2021. Occurrence, toxicity and risk assessment of plastic additives in Besos river, Spain. *Chemosphere.* 263(128022):128022. doi:10.1016/j.chemosphere.2020.128022. <http://dx.doi.org/10.1016/j.chemosphere.2020.128022>.
- Bolpagni R, Poikane S, Laini A, Bagella S, Bartoli M, Cantonati M. 2019. Ecological and conservation value of small standing-water ecosystems: A systematic review of current knowledge and future challenges. *Water (Basel).* 11(3):402. doi:10.3390/w11030402. <http://dx.doi.org/10.3390/w11030402>.
- Brock TCM, Hammers-Wirtz M, Hommen U, Preuss TG, Ratte H-T, Roessink I, Strauss T, Van den Brink PJ. 2015. The minimum detectable difference (MDD) and the interpretation of treatment-related effects of pesticides in experimental ecosystems. *Environ Sci Pollut Res Int.* 22(2):1160–1174. doi:10.1007/s11356-014-3398-2. <http://dx.doi.org/10.1007/s11356-014-3398-2>.
- Brondizio ES, Settele J, Díaz S, Ngo HT. 2019. Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services IPBES secretariat, Bonn, Germany.

- Browne MA, Niven SJ, Galloway TS, Rowland SJ, Thompson RC. 2013. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. *Curr Biol.* 23(23):2388–2392. doi:10.1016/j.cub.2013.10.012. <http://dx.doi.org/10.1016/j.cub.2013.10.012>.
- Bruck S, Ford AT. 2018. Chronic ingestion of polystyrene microparticles in low doses has no effect on food consumption and growth to the intertidal amphipod *Echinogammarus marinus*? *Environ Pollut.* 233:1125–1130. doi:10.1016/j.envpol.2017.10.015. <http://dx.doi.org/10.1016/j.envpol.2017.10.015>.
- Bucci K, Tulio M, Rochman CM. 2020. What is known and unknown about the effects of plastic pollution: A meta-analysis and systematic review. *Ecol Appl.* 30(2):e02044. doi:10.1002/eap.2044. <http://dx.doi.org/10.1002/eap.2044>.
- Bundschuh M, Englert D, Rosenfeldt RR, Bundschuh R, Feckler A, Lüderwald S, Seitz F, Zubrod JP, Schulz R. 2019. Nanoparticles transported from aquatic to terrestrial ecosystems via emerging aquatic insects compromise subsidy quality. *Sci Rep.* 9(1):15676. doi:10.1038/s41598-019-52096-7. <http://dx.doi.org/10.1038/s41598-019-52096-7>.
- Campbell, N.A., Reece, J.B., Urry, L.A., Cain M.L., Wasserman, S.A., Minorsky, P.V., Jackson, R.B. 2009. *Biologie*. München: Always learning Pearson.
- Cao Q, Sun W, Yang T, Zhu Z, Jiang Y, Hu W, Wei W, Zhang Y, Yang H. 2022. The toxic effects of polystyrene microplastic on freshwater algae *Chlorella pyrenoidosa* depends on the different size of polystyrene microplastic. *Chemosphere.* 308(Pt 1):136135. doi:10.1016/j.chemosphere.2022.136135. <http://dx.doi.org/10.1016/j.chemosphere.2022.136135>.
- Caquet T. 2002. Use of aquatic mesocosms in ecotoxicology: State of the art and perspectives. *Radioprotection.* 37(C1):C1-173-C1-177. doi:10.1051/radiopro/2002033. <http://dx.doi.org/10.1051/radiopro/2002033>.
- Caquet T, Lagadic L, Sheffield SR. 2000. Mesocosms in Ecotoxicology (1): Outdoor Aquatic Systems. In: *Reviews of Environmental Contamination and Toxicology*. New York, NY: Springer New York. S. 1–38.
- Carde RT, Resh VH. 2009. *Encyclopedia of insects*. 2. Aufl. San Diego, CA, USA: Academic Press.
- Caspers H. 1984. OECD: Eutrophication of Waters. Monitoring, Assessment and Control. — 154 pp. Paris: Organisation for Economic Co-Operation and Development 1982. (Publié en français sous le titre »Eutrophication des Eaux. Méthodes de Surveillance, d’Evaluation et de Lutte«). *Int Rev Gesamt Hydrobiol.* 69(2):200–200. doi:10.1002/iroh.19840690206. <http://dx.doi.org/10.1002/iroh.19840690206>.
- Castellano S, Scarascia Mugnozza G, Russo G, Briassoulis D, Mistriotis A, Hemming S, Waaijenberg D. 2008. Plastic nets in agriculture: A general review of types and applications. *Appl Eng Agric.* 24(6):799–808. doi:10.13031/2013.25368. <http://dx.doi.org/10.13031/2013.25368>.
- Castro-Castellon AT, Horton AA, Hughes JMR, Rampley C, Jeffers ES, Bussi G, Whitehead P. 2022. Ecotoxicity of microplastic to freshwater biota: Considering exposure and hazard across trophic levels. *Sci Total Environ.* 816(151638):151638. doi:10.1016/j.scitotenv.2021.151638. <http://dx.doi.org/10.1016/j.scitotenv.2021.151638>.

- Chapman PM. 2002. Integrating toxicology and ecology: putting the “eco” into ecotoxicology. *Mar Pollut Bull.* 44(1):7–15. doi:10.1016/s0025-326x(01)00253-3. [http://dx.doi.org/10.1016/s0025-326x\(01\)00253-3](http://dx.doi.org/10.1016/s0025-326x(01)00253-3).
- Chen Y, Tang H, Cheng Y, Huang T, Xing B. 2023. Interaction between microplastic and humic acid and its effect on their properties as revealed by molecular dynamics simulations. *J Hazard Mater.* 455(131636):131636. doi:10.1016/j.jhazmat.2023.131636. <http://dx.doi.org/10.1016/j.jhazmat.2023.131636>.
- Chia RW, Lee J-Y, Lee M, Lee G-S, Jeong C-D. 2023. Role of soil microplastic pollution in climate change. *Sci Total Environ.* 887(164112):164112. doi:10.1016/j.scitotenv.2023.164112. <http://dx.doi.org/10.1016/j.scitotenv.2023.164112>.
- Choi J-H, Lee J-H, Jo A-H, Choi YJ, Choi CY, Kang J-C, Kim J-H. 2023. Microplastic polyamide toxicity: Neurotoxicity, stress indicators and immune responses in crucian carp, *Carassius carassius*. *Ecotoxicol Environ Saf.* 265(115469):115469. doi:10.1016/j.ecoenv.2023.115469. <http://dx.doi.org/10.1016/j.ecoenv.2023.115469>.
- Cockroft RJG, Jenkins WR, Irwin AG, Norman S, Brown KC. 2022. Emergence timing and voltinism of phantom midges, *Chaoborus* spp., in the UK. *Web Ecol.* 22(2):101–108. doi:10.5194/we-22-101-2022. <http://dx.doi.org/10.5194/we-22-101-2022>.
- Cole M, Lindeque P, Fileman E, Halsband C, Goodhead R, Moger J, Galloway TS. 2013. Microplastic ingestion by zooplankton. *Environ Sci Technol.* 47(12):6646–6655. doi:10.1021/es400663f. <http://dx.doi.org/10.1021/es400663f>.
- Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food Text with EEA relevance. 2011.
- Connors KA, Dyer SD, Belanger SE. 2017. Advancing the quality of environmental microplastic research: Advancing the quality of environmental microplastic research. *Environ Toxicol Chem.* 36(7):1697–1703. doi:10.1002/etc.3829. <http://dx.doi.org/10.1002/etc.3829>.
- Corbet PS. 1964. Temporal patterns of emergence in aquatic insects. *Can Entomol.* 96(1–2):264–279. doi:10.4039/ent96264-1. <http://dx.doi.org/10.4039/ent96264-1>.
- Cózar A, Echevarría F, González-Gordillo JI, Irigoien X, Ubeda B, Hernández-León S, Palma AT, Navarro S, García-de-Lomas J, Ruiz A, u. a. 2014. Plastic debris in the open ocean. *Proc Natl Acad Sci U S A.* 111(28):10239–10244. doi:10.1073/pnas.1314705111. <http://dx.doi.org/10.1073/pnas.1314705111>.
- Cunningham EM, Sigwart JD. 2019. Environmentally accurate microplastic levels and their absence from exposure studies. *Integr Comp Biol.* 59(6):1485–1496. doi:10.1093/icb/icz068. <http://dx.doi.org/10.1093/icb/icz068>.
- Cuthbert RN, Al-Jaibachi R, Dalu T, Dick JTA, Callaghan A. 2019. The influence of microplastic on trophic interaction strengths and oviposition preferences of dipterans. *Sci Total Environ.* 651(Pt 2):2420–2423. doi:10.1016/j.scitotenv.2018.10.108. <http://dx.doi.org/10.1016/j.scitotenv.2018.10.108>.
- Dahms HTJ, van Rensburg GJ, Greenfield R. 2020. The microplastic profile of an urban African stream. *Sci Total Environ.* 731(138893):138893. doi:10.1016/j.scitotenv.2020.138893. <http://dx.doi.org/10.1016/j.scitotenv.2020.138893>.

- Daily J, Tyler AC, Hoffman MJ. 2022. Modeling three-dimensional transport of microplastic and impacts of biofouling in Lake Erie and Lake Ontario. *J Great Lakes Res.* 48(5):1180–1190. doi:10.1016/j.jglr.2022.07.001. <http://dx.doi.org/10.1016/j.jglr.2022.07.001>.
- Daniel D, Vieira M, da Costa JP, Girão AV, Nunes B. 2024. Effects of microplastic on key reproductive and biochemical endpoints of the freshwater microcrustacean *Daphnia magna*. *Comp Biochem Physiol C Toxicol Pharmacol.* 281(109917):109917. doi:10.1016/j.cbpc.2024.109917. <http://dx.doi.org/10.1016/j.cbpc.2024.109917>.
- DIN 38409-60 Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung – Summarische Wirkungs- und Stoffkenngrößen (Gruppe H) – Teil 60: Bestimmung von Chlorophyll-a und -b in Oberflächenwasser (H 60). 2019. <https://dx.doi.org/10.31030/3116374>.
- Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. 2001.
- Dobinski W. 2006. Ice and environment: A terminological discussion. *Earth Sci Rev.* 79(3–4):229–240. doi:10.1016/j.earscirev.2006.07.003. <http://dx.doi.org/10.1016/j.earscirev.2006.07.003>.
- Donner J. 1973. Rädertiere: (Rotatorien), 4. Aufl., 5. Tsd ed. Einführung in die Kleinlebewelt. Stuttgart: Franckh-Kosmos Verlags.
- Downing JA, Cole JJ, Middelburg JJ, Striegl RG, Duarte CM, Kortelainen P, Prairie YT, Laube KA. 2008. Sediment organic carbon burial in agriculturally eutrophic impoundments over the last century: SEDIMENT ORGANIC CARBON BURIAL. *Global Biogeochem Cycles.* 22(1). doi:10.1029/2006gb002854. <http://dx.doi.org/10.1029/2006gb002854>.
- Du Z, Li G, Ding S, Song W, Zhang M, Jia R, Chu W. 2023. Effects of UV-based oxidation processes on the degradation of microplastic: Fragmentation, organic matter release, toxicity and disinfection byproduct formation. *Water Res.* 237(119983):119983. doi:10.1016/j.watres.2023.119983. <http://dx.doi.org/10.1016/j.watres.2023.119983>.
- Dudgeon D, Arthington AH, Gessner MO, Kawabata Z-I, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard A-H, Soto D, Stiassny MLJ, u. a. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol Rev Camb Philos Soc.* 81(2):163–182. doi:10.1017/S1464793105006950. <http://dx.doi.org/10.1017/S1464793105006950>.
- Duft M, Schmitt C, Bachmann J, Brandelik C, Schulte-Oehlmann U, Oehlmann J. 2007. Prosobranch snails as test organisms for the assessment of endocrine active chemicals--an overview and a guideline proposal for a reproduction test with the freshwater mudsnail *Potamopyrgus antipodarum*. *Ecotoxicology.* 16(1):169–182. doi:10.1007/s10646-006-0106-0. <http://dx.doi.org/10.1007/s10646-006-0106-0>.
- Duft M, Schulte-Oehlmann U, Weltje L, Tillmann M, Oehlmann J. 2003. Stimulated embryo production as a parameter of estrogenic exposure via sediments in the freshwater mudsnail *Potamopyrgus antipodarum*. *Aquat Toxicol.* 64(4):437–449. doi:10.1016/s0166-445x(03)00102-4. [http://dx.doi.org/10.1016/s0166-445x\(03\)00102-4](http://dx.doi.org/10.1016/s0166-445x(03)00102-4).
- Eastham LES. 1958. THE ABDOMINAL MUSCULATURE OF NYMPHAL *CHLOEON DIPTERUM* L. (INSECTA: EPHEMEROPTERA) IN RELATION TO GILL MOVEMENT AND SWIMMING. *Proc Zool Soc Lond* (1944). 131(2):279–291. doi:10.1111/j.1096-3642.1958.tb00688.x. <http://dx.doi.org/10.1111/j.1096-3642.1958.tb00688.x>.

- Einsle U. 1993. Süßwasserfauna von Mitteleuropa / Crustacea: Copepoda: Calanoida und Cyclopoida. Schwoerbel J, Zwick P, Herausgeber. München: Urban & Fischer in Elsevier.
- Ennis WB Jr, Dowler WM, Klassen W. 1975. Crop protection to increase food supplies. *Science*. 188(4188):593–598. doi:10.1126/science.188.4188.593. <http://dx.doi.org/10.1126/science.188.4188.593>.
- Eriksen M, Lebreton LCM, Carson HS, Thiel M, Moore CJ, Borroero JC, Galgani F, Ryan PG, Reisser J. 2014. Plastic pollution in the world's oceans: More than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *PLoS One*. 9(12):e111913. doi:10.1371/journal.pone.0111913. <http://dx.doi.org/10.1371/journal.pone.0111913>.
- European Food Safety Authority (EFSA). 2006. Opinion of the Scientific Panel on Plant protection products and their residues (PPR) related to the aquatic risk assessment for cyprodinil and the use of a mesocosm study in particular: Opinion of the Scientific Panel on Plant protection products and their residues (PPR) related to the aquatic risk assessment for cyprodinil and the us. *EFSA J*. 4(3):329. doi:10.2903/j.efsa.2006.329. <http://dx.doi.org/10.2903/j.efsa.2006.329>.
- European Food Safety Authority (EFSA). 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters <https://doi.org/10.2903/j.efsa.2013.3290>.
- Fagiano V, Alomar C, Compa M, Soto-Navarro J, Jordá G, Deudero S. 2022. Neustonic microplastic and zooplankton in coastal waters of Cabrera Marine Protected Area (Western Mediterranean Sea). *Sci Total Environ*. 804(150120):150120. doi:10.1016/j.scitotenv.2021.150120. <http://dx.doi.org/10.1016/j.scitotenv.2021.150120>.
- Fath A. 2019. Mikroplastik kompakt: Wissenswertes für alle. 1. Aufl. Wiesbaden: Springer Spektrum.
- Fent K. 2013. Ökotoxikologie: Umweltchemie - Toxikologie - Ökologie. Fent K, Herausgeber. Stuttgart: Thieme.
- Fukuoka T, Yamane M, Kinoshita C, Narazaki T, Marshall GJ, Abernathy KJ, Miyazaki N, Sato K. 2016. The feeding habit of sea turtles influences their reaction to artificial marine debris. *Sci Rep*. 6:28015. doi:10.1038/srep28015. <http://dx.doi.org/10.1038/srep28015>.
- Gerhardt V, Bodemer U. 1998. Delayed fluorescence excitation spectroscopy: a method for automatic determination of phytoplankton composition of freshwaters and sediments (interstitial) of algal composition of benthos. *Limnologia*. 28(3):313–322.
- Gerhardt V, Bodemer U. 2000. Delayed fluorescence excitation spectroscopy: a method for determining phytoplankton composition. *advances in limnol.*(55):101–120.
- Geyer R, Jambeck JR, Law KL. 2017. Production, use, and fate of all plastics ever made. *Sci Adv*. 3(7):e1700782. doi:10.1126/sciadv.1700782. <http://dx.doi.org/10.1126/sciadv.1700782>.
- Gibson CE, Wang G, Foy RH. 2000. Silica and diatom growth in Lough Neagh: the importance of internal recycling: Silica and diatom growth in Lough Neagh. *Freshw Biol*. 45(3):285–293. doi:10.1111/j.1365-2427.2000.00624.x. <http://dx.doi.org/10.1111/j.1365-2427.2000.00624.x>.
- Gilpin LC, Davidson K, Roberts E. 2004. The influence of changes in nitrogen: silicon ratios on diatom growth dynamics. *J Sea Res*. 51(1):21–35. doi:10.1016/j.seares.2003.05.005. <http://dx.doi.org/10.1016/j.seares.2003.05.005>.

- Godoy V, Martín-Lara MA, Calero M, Blázquez G. 2020. The relevance of interaction of chemicals/pollutants and microplastic samples as route for transporting contaminants. *Process Saf Environ Prot.* 138:312–323. doi:10.1016/j.psep.2020.03.033. <http://dx.doi.org/10.1016/j.psep.2020.03.033>.
- Griggs D, Stafford-Smith M, Gaffney O, Rockström J, Ohman MC, Shyamsundar P, Steffen W, Glaser G, Kanie N, Noble I. 2013. Policy: Sustainable development goals for people and planet: Policy. *Nature.* 495(7441):305–307. doi:10.1038/495305a. <http://dx.doi.org/10.1038/495305a>.
- Guo, J.-X., Pan, J., Wang, J., Wang, F., & Shi, H.-X. (2019). Study of the adsorption of endocrine disruptor compounds on typical filter materials using a quartz crystal microbalance. *Environmental Science and Pollution Research International*, 26(20), 20499–20509. <https://doi.org/10.1007/s11356-019-05361-1>
- Hall DJ, Cooper WE, Werner EE. 1970. An experimental approach to the production dynamics and structure of freshwater animal communities1: Dynamics and structure of aquatic communities. *Limnol Oceanogr.* 15(6):839–928. doi:10.4319/lo.1970.15.6.0839. <http://dx.doi.org/10.4319/lo.1970.15.6.0839>.
- Hämer J, Gutow L, Köhler A, Saborowski R. 2014. Fate of microplastic in the marine isopod *Idotea emarginata*. *Environ Sci Technol.* 48(22):13451–13458. doi:10.1021/es501385y. <http://dx.doi.org/10.1021/es501385y>.
- Harper MP, Peckarsky BL. 2006. Emergence cues of a mayfly in a high-altitude stream ecosystem: potential response to climate change. *Ecol Appl.* 16(2):612–621. doi:10.1890/1051-0761(2006)016[0612:ecoami]2.0.co;2. [http://dx.doi.org/10.1890/1051-0761\(2006\)016\[0612:ecoami\]2.0.co;2](http://dx.doi.org/10.1890/1051-0761(2006)016[0612:ecoami]2.0.co;2).
- Hasan J, Siddik MA, Ghosh AK, Mesbah SB, Sadat MA, Shahjahan M. 2023. Increase in temperature increases ingestion and toxicity of polyamide microplastic in Nile tilapia. *Chemosphere.* 327(138502):138502. doi:10.1016/j.chemosphere.2023.138502. <http://dx.doi.org/10.1016/j.chemosphere.2023.138502>.
- Henriksen CI, Saiz E, Calbet A, Hansen BW. 2007. Feeding activity and swimming patterns of *Acartia grani* and *Oithona davisae* nauplii in the presence of motile and non-motile prey. *Mar Ecol Prog Ser.* 331:119–129. doi:10.3354/meps331119. <http://dx.doi.org/10.3354/meps331119>.
- Hildebrandt L, Nack FL, Zimmermann T, Pröfrock D. 2021. Microplastic as a Trojan horse for trace metals. *J Hazard Mater Lett.* 2(100035):100035. doi:10.1016/j.hazl.2021.100035. <http://dx.doi.org/10.1016/j.hazl.2021.100035>.
- Hu L, Zhao Y, Xu H. 2022. Trojan horse in the intestine: A review on the biotoxicity of microplastic combined environmental contaminants. *J Hazard Mater.* 439(129652):129652. doi:10.1016/j.jhazmat.2022.129652. <http://dx.doi.org/10.1016/j.jhazmat.2022.129652>.
- Hurley R, Woodward J, Rothwell JJ. 2018. Microplastic contamination of river beds significantly reduced by catchment-wide flooding. *Nat Geosci.* 11(4):251–257. doi:10.1038/s41561-018-0080-1. <http://dx.doi.org/10.1038/s41561-018-0080-1>.
- Hurley RR, Woodward JC, Rothwell JJ. 2017. Ingestion of microplastic by freshwater Tubifex worms. *Environ Sci Technol.* 51(21):12844–12851. doi:10.1021/acs.est.7b03567. <http://dx.doi.org/10.1021/acs.est.7b03567>.

- Imhof HK, Ivleva NP, Schmid J, Niessner R, Laforsch C. 2013. Contamination of beach sediments of a subalpine lake with microplastic particles. *Curr Biol.* 23(19):R867-8. doi:10.1016/j.cub.2013.09.001. <http://dx.doi.org/10.1016/j.cub.2013.09.001>.
- Imhof HK, Laforsch C. 2016. Hazardous or not – Are adult and juvenile individuals of *Potamopyrgus antipodarum* affected by non buoyant microplastic particles? *Environmental po.*(49):1–19.
- Jaikumar G, Baas J, Brun NR, Vijver MG, Bosker T. 2018. Acute sensitivity of three Cladoceran species to different types of microplastic in combination with thermal stress. *Environ Pollut.* 239:733–740. doi:10.1016/j.envpol.2018.04.069. <http://dx.doi.org/10.1016/j.envpol.2018.04.069>.
- Jambeck JR, Geyer R, Wilcox C, Siegler TR, Perryman M, Andrady A, Narayan R, Law KL. 2015. Marine pollution. Plastic waste inputs from land into the ocean. *Science.* 347(6223):768–771. doi:10.1126/science.1260352. <http://dx.doi.org/10.1126/science.1260352>.
- James S, Boone M. 2005. Aquatic and terrestrial mesocosms in amphibian ecotoxicology. *Appl Herpetol.* 2(3):231–257. doi:10.1163/1570754054507442. <http://dx.doi.org/10.1163/1570754054507442>.
- Jemec A, Horvat P, Kunej U, Bele M, Kržan A. 2016. Uptake and effects of microplastic textile fibers on freshwater crustacean *Daphnia magna*. *Environ Pollut.* 219:201–209. doi:10.1016/j.envpol.2016.10.037. <http://dx.doi.org/10.1016/j.envpol.2016.10.037>.
- Jepsen D, Zimmermann T, Spengler L, Rödiger L, Bliklen R, Wagner J, Struck K, Hiestermann L, Schulz H. 2020. Kunststoffe in der Umwelt - Erarbeitung einer Systematik für erste Schätzungen zum Verbleib von Abfällen und anderen Produkten aus Kunststoffen in verschiedenen Umweltmedien. 198/2020: Umweltbundesamt.
- Jin M, Liu J, Yu J, Zhou Q, Wu W, Fu L, Yin C, Fernandez C, Karimi-Maleh H. 2022. Current development and future challenges in microplastic detection techniques: A bibliometrics-based analysis and review. *Sci Prog.* 105(4):368504221132151. doi:10.1177/00368504221132151. <http://dx.doi.org/10.1177/00368504221132151>.
- Junck, J., Diagboya, P. N., Peqini, A., Rohnke, M., & Düring, R.-A. (2024). Mechanistic interpretation of the sorption of terbuthylazine pesticide onto aged microplastics. *Environmental Pollution (Barking, Essex: 1987)*, 345(123502), 123502. <https://doi.org/10.1016/j.envpol.2024.123502>
- Kaandorp MLA, Lobelle D, Kehl C, Dijkstra HA, van Sebille E. 2023. Global mass of buoyant marine plastics dominated by large long-lived debris. *Nature geoscience.* 2023(16):689–694.
- Khosrovyan A, Kahru A. 2021. Evaluation of the potential toxicity of UV-weathered virgin polyamide microplastic to non-biting midge *Chironomus riparius*. *Environ Pollut.* 287(117334):117334. doi:10.1016/j.envpol.2021.117334. <http://dx.doi.org/10.1016/j.envpol.2021.117334>.
- Khosrovyan A, Kahru A. 2022. Virgin and UV-weathered polyamide microplastic posed no effect on the survival and reproduction of *Daphnia magna*. *PeerJ.* 10(e13533):e13533. doi:10.7717/peerj.13533. <http://dx.doi.org/10.7717/peerj.13533>.
- Kinzelbach R. 1995. Neozoans in european waters — Exemplifying the worldwide process of invasion and species mixing. *Experientia.* 51(5):526–538. doi:10.1007/bf02143203. <http://dx.doi.org/10.1007/bf02143203>.

- Klein S, Worch E, Knepper TP. 2015. Occurrence and spatial distribution of microplastic in river shore sediments of the Rhine-main area in Germany. *Environ Sci Technol.* 49(10):6070–6076. doi:10.1021/acs.est.5b00492. <http://dx.doi.org/10.1021/acs.est.5b00492>.
- Koelmans AA, Mohamed Nor NH, Hermesen E, Kooi M, Mintenig SM, De France J. 2019. Microplastic in freshwaters and drinking water: Critical review and assessment of data quality. *Water Res.* 155:410–422. doi:10.1016/j.watres.2019.02.054. <http://dx.doi.org/10.1016/j.watres.2019.02.054>.
- Kowalski N, Reichardt AM, Waniek JJ. 2016. Sinking rates of microplastic and potential implications of their alteration by physical, biological, and chemical factors. *Mar Pollut Bull.* 109(1):310–319. doi:10.1016/j.marpolbul.2016.05.064. <http://dx.doi.org/10.1016/j.marpolbul.2016.05.064>.
- Kozioł A, Paso KG, Kuciel S. 2022. Properties and recyclability of abandoned fishing net-based plastic debris. *Catalysts.* 12(9):948. doi:10.3390/catal12090948. <http://dx.doi.org/10.3390/catal12090948>.
- Kvale K, Prowe AEF, Chien C-T, Landolfi A, Oschlies A. 2021. Zooplankton grazing of microplastic can accelerate global loss of ocean oxygen. *Nat Commun.* 12(1):2358. doi:10.1038/s41467-021-22554-w. <http://dx.doi.org/10.1038/s41467-021-22554-w>.
- Lagarde F, Olivier O, Zanella M, Daniel P, Hiard S, Caruso A. 2016. Microplastic interactions with freshwater microalgae: Hetero-aggregation and changes in plastic density appear strongly dependent on polymer type. *Environ Pollut.* 215:331–339. doi:10.1016/j.envpol.2016.05.006. <http://dx.doi.org/10.1016/j.envpol.2016.05.006>.
- Lambert S, Sinclair C, Boxall A. 2014. Occurrence, degradation, and effect of polymer-based materials in the environment. *Rev Environ Contam Toxicol.* 227:1–53. doi:10.1007/978-3-319-01327-5\_1. [http://dx.doi.org/10.1007/978-3-319-01327-5\\_1](http://dx.doi.org/10.1007/978-3-319-01327-5_1).
- Lambert S, Wagner M. 2018. Microplastic are contaminants of emerging concern in freshwater environments: An overview. In: *The Handbook of Environmental Chemistry*. Cham: Springer International Publishing. S. 1–23.
- Länderarbeitsgemeinschaft Wasser (LAWA). 1998. Beurteilung der Trophie von Seen.
- Landner L, Blanck H, Heyman U, Lundgren A, Notini M, Rosemarin A, Sundelin B. 1989. Community testing, microcosm and mesocosm experiments: Ecotoxicological tools with high ecological realism. In: *Springer Series on Environmental Management*. Berlin, Heidelberg: Springer Berlin Heidelberg. S. 216–254.
- Lebreton L, Slat B, Ferrari F, Sainte-Rose B, Aitken J, Marthouse R, Hajbane S, Cunsolo S, Schwarz A, Levivier A, u. a. 2018. Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. *Sci Rep.* 8(1):4666. doi:10.1038/s41598-018-22939-w. <http://dx.doi.org/10.1038/s41598-018-22939-w>.
- Lechner A, Keckeis H, Lumesberger-Loisl F, Zens B, Krusch R, Tritthart M, Glas M, Schludermann E. 2014. The Danube so colourful: a potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. *Environ Pollut.* 188:177–181. doi:10.1016/j.envpol.2014.02.006. <http://dx.doi.org/10.1016/j.envpol.2014.02.006>.
- Lehmann R, Bachmann J, Karaoglan B, Lacker J, Lurman G, Polleichtner C, Ratte HT, Ratte M. 2018. The CPCAT as a novel tool to overcome the shortcomings of NOEC/LOEC statistics in ecotoxicology: a simulation study to evaluate the statistical power. *Environ Sci Eur.* 30(1):50. doi:10.1186/s12302-018-0178-5. <http://dx.doi.org/10.1186/s12302-018-0178-5>.

- Leiser, R., Wu, G.-M., Neu, T. R., & Wendt-Potthoff, K. (2020). Biofouling, metal sorption and aggregation are related to sinking of microplastic in a stratified reservoir. *Water Research*, 176(115748), 115748. <https://doi.org/10.1016/j.watres.2020.115748>
- Lenz R, Enders K, Nielsen TG. 2016. Microplastic exposure studies should be environmentally realistic. *Proc Natl Acad Sci U S A*. 113(29):E4121-2. doi:10.1073/pnas.1606615113. <http://dx.doi.org/10.1073/pnas.1606615113>.
- Leonard S V, Liddle CR, Atherall CA, Chapman E, Watkins M, D J Calaminus S, Rotchell JM. 2024. Microplastic in human blood: Polymer types, concentrations and characterisation using  $\mu$ FTIR. *Environ Int*. 188(108751):108751. doi:10.1016/j.envint.2024.108751. <http://dx.doi.org/10.1016/j.envint.2024.108751>.
- Lewis-Phillips J, Brooks SJ, Sayer CD, Patmore IR, Hilton GM, Harrison A, Robson H, Axmacher JC. 2020. Ponds as insect chimneys: Restoring overgrown farmland ponds benefits birds through elevated productivity of emerging aquatic insects. *Biol Conserv*. 241(108253):108253. doi:10.1016/j.biocon.2019.108253. <http://dx.doi.org/10.1016/j.biocon.2019.108253>.
- Lichtenberg R. 1973. Die Entwicklung einiger charakteristischer Benthosorganismen des Hallateiches südlich von Wien'. *Annalen des Naturhistorischen Museums in Wien*. S. 77. Bd pp 305-311.
- Lindeque PK, Cole M, Coppock RL, Lewis CN, Miller RZ, Watts AJR, Wilson-McNeal A, Wright SL, Galloway TS. 2020. Are we underestimating microplastic abundance in the marine environment? A comparison of microplastic capture with nets of different mesh-size. *Environ Pollut*. 265(Pt A):114721. doi:10.1016/j.envpol.2020.114721. <http://dx.doi.org/10.1016/j.envpol.2020.114721>.
- Lira AFA, DeSouza AM. 2016. Microhabitat use of harvestmen (Arachnida: Opiliones) assemblage in the highland Brazilian Atlantic rainforest. *Can J Zool*. 94(5):323–327. doi:10.1139/cjz-2015-0219. <http://dx.doi.org/10.1139/cjz-2015-0219>.
- Loerracher A-K, Schmidt J, Ebke P, Schmolke A, Abi-Akar F, Galic N, Ashauer R. 2023. Characterization of patterns and variability in the dynamics of outdoor aquatic mesocosms: exploring the capabilities and challenges in data supporting aquatic system models. *Ecotoxicology*. 32(6):782–801. doi:10.1007/s10646-023-02685-0. <http://dx.doi.org/10.1007/s10646-023-02685-0>.
- Long M, Moriceau B, Gallinari M, Lambert C, Huvet A, Paul-Pont I, Hégaret H, Soudant P. 2017. On the potential role of phytoplankton aggregates in microplastic sedimentation. In: *Fate and Impact of Microplastic in Marine Ecosystems*. Elsevier. S. 71.
- Ma C, Shi H, Slaveykova VI. 2024. Entanglement of *Daphnia magna* by Fibrous Microplastic through “Hook and Loop” Action. *Environmental Science & Technology Letters*. 11(5):433–437.
- Mahon AM, O’Connell B, Healy MG, O’Connor I, Officer R, Nash R, Morrison L. 2017. Microplastic in sewage sludge: Effects of treatment. *Environ Sci Technol*. 51(2):810–818. doi:10.1021/acs.est.6b04048. <http://dx.doi.org/10.1021/acs.est.6b04048>.
- Mao R, Song J, Yan P, Ouyang Z, Wu R, Liu S, Guo X. 2021. Horizontal and vertical distribution of microplastic in the Wuliangshuai Lake sediment, northern China. *Sci Total Environ*. 754(142426):142426. doi:10.1016/j.scitotenv.2020.142426. <http://dx.doi.org/10.1016/j.scitotenv.2020.142426>.
- Martin Wagner, Scott Lambert. 2020. *Freshwater microplastic: Emerging environmental contaminants?* Saint Philip Street Press.

- Martin, Y. E., & Johnson, E. A. 2012. Biogeosciences survey: Studying interactions of the biosphere with the lithosphere, hydrosphere and atmosphere. *Progress in Physical Geography: Earth and Environment*. 2012(36(6)):833–852.
- Martínez A, Barbosa A. 2024. Chemical reactivity theory to analyze possible toxicity of microplastic: Polyethylene and polyester as examples. *PLoS One*. 19(3):e0285515. doi:10.1371/journal.pone.0285515. <http://dx.doi.org/10.1371/journal.pone.0285515>.
- Martínez Rodríguez A, Kratina P, Jones JI. 2024. Microplastic pollution and nutrient enrichment shift the diet of freshwater macroinvertebrates. *Environ Pollut*. 359(124540):124540. doi:10.1016/j.envpol.2024.124540. <http://dx.doi.org/10.1016/j.envpol.2024.124540>.
- Mason SA, Garneau D, Sutton R, Chu Y, Ehmman K, Barnes J, Fink P, Papazissimos D, Rogers DL. 2016. Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. *Environ Pollut*. 218:1045–1054. doi:10.1016/j.envpol.2016.08.056. <http://dx.doi.org/10.1016/j.envpol.2016.08.056>.
- McNair HM, Brzezinski MA, Krause JW. 2018. Diatom populations in an upwelling environment decrease silica content to avoid growth limitation: Diatom physiology and silicon stress. *Environ Microbiol*. 20(11):4184–4193. doi:10.1111/1462-2920.14431. <http://dx.doi.org/10.1111/1462-2920.14431>.
- Meijer LJJ, van Emmerik T, van der Ent R, Schmidt C, Lebreton L. 2021. More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean. *Sci Adv*. 7(18). doi:10.1126/sciadv.aaz5803. <http://dx.doi.org/10.1126/sciadv.aaz5803>.
- Mejías, C., Martín, J., Santos, J. L., Aparicio, I., & Alonso, E. (2023). Adsorption of perfluoroalkyl substances on polyamide microplastics: Effect of sorbent and influence of environmental factors. *Environmental Research*, 216(Pt 4), 114834. <https://doi.org/10.1016/j.envres.2022.114834>
- Michler-Kozma DN, Kruckenfellner L, Heitkamp A, Ebke KP, Gabel F. 2022. Uptake and transfer of polyamide microplastic in a freshwater mesocosm study. *Water (Basel)*. 14(6):887. doi:10.3390/w14060887. <http://dx.doi.org/10.3390/w14060887>.
- Millennium Ecosystem Assessment 2005 Ecosystem and Human Well-Being: Biodiversity Synthesis. Washington D.C.: Island Press.
- Miller ME, Hamann M, Kroon FJ. 2020. Bioaccumulation and biomagnification of microplastic in marine organisms: A review and meta-analysis of current data. *PLoS One*. 15(10):e0240792. doi:10.1371/journal.pone.0240792. <http://dx.doi.org/10.1371/journal.pone.0240792>.
- Mora-Teddy AK, Closs GP, Matthaei CD. 2024. Microplastic and riverine macroinvertebrate communities in a multiple-stressor context: A mesocosm approach. *Sci Total Environ*. 951(175456):175456. doi:10.1016/j.scitotenv.2024.175456. <http://dx.doi.org/10.1016/j.scitotenv.2024.175456>.
- Muñiz-González A-B, Silva CJM, Patricio Silva AL, Campos D, Pestana JLT, Martínez-Guitarte J-L. 2021. Suborganismal responses of the aquatic midge *Chironomus riparius* to polyethylene microplastic. *Sci Total Environ*. 783(146981):146981. doi:10.1016/j.scitotenv.2021.146981. <http://dx.doi.org/10.1016/j.scitotenv.2021.146981>.
- Naidoo T, Glassom D. 2019. Decreased growth and survival in small juvenile fish, after chronic exposure to environmentally relevant concentrations of microplastic. *Mar Pollut Bull*. 145:254–259. doi:10.1016/j.marpolbul.2019.02.037. <http://dx.doi.org/10.1016/j.marpolbul.2019.02.037>.

Naqash N, Prakash S, Kapoor D, Singh R. 2020. Interaction of freshwater microplastic with biota and heavy metals: a review. *Environ Chem Lett.* 18(6):1813–1824. doi:10.1007/s10311-020-01044-3. <http://dx.doi.org/10.1007/s10311-020-01044-3>.

Natarajan L, Soupam D, Dey S, Chandrasekaran N, Kundu R, Paul S, Mukherjee A. 2022. Toxicity of polystyrene microplastic in freshwater algae *Scenedesmus obliquus*: Effects of particle size and surface charge. *Toxicol Rep.* 9:1953–1961. doi:10.1016/j.toxrep.2022.10.013. <http://dx.doi.org/10.1016/j.toxrep.2022.10.013>.

Odum EP. 1984. The Mesocosm. *Bioscience.* 34(9):558–562. doi:10.2307/1309598. <http://dx.doi.org/10.2307/1309598>.

OECD. 2022. Plastic pollution is growing relentlessly as waste management and recycling fall short, says OECD. *Oecd.org*. [accessed 2025 March 13]. <https://www.oecd.org/en/about/news/press-releases/2022/02/plastic-pollution-is-growing-relentlessly-as-waste-management-and-recycling-fall-short.html>.

OECD Guideline for the Testing of Chemicals, No. 210, "Fish, Early-lif Stage Toxicity Test"; adopted July 26, 2013.

OECD Guideline for the Testing of Chemicals, No. 211, "Daphnia magna Reproduction Test"; adopted October 2, 2012.

OECD Guideline for the Testing of Chemicals, No. 218, "Sediment-Water Chironomid Toxicity Using Spiked Sediment"; adopted July 4, 2023.

OECD Guideline for the Testing of Chemicals, No. 242, "Potamopyrgus antipodarum Reproduction Test"; adopted July 29, 2016.

OECD Guideline for the Testing of Chemicals, No. 305, "Bioaccumulation in Fish: Aqueous and Dietary Exposure"; adopted October 2, 2012.

OECD Guideline for the Testing of Chemicals, No. 308, "Aerobic and Anaerobic Transformation in Aquatic Sediment Systems"; adopted April 24, 2002.

OECD Guideline for the Testing of Chemicals, No. 309, "Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test"; adopted November 23, 2004.

OECD Guideline for the Testing of Chemicals, No. 416, "Two-Generation Reproduction Toxicity"; adopted January 22, 2001.

OECD Guideline for the Testing of Chemicals, No. 443, "Extended One-Generation Reproductive Toxicity Study"; adopted June 27, 2018.

Oerke E-C, Dehne H-W. 2004. Safeguarding production—losses in major crops and the role of crop protection. *Crop Prot.* 23(4):275–285. doi:10.1016/j.cropro.2003.10.001. <http://dx.doi.org/10.1016/j.cropro.2003.10.001>.

Oertli B, Céréghino R, Hull A, Miracle R. 2009. Pond conservation: from science to practice. *Hydrobiologia.* 634(1):1–9. doi:10.1007/s10750-009-9891-9. <http://dx.doi.org/10.1007/s10750-009-9891-9>.

Öffentlichkeitsarbeit RKU. 2018 Aug 16. Talsperre Bautzen. Sachsen.de. [accessed 2025 March 14]. [https://www.wasserwirtschaft.sachsen.de/TS\\_Bautzen.html](https://www.wasserwirtschaft.sachsen.de/TS_Bautzen.html).

- Ogonowski M, Schür C, Jarsén Å, Gorokhova E. 2016. The effects of natural and anthropogenic microparticles on individual fitness in *Daphnia magna*. *PLoS One*. 11(5):e0155063. doi:10.1371/journal.pone.0155063. <http://dx.doi.org/10.1371/journal.pone.0155063>.
- Pandey P, Dhiman M, Kansal A, Subudhi SP. 2023. Plastic waste management for sustainable environment: techniques and approaches. *Waste Dispos Sustain Energy*. 5(2):1–18. doi:10.1007/s42768-023-00134-6. <http://dx.doi.org/10.1007/s42768-023-00134-6>.
- Parvez MS, Ullah H, Faruk O, Simon E, Czédli H. 2024. Role of microplastic in global warming and climate change: A review. *Water Air Soil Pollut*. 235(3). doi:10.1007/s11270-024-07003-w. <http://dx.doi.org/10.1007/s11270-024-07003-w>.
- Patrício Silva AL, Prata JC, Walker TR, Duarte AC, Ouyang W, Barcelò D, Rocha-Santos T. 2021. Increased plastic pollution due to COVID-19 pandemic: Challenges and recommendations. *Chem Eng J*. 405(126683):126683. doi:10.1016/j.cej.2020.126683. <http://dx.doi.org/10.1016/j.cej.2020.126683>.
- Peckarsky BL, Taylor BW, McIntosh AR, McPeck MA, Lytle DA. 2001. Variation in mayfly size at metamorphosis as a developmental response to risk of predation. *Ecology*. 82(3):740. doi:10.2307/2680193. <http://dx.doi.org/10.2307/2680193>.
- Pepper IL, Gerba CP, Brusseau ML. 2014. *Environmental and Pollution Science*. 2. Aufl. Academic Press.
- Pérez GL, Torremorell A, Mugni H, Rodríguez P, Solange Vera M, do Nascimento M, Allende L, Bustingorry J, Escaray R, Ferraro M, u. a. 2007. Effects of the herbicide Roundup on freshwater microbial communities: a mesocosm study. *Ecol Appl*. 17(8):2310–2322. doi:10.1890/07-0499.1. <http://dx.doi.org/10.1890/07-0499.1>.
- Plastics – the Facts 2020. [Plasticseurope.org](https://plasticseurope.org). [accessed 2025 March 6]. [https://plasticseurope.org/wp-content/uploads/2021/09/Plastics\\_the\\_facts-WEB-2020\\_versionJun21\\_final.pdf](https://plasticseurope.org/wp-content/uploads/2021/09/Plastics_the_facts-WEB-2020_versionJun21_final.pdf).
- [Plasticseurope.org](https://plasticseurope.org). [accessed 2025 March 6]. <https://plasticseurope.org/wp-content/uploads/2021/10/2009-Compelling-facts.pdf>.
- Polhill L, de Bruijn R, Amaral-Zettler L, Praetorius A, van Wezel A. 2022. *Daphnia magna*'s Favorite Snack: Biofouled Plastics. *Environ Toxicol Chem*. 41(8):1977–1981. doi:10.1002/etc.5393. <http://dx.doi.org/10.1002/etc.5393>.
- Ponder WF. 1988. POTAMOPYRGUS ANTIPODARUM—A MOLLUSCAN COLONISER OF EUROPE AND AUSTRALIA. *J Molluscan Stud*. 54(3):271–285. doi:10.1093/mollus/54.3.271. <http://dx.doi.org/10.1093/mollus/54.3.271>.
- Ragusa A, Svelato A, Santacroce C, Catalano P, Notarstefano V, Carnevali O, Papa F, Rongioletti MCA, Baiocco F, Draghi S, u. a. 2021. Plasticenta: First evidence of microplastic in human placenta. *Environ Int*. 146(106274):106274. doi:10.1016/j.envint.2020.106274. <http://dx.doi.org/10.1016/j.envint.2020.106274>.
- Rani-Borges B, Moschini-Carlos V, Pompêo M. 2021. Microplastic and freshwater microalgae: what do we know so far? *Aquat Ecol*. 55(2):363–377. doi:10.1007/s10452-021-09834-9. <http://dx.doi.org/10.1007/s10452-021-09834-9>.

Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. 2009.

Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. 2024.

Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. 2024.

Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. 2015.

Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC. 2017.

Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU. 2017.

Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC (. 2018.

Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products Text with EEA relevance. 2012.

Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004 Text with EEA relevance. 2011a.

Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004 Text with EEA relevance. 2011b.

Rehse S, Kloas W, Zarfl C. 2016. Short-term exposure with high concentrations of pristine microplastic particles leads to immobilisation of *Daphnia magna*. *Chemosphere*. 153:91–99. doi:10.1016/j.chemosphere.2016.02.133. <http://dx.doi.org/10.1016/j.chemosphere.2016.02.133>.

- Rehse S, Kloas W, Zarfl C. 2018. Microplastic reduce short-term effects of environmental contaminants. Part I: Effects of bisphenol A on freshwater zooplankton are lower in presence of polyamide particles. *Int J Environ Res Public Health*. 15(2). doi:10.3390/ijerph15020280. <http://dx.doi.org/10.3390/ijerph15020280>.
- Reichert J, Schellenberg J, Schubert P, Wilke T. 2018. Responses of reef building corals to microplastic exposure. *Environ Pollut*. 237:955–960. doi:10.1016/j.envpol.2017.11.006. <http://dx.doi.org/10.1016/j.envpol.2017.11.006>.
- Rillig MC, Kim SW, Zhu Y-G. 2024. The soil plastisphere. *Nat Rev Microbiol*. 22(2):64–74. doi:10.1038/s41579-023-00967-2. <http://dx.doi.org/10.1038/s41579-023-00967-2>.
- Rodrigues MO, Abrantes N, Gonçalves FJM, Nogueira H, Marques JC, Gonçalves AMM. 2018. Spatial and temporal distribution of microplastic in water and sediments of a freshwater system (Antuã River, Portugal). *Sci Total Environ*. 633:1549–1559. doi:10.1016/j.scitotenv.2018.03.233. <http://dx.doi.org/10.1016/j.scitotenv.2018.03.233>.
- Rodríguez-Torres R, Almeda R, Kristiansen M, Rist S, Winding MS, Nielsen TG. 2020. Ingestion and impact of microplastic on arctic *Calanus* copepods. *Aquat Toxicol*. 228(105631):105631. doi:10.1016/j.aquatox.2020.105631. <http://dx.doi.org/10.1016/j.aquatox.2020.105631>.
- Romero-Blanco A, Remón-Elola A, Alonso Á. 2021. Assessment of the effects of environmental concentrations of microplastic on the aquatic snail *Potamopyrgus antipodarum*. *Water Air Soil Pollut*. 232(10). doi:10.1007/s11270-021-05379-7. <http://dx.doi.org/10.1007/s11270-021-05379-7>.
- de Ruijter VN, Hof M, Kotorou P, van Leeuwen J, van den Heuvel-Greve MJ, Roessink I, Koelmans AA. 2023. Microplastic effect tests should use a standard heterogeneous mixture: Multifarious impacts among 16 benthic invertebrate species detected under ecologically relevant test conditions. *Environ Sci Technol*. 57(48):19430–19441. doi:10.1021/acs.est.3c06829. <http://dx.doi.org/10.1021/acs.est.3c06829>.
- Sacha GA, Saffell-Clemmer W, Abram K, Akers MJ. 2010. Practical fundamentals of glass, rubber, and plastic sterile packaging systems. *Pharm Dev Technol*. 15(1):6–34. doi:10.3109/10837450903511178. <http://dx.doi.org/10.3109/10837450903511178>.
- Salomon M, Markus T, Herausgeber. 2019. Handbook on marine environment protection: Science, impacts and sustainable management. Cham ZG, Schweiz: Springer Nature.
- Santos RG, Machovsky-Capuska GE, Andrades R. 2021. Plastic ingestion as an evolutionary trap: Toward a holistic understanding. *Science*. 373(6550):56–60. doi:10.1126/science.abh0945. <http://dx.doi.org/10.1126/science.abh0945>.
- Scherer C, Brennholt N, Reifferscheid G, Wagner M. 2017. Feeding type and development drive the ingestion of microplastic by freshwater invertebrates. *Sci Rep*. 7(1). doi:10.1038/s41598-017-17191-7. <http://dx.doi.org/10.1038/s41598-017-17191-7>.
- Scherer C, Weber A, Lambert S, Wagner M. 2018. Interactions of microplastic with freshwater Biota. In: *The Handbook of Environmental Chemistry*. Cham: Springer International Publishing. S. 153–180.
- Scherer C, Wolf R, Völker J, Stock F, Brennholt N, Reifferscheid G, Wagner M. 2020. Toxicity of microplastic and natural particles in the freshwater dipteran *Chironomus riparius*: Same same but different? *Sci Total Environ*. 711(134604):134604. doi:10.1016/j.scitotenv.2019.134604. <http://dx.doi.org/10.1016/j.scitotenv.2019.134604>.

- Schmitt C, Balaam J, Leonards P, Brix R, Streck G, Tuikka A, Bervoets L, Brack W, van Hattum B, Meire P, u. a. 2010. Characterizing field sediments from three European river basins with special emphasis on endocrine effects - A recommendation for *Potamopyrgus antipodarum* as test organism. *Chemosphere*. 80(1):13–19. doi:10.1016/j.chemosphere.2010.03.050. <http://dx.doi.org/10.1016/j.chemosphere.2010.03.050>.
- Schwarz AE, Lensen SMC, Langeveld E, Parker LA, Urbanus JH. 2023. Plastics in the global environment assessed through material flow analysis, degradation and environmental transportation. *Sci Total Environ*. 875(162644):162644. doi:10.1016/j.scitotenv.2023.162644. <http://dx.doi.org/10.1016/j.scitotenv.2023.162644>.
- Setälä O, Fleming-Lehtinen V, Lehtiniemi M. 2014. Ingestion and transfer of microplastic in the planktonic food web. *Environ Pollut*. 185:77–83. doi:10.1016/j.envpol.2013.10.013. <http://dx.doi.org/10.1016/j.envpol.2013.10.013>.
- Sharma KV, Sarvalingam BK, Marigoudar SR. 2021. A review of mesocosm experiments on heavy metals in marine environment and related issues of emerging concerns. *Environ Sci Pollut Res Int*. 28(2):1304–1316. doi:10.1007/s11356-020-11121-3. <http://dx.doi.org/10.1007/s11356-020-11121-3>.
- Simakova A, Varenitsina A, Babkina I, Andreeva Y, Bagirov R, Yartsev V, Frank Y. 2022. Ontogenetic transfer of microplastic in bloodsucking mosquitoes *Aedes aegypti* L. (Diptera: Culicidae) is a potential pathway for particle distribution in the environment. *Water (Basel)*. 14(12):1852. doi:10.3390/w14121852. <http://dx.doi.org/10.3390/w14121852>.
- Spivak AC, Vanni MJ, Mette EM. 2011. Moving on up: can results from simple aquatic mesocosm experiments be applied across broad spatial scales?: Scaling pelagic mesocosm experiments. *Freshw Biol*. 56(2):279–291. doi:10.1111/j.1365-2427.2010.02495.x. <http://dx.doi.org/10.1111/j.1365-2427.2010.02495.x>.
- Stanković J, Milošević D, Jovanović B, Savić-Zdravković D, Petrović A, Raković M, Stanković N, Stojković Piperac M. 2022. In situ effects of a microplastic mixture on the community structure of benthic macroinvertebrates in a freshwater pond. *Environ Toxicol Chem*. 41(4):888–895. doi:10.1002/etc.5119. <http://dx.doi.org/10.1002/etc.5119>.
- Stanković J, Milošević D, Savić-Zdravković D, Yalçın G, Yıldız D, Beklioğlu M, Jovanović B. 2020. Exposure to a microplastic mixture is altering the life traits and is causing deformities in the non-biting midge *Chironomus riparius* Meigen (1804). *Environ Pollut*. 262(114248):114248. doi:10.1016/j.envpol.2020.114248. <http://dx.doi.org/10.1016/j.envpol.2020.114248>.
- Stride B, Abolfathi S, Bending GD, Pearson J. 2024. Quantifying microplastic dispersion due to density effects. *J Hazard Mater*. 466(133440):133440. doi:10.1016/j.jhazmat.2024.133440. <http://dx.doi.org/10.1016/j.jhazmat.2024.133440>.
- Šupina J, Bojková J, Boukal DS. 2016. Influence of food availability, predation risk and initial body size on growth and maturation of *Cloeon dipterum* (Ephemeroptera: Baetidae). *Zoosymposia*. 11:53–64. doi:10.11646/zoosymposia.11.1.9. <http://dx.doi.org/10.11646/zoosymposia.11.1.9>.
- Syberg K, Nielsen MB, Westergaard Clausen LP, van Calster G, van Wezel A, Rochman C, Koelmans AA, Cronin R, Pahl S, Hansen SF. 2021. Regulation of plastic from a circular economy perspective. *Curr Opin Green Sustain Chem*. 29(100462):100462. doi:10.1016/j.cogsc.2021.100462. <http://dx.doi.org/10.1016/j.cogsc.2021.100462>.

- Tang Y, Liu Y, Chen Y, Zhang W, Zhao J, He S, Yang C, Zhang T, Tang C, Zhang C, u. a. 2021. A review: Research progress on microplastic pollutants in aquatic environments. *Sci Total Environ.* 766(142572):142572. doi:10.1016/j.scitotenv.2020.142572. <http://dx.doi.org/10.1016/j.scitotenv.2020.142572>.
- Teymourian T, Teymoorian T, Kowsari E, Ramakrishna S. 2021. Challenges, strategies, and recommendations for the huge surge in plastic and medical waste during the global COVID-19 pandemic with circular economy approach. *Mater Circ Econ.* 3(1). doi:10.1007/s42824-021-00020-8. <http://dx.doi.org/10.1007/s42824-021-00020-8>.
- Townsend CR, Begon M, Harper JL. 2009. *Ökologie*. 2. Aufl. Hoffmeister TS, Steidle J, Thomas F, Übersetzer. Berlin: Springer.
- Trestrail C, Walpitagama M, Miranda A, Nugegoda D, Shimeta J. 2021. Microplastic alter digestive enzyme activities in the marine bivalve, *Mytilus galloprovincialis*. *Sci Total Environ.* 779(146418):146418. doi:10.1016/j.scitotenv.2021.146418. <http://dx.doi.org/10.1016/j.scitotenv.2021.146418>.
- Valentine, K., Cross, R., Cox, R., Woodmancy, G., & Boxall, A. B. A. (2022). Caddisfly larvae are a driver of plastic litter breakdown and microplastic formation in freshwater environments. *Environmental Toxicology and Chemistry*, 41(12), 3058–3069. <https://doi.org/10.1002/etc.5496>
- Vallenduuk HJ, Moller HK. 2007. *Chironomidae Larvae, Vol. 1: Tanypodinae: General Ecology and Tanypodinae*. Knnv Publishing. <http://dx.doi.org/10.1163/9789004278035>.
- Van Cauwenberghe L, Claessens M, Vandegehuchte MB, Mees J, Janssen CR. 2013. Assessment of marine debris on the Belgian Continental Shelf. *Mar Pollut Bull.* 73(1):161–169. doi:10.1016/j.marpolbul.2013.05.026. <http://dx.doi.org/10.1016/j.marpolbul.2013.05.026>.
- Van den Brink PJ, Hattink J, Bransen F, Donke V, Brock T. 2000. Impact of the fungicide carbenda-zim in freshwater microcosms. II. Zooplankton, primary producers and final conclusions. *Aquat Toxicol.* 48:251–264.
- Van den Brink PJ, Tarazona JV, Solomon KR, Knacker T, Van den Brink NW, Brock TCM, Hoogland JP. 2005. The use of terrestrial and aquatic microcosms and mesocosms for the ecological risk assessment of veterinary medicinal products. *Environ Toxicol Chem.* 24(4):820–829. doi:10.1897/04-268r.1. <http://dx.doi.org/10.1897/04-268r.1>.
- Van Wijngaarden RPA, Brock TCM, Van den Brink PJ. 2005. Threshold levels for effects of insecticides in freshwater ecosystems: a review. *Ecotoxicology.* 14(3):355–380. doi:10.1007/s10646-004-6371-x. <http://dx.doi.org/10.1007/s10646-004-6371-x>.
- Voigt M, Koste W. 1978. *Rotatoria: Die Radertiere Mitteleuropas : Überordnung Monogononta : ein Bestimmungswerk*, 2.th ed. Berlin: Borntraeger.
- Vroom RJE, Koelmans AA, Besseling E, Halsband C. 2017. Aging of microplastic promotes their ingestion by marine zooplankton. *Environ Pollut.* 231:987–996. doi:10.1016/j.envpol.2017.08.088. <http://dx.doi.org/10.1016/j.envpol.2017.08.088>.
- Waldschläger K. 2019. *Mikroplastik in der aquatischen Umwelt: Quellen, Senken und Transportpfade*. 1. Aufl. Wiesbaden: Springer Vieweg.

- Waldschläger K, Schüttrumpf H. 2019. Effects of particle properties on the settling and rise velocities of microplastic in freshwater under laboratory conditions. *Environ Sci Technol*. 53(4):1958–1966. doi:10.1021/acs.est.8b06794. <http://dx.doi.org/10.1021/acs.est.8b06794>.
- Wang Y, Zhang D, Zhang M, Mu J, Ding G, Mao Z, Cao Y, Jin F, Cong Y, Wang L, u. a. 2019. Effects of ingested polystyrene microplastic on brine shrimp, *Artemia parthenogenetica*. *Environ Pollut*. 244:715–722. doi:10.1016/j.envpol.2018.10.024. <http://dx.doi.org/10.1016/j.envpol.2018.10.024>.
- Weber A, Scherer C, Brennholt N, Reifferscheid G, Wagner M. 2018. PET microplastic do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate *Gammarus pulex*. *Environ Pollut*. 234:181–189. doi:10.1016/j.envpol.2017.11.014. <http://dx.doi.org/10.1016/j.envpol.2017.11.014>.
- Weis JS, Palmquist KH. 2021. Reality check: Experimental studies on microplastic lack realism. *Appl Sci (Basel)*. 11(18):8529. doi:10.3390/app11188529. <http://dx.doi.org/10.3390/app11188529>.
- Weiss L, Ludwig W, Heussner S, Canals M, Ghiglione J-F, Estournel C, Constant M, Kerhervé P. 2021. The missing ocean plastic sink: Gone with the rivers. *Science*. 373(6550):107–111. doi:10.1126/science.abe0290. <http://dx.doi.org/10.1126/science.abe0290>.
- Welden NAC, Cowie PR. 2016. Long-term microplastic retention causes reduced body condition in the langoustine, *Nephrops norvegicus*. *Environ Pollut*. 218:895–900. doi:10.1016/j.envpol.2016.08.020. <http://dx.doi.org/10.1016/j.envpol.2016.08.020>.
- Weltweite Kunststoffproduktion bis 2023. Statista. [accessed 2025 March 13]. <https://de.statista.com/statistik/daten/studie/167099/umfrage/weltproduktion-von-kunststoff-seit-1950/>.
- Wendt-Potthoff, K., Drago, C., Ebke, P., Fueser, H., Gabel, F., Hägerbäumer, A., Höss, S., Jongsma, R., Kruckenfellner, L., Leiser, R., Michler-Kozma, D., Philipp, B., Rauchschalbe, M.-T., Traunspurger, W., Völkner, C., Weithoff, G., & Wieprecht, M. (2022). *Mikroplastik in Talsperren und Staubereichen: Sedimentation, Verbreitung, Wirkung (MikroPlaTaS)*.
- Wu C, Zhang K, Xiong X. 2018. Microplastic pollution in inland waters focusing on Asia. In: *The Handbook of Environmental Chemistry*. Cham: Springer International Publishing. S. 85–99.
- Wu X, Liu P, Gong Z, Wang H, Huang H, Shi Y, Zhao X, Gao S. 2021. Humic acid and fulvic acid hinder long-term weathering of microplastic in lake water. *Environ Sci Technol*. 55(23):15810–15820. doi:10.1021/acs.est.1c04501. <http://dx.doi.org/10.1021/acs.est.1c04501>.
- Wu Y, Guo P, Zhang X, Zhang Y, Xie S, Deng J. 2019. Effect of microplastic exposure on the photosynthesis system of freshwater algae. *J Hazard Mater*. 374:219–227. doi:10.1016/j.jhazmat.2019.04.039. <http://dx.doi.org/10.1016/j.jhazmat.2019.04.039>.
- Yan J, Yao S, Zhang T, Ye Z, Weng J, Hu X, Zhang K. 2022. Microplastic and their impacts on organisms and trophic chains. *Water (Basel)*. 14(24):4069. doi:10.3390/w14244069. <http://dx.doi.org/10.3390/w14244069>.
- Yang CZ, Yaniger SI, Jordan VC, Klein DJ, Bittner GD. 2011. Most plastic products release estrogenic chemicals: a potential health problem that can be solved. *Environ Health Perspect*. 119(7):989–996. doi:10.1289/ehp.1003220. <http://dx.doi.org/10.1289/ehp.1003220>.

- Yıldız D, Yalçın G, Jovanović B, Boukal DS, Vebrová L, Riha D, Stanković J, Savić-Zdraković D, Metin M, Akyürek YN, u. a. 2022. Effects of a microplastic mixture differ across trophic levels and taxa in a freshwater food web: In situ mesocosm experiment. *Sci Total Environ.* 836(155407):155407. doi:10.1016/j.scitotenv.2022.155407. <http://dx.doi.org/10.1016/j.scitotenv.2022.155407>.
- Zalasiewicz J, Williams M, Waters CN, Barnosky AD, Palmesino J, Rönnskog A-S, Edgeworth M, Neal C, Cearreta A, Ellis EC, u. a. 2017. Scale and diversity of the physical technosphere: A geological perspective. *Anthr Rev.* 4(1):9–22. doi:10.1177/2053019616677743. <http://dx.doi.org/10.1177/2053019616677743>.
- Zangmeister CD, Radney JG, Benkstein KD, Kalanyan B. 2022. Common single-use consumer plastic products release trillions of sub-100 nm nanoparticles per liter into water during normal use. *Environ Sci Technol.* 56(9):5448–5455. doi:10.1021/acs.est.1c06768. <http://dx.doi.org/10.1021/acs.est.1c06768>.
- Zeenat, Elahi A, Bukhari DA, Shamim S, Rehman A. 2021. Plastics degradation by microbes: A sustainable approach. *J King Saud Univ Sci.* 33(6):101538. doi:10.1016/j.jksus.2021.101538. <http://dx.doi.org/10.1016/j.jksus.2021.101538>.
- Zepernick BN, Gann ER, Martin RM, Pound HL, Krausfeldt LE, Chaffin JD, Wilhelm SW. 2021. Elevated pH conditions associated with *Microcystis* spp. Blooms decrease viability of the cultured diatom *Fragilaria crotonensis* and natural diatoms in Lake Erie. *Front Microbiol.* 12:598736. doi:10.3389/fmicb.2021.598736. <http://dx.doi.org/10.3389/fmicb.2021.598736>.
- Zhang Q, Gong K, Shao X, Liang W, Zhang W, Peng C. 2023. Effect of polyethylene, polyamide, and polylactic acid microplastic on Cr accumulation and toxicity to cucumber (*Cucumis sativus* L.) in hydroponics. *J Hazard Mater.* 450(131022):131022. doi:10.1016/j.jhazmat.2023.131022. <http://dx.doi.org/10.1016/j.jhazmat.2023.131022>.
- Zhang R-F, Guo Y-J, Lai Y-W, Zhao T-T, Li G-L, Yan Z-G, Wang Y-J, Rillig MC. 2024. Microplastic promote the invasiveness of invasive alien species under fluctuating water regime. *J Appl Ecol.* 61(9):2281–2293. doi:10.1111/1365-2664.14726. <http://dx.doi.org/10.1111/1365-2664.14726>.
- Zhang X, Chen J, Li J. 2020. The removal of microplastic in the wastewater treatment process and their potential impact on anaerobic digestion due to pollutants association. *Chemosphere.* 251(126360):126360. doi:10.1016/j.chemosphere.2020.126360. <http://dx.doi.org/10.1016/j.chemosphere.2020.126360>.
- Zhang X, Shen Z, Wu J, Su M, Zheng L, Xie M, Hong H, Huang X, Lu H. 2024. High salinity restrains microplastic transport and increases the risk of pollution in coastal wetlands. *Water Res.* 267(122463):122463. doi:10.1016/j.watres.2024.122463. <http://dx.doi.org/10.1016/j.watres.2024.122463>.

## **VIII. Appendix**

Appendix A Material Data Sheets Silica and Polyamide

Appendix B Application logs

Appendix C Weather data of DWD-Station 7410 Neu-Ulrichstein

Appendix D Water level

Appendix E Raw data tables

Appendix F Pictures

Appendix G EMT<sub>x</sub> validation graph

Appendix H Outer Pond chemical analysis

Appendix I Outer Pond logbook

Appendix J DOC/TOC



## Spezifikation

### Siliciumdioxid (Rieselhilfe) (Lebensmittelqualität)

Artikel-Nr.:	S100252
Summenformel:	SiO <sub>2</sub>
Molare Masse:	60,10g/mol
CAS:	7631-86-9; 112926-00-8
EG-Nr.:	231-545-4
Lagerklasse:	10 - 13

Prüfmerkmal	Wert
Trocknungsverlust (2 h, 105 °C)	max. 7,0%
pH (5% in Wasser)	5,5-7,5
Siebrückstand (45 µm, Brause)	max. 1,5%
BET-Oberfläche	170-210 m <sup>2</sup> /g
DOA-Absorption (bezogen auf Originalsubstanz)	225-255 ml/100g
Teilchengröße (d50, Coulter LS 230)	11,5-15,5 µm
Glühverlust (2h, 1000 °C)	max. 8,5%
Gehalt (SiO <sub>2</sub> )	min. 97%
Natriumsulfat	max. 5,0%
Arsen (As)	max. 3 ppm
Blei (Pb)	max. 5 ppm
Quecksilber (Hg)	max. 1 ppm
Cadmium (Cd)	max. 2 ppm
Dioxid (WHO-PCDD/F-TEQ)	max. 0,75 ng/kg
Dioxin / dioxinähnliche PCBs)	max. 1,50 ng/kg
Nicht dioxinähnliche PCBs	max. 10 µg/kg

Wir garantieren die Einhaltung der spezifizierten Merkmale. Diese Spezifikation stellt keine Zusicherung von Eigenschaften für einen bestimmten Einsatzzweck dar. Wir weisen ausdrücklich darauf hin, dass der Empfänger verpflichtet ist, die Ware vor Verwendung zu prüfen.

S3 Handel und Dienstleistungen UG (haftungsbeschränkt)  
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 32549 Bad Oeynhausen  
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 Fax: +49 (0) 57 31 – 245 11 799  
 Web: www.s3-chemicals.de

Produktinformation  
 Referenzdatum: 30.03.2023



Seite: 1/6

## Sicherheitsdatenblatt gemäß 1907/2006/EG, Artikel 31

Druckdatum: 29.05.2019

überarbeitet am: 29.05.2019

### 1 Bezeichnung des Stoffs beziehungsweise des Gemischs und des Unternehmens

- **Produktidentifikator**
- **Handelsname:** Siliciumdioxid (amorph, chemisch)
- **Artikelnummer:** S100252
- **CAS-Nummer:** 7631-86-9
- **EG-Nummer:** 231-545-4
- **Registrierungsnummer** 01-2119379499-16-0000
- **Relevante identifizierte Verwendungen des Stoffs oder Gemischs und Verwendungen, von denen abgeraten wird**  
Keine weiteren relevanten Informationen verfügbar.
- **Verwendung des Stoffes / des Gemisches**  
Verwendung in Nahrungsergänzungsmitteln  
Herstellung von Stoffen  
Laborchemikalie  
Chemikalie für verschiedene Anwendungen  
Chemische Analytik  
Lebensmittelzusatz  
Rieselhilfe
- **Einzelheiten zum Lieferanten, der das Sicherheitsdatenblatt bereitstellt**
- **Hersteller/Lieferant:**  
S3 Handel und Dienstleistungen UG  
Klinkerwerkstraße 9  
32549 Bad Oeynhausen  
Deutschland
- **Auskunftgebender Bereich:**  
Medizinische Notfallauskunft bei Vergiftungen:  
Giftinformationszentrum Mainz - 24h - Tel.: +49 (0) 6131 19240  
(Beratung in deutscher oder englischer Sprache)
- **Notrufnummer:** +49 (0) 6131 19240

### 2 Mögliche Gefahren

- **Einstufung des Stoffs oder Gemischs**
- **Einstufung gemäß Verordnung (EG) Nr. 1272/2008**  
Der Stoff ist gemäß CLP-Verordnung nicht eingestuft.
- **Kennzeichnungselemente**
- **Kennzeichnung gemäß Verordnung (EG) Nr. 1272/2008 entfällt**
- **Gefahrenpiktogramme entfällt**
- **Signalwort entfällt**
- **Gefahrenhinweise entfällt**
- **Sonstige Gefahren**
- **Ergebnisse der PBT- und vPvB-Beurteilung**
- **PBT:** Nicht anwendbar.
- **vPvB:** Nicht anwendbar.

DE

(Fortsetzung auf Seite 2)

## Sicherheitsdatenblatt gemäß 1907/2006/EG, Artikel 31

Druckdatum: 29.05.2019

überarbeitet am: 29.05.2019

**Handelsname: Siliciumdioxid (amorph, chemisch)**

(Fortsetzung von Seite 1)

### 3 Zusammensetzung/Angaben zu Bestandteilen

- **Chemische Charakterisierung: Stoffe**
- **CAS-Nr. Bezeichnung**  
7631-86-9 Siliciumdioxid
- **Identifikationsnummer(n)**
- **EG-Nummer: 231-545-4**

### 4 Erste-Hilfe-Maßnahmen

- **Beschreibung der Erste-Hilfe-Maßnahmen**
- **Allgemeine Hinweise:** Keine besonderen Maßnahmen erforderlich.
- **Nach Einatmen:** Frischluftzufuhr, bei Beschwerden Arzt aufsuchen.
- **Nach Hautkontakt:** Im allgemeinen ist das Produkt nicht hautreizend.
- **Nach Augenkontakt:**  
Augen bei geöffnetem Lidspalt mehrere Minuten mit fließendem Wasser spülen.
- **Nach Verschlucken:** Bei anhaltenden Beschwerden Arzt konsultieren.
- **Hinweise für den Arzt:**
- **Wichtigste akute und verzögert auftretende Symptome und Wirkungen**  
Keine weiteren relevanten Informationen verfügbar.
- **Hinweise auf ärztliche Soforthilfe oder Spezialbehandlung**  
Keine weiteren relevanten Informationen verfügbar.

### 5 Maßnahmen zur Brandbekämpfung

- **Löschmittel**
- **Geeignete Löschmittel:** Feuerlöschmaßnahmen auf die Umgebung abstimmen.
- **Besondere vom Stoff oder Gemisch ausgehende Gefahren**  
Keine weiteren relevanten Informationen verfügbar.
- **Hinweise für die Brandbekämpfung**
- **Besondere Schutzausrüstung:** Keine besonderen Maßnahmen erforderlich.

### 6 Maßnahmen bei unbeabsichtigter Freisetzung

- **Personenbezogene Vorsichtsmaßnahmen, Schutzausrüstungen und in Notfällen anzuwendende Verfahren**  
Nicht erforderlich.
- **Umweltschutzmaßnahmen:** Keine besonderen Maßnahmen erforderlich.
- **Methoden und Material für Rückhaltung und Reinigung:** Mechanisch aufnehmen.
- **Verweis auf andere Abschnitte**  
Informationen zur sicheren Handhabung siehe Abschnitt 7.  
Informationen zur persönlichen Schutzausrüstung siehe Abschnitt 8.  
Informationen zur Entsorgung siehe Abschnitt 13.

### 7 Handhabung und Lagerung

- **Handhabung:**
- **Schutzmaßnahmen zur sicheren Handhabung** Keine besonderen Maßnahmen erforderlich.
- **Hinweise zum Brand- und Explosionsschutz:** Keine besonderen Maßnahmen erforderlich.
- **Bedingungen zur sicheren Lagerung unter Berücksichtigung von Unverträglichkeiten**
- **Lagerung:**
- **Anforderung an Lagerräume und Behälter:** Keine besonderen Anforderungen.
- **Zusammenlagerungshinweise:** Nicht erforderlich.
- **Weitere Angaben zu den Lagerbedingungen:** Keine.
- **Lagerklasse:** 10-13

(Fortsetzung auf Seite 3)

## Sicherheitsdatenblatt gemäß 1907/2006/EG, Artikel 31

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(Fortsetzung von Seite 2)

- **Klassifizierung nach Betriebssicherheitsverordnung (BetrSichV):** -
- **Spezifische Endanwendungen** Keine weiteren relevanten Informationen verfügbar.

### 8 Begrenzung und Überwachung der Exposition/Persönliche Schutzausrüstungen

- **Zusätzliche Hinweise zur Gestaltung technischer Anlagen:**  
Keine weiteren Angaben, siehe Abschnitt 7.

- **Zu überwachende Parameter**

- **Bestandteile mit arbeitsplatzbezogenen, zu überwachenden Grenzwerten:**

**7631-86-9 Siliciumdioxid**

AGW Langzeitwert: 4 E mg/m<sup>3</sup>  
DFG, 2, Y

- **Zusätzliche Hinweise:** Als Grundlage dienen die bei der Erstellung gültigen Listen.
- **Begrenzung und Überwachung der Exposition**
- **Persönliche Schutzausrüstung:**
- **Allgemeine Schutz- und Hygienemaßnahmen:**  
Die üblichen Vorsichtsmaßnahmen beim Umgang mit Chemikalien sind zu beachten.
- **Atemschutz:** Nicht erforderlich.
- **Handschutz:**  
Das Handschuhmaterial muss undurchlässig und beständig gegen das Produkt / den Stoff / die Zubereitung sein.  
Aufgrund fehlender Tests kann keine Empfehlung zum Handschuhmaterial für das Produkt / die Zubereitung / das Chemikaliengemisch abgegeben werden.  
Auswahl des Handschuhmaterials unter Beachtung der Durchbruchzeiten, Permeationsraten und der Degradation.
- **Handschuhmaterial**  
Die Auswahl eines geeigneten Handschuhs ist nicht nur vom Material, sondern auch von weiteren Qualitätsmerkmalen abhängig und von Hersteller zu Hersteller unterschiedlich.
- **Durchdringungszeit des Handschuhmaterials**  
Die genaue Durchbruchzeit ist beim Schutzhandschuhhersteller zu erfahren und einzuhalten.
- **Augenschutz:** Nicht erforderlich.

### 9 Physikalische und chemische Eigenschaften

- **Angaben zu den grundlegenden physikalischen und chemischen Eigenschaften**

- **Allgemeine Angaben**

- **Aussehen:**

<b>Form:</b>	Pulver
<b>Farbe:</b>	Weiß
<b>Geruch:</b>	Geruchlos
<b>Geruchsschwelle:</b>	Nicht bestimmt.

- **pH-Wert:** 6

- **Zustandsänderung**

<b>Schmelzpunkt/Gefrierpunkt:</b>	1.700 °C
<b>Siedebeginn und Siedebereich:</b>	2.230 °C

- **Flammpunkt:** Nicht anwendbar.

- **Entzündbarkeit (fest, gasförmig):** Der Stoff ist nicht entzündlich.

- **Zündtemperatur:** >370 °C

- **Zersetzungstemperatur:** Nicht bestimmt.

(Fortsetzung auf Seite 4)

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## Sicherheitsdatenblatt gemäß 1907/2006/EG, Artikel 31

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**Handelsname: Siliciumdioxid (amorph, chemisch)**

(Fortsetzung von Seite 3)

· <b>Selbstentzündungstemperatur:</b>	Nicht bestimmt.
· <b>Explosive Eigenschaften:</b>	Das Produkt ist nicht explosionsgefährlich.
· <b>Explosionsgrenzen:</b>	
<b>Untere:</b>	Nicht bestimmt.
<b>Obere:</b>	Nicht bestimmt.
· <b>Dampfdruck:</b>	Nicht anwendbar.
· <b>Dichte bei 20 °C:</b>	1,9 g/cm <sup>3</sup>
· <b>Relative Dichte</b>	Nicht bestimmt.
· <b>Dampfdichte</b>	Nicht anwendbar.
· <b>Verdampfungsgeschwindigkeit</b>	Nicht anwendbar.
· <b>Löslichkeit in / Mischbarkeit mit Wasser bei 20 °C:</b>	0,001 g/l
· <b>Verteilungskoeffizient: n-Octanol/Wasser:</b>	Nicht bestimmt.
· <b>Viskosität:</b>	
<b>Dynamisch:</b>	Nicht anwendbar.
<b>Kinematisch:</b>	Nicht anwendbar.
· <b>Sonstige Angaben</b>	Keine weiteren relevanten Informationen verfügbar.

### 10 Stabilität und Reaktivität

- **Reaktivität** Keine weiteren relevanten Informationen verfügbar.
- **Chemische Stabilität**
- **Thermische Zersetzung / zu vermeidende Bedingungen:**  
Keine Zersetzung bei bestimmungsgemäßer Verwendung.
- **Möglichkeit gefährlicher Reaktionen** Keine gefährlichen Reaktionen bekannt.
- **Zu vermeidende Bedingungen** Keine weiteren relevanten Informationen verfügbar.
- **Unverträgliche Materialien:** Keine weiteren relevanten Informationen verfügbar.
- **Gefährliche Zersetzungsprodukte:** Keine gefährlichen Zersetzungsprodukte bekannt.

### 11 Toxikologische Angaben

- **Angaben zu toxikologischen Wirkungen**
- **Akute Toxizität** Aufgrund der verfügbaren Daten sind die Einstufungskriterien nicht erfüllt.

**Einstufungsrelevante LD/LC50-Werte:**
**7631-86-9 Siliciumdioxid**

Oral LD50 10.000 mg/kg (rat)

- **Primäre Reizwirkung:**
- **Ätz-/Reizwirkung auf die Haut**  
Aufgrund der verfügbaren Daten sind die Einstufungskriterien nicht erfüllt.
- **Schwere Augenschädigung/-reizung**  
Aufgrund der verfügbaren Daten sind die Einstufungskriterien nicht erfüllt.
- **Sensibilisierung der Atemwege/Haut**  
Aufgrund der verfügbaren Daten sind die Einstufungskriterien nicht erfüllt.
- **CMR-Wirkungen (krebserzeugende, erbgutverändernde und fortpflanzungsgefährdende Wirkung)**
- **Keimzell-Mutagenität** Aufgrund der verfügbaren Daten sind die Einstufungskriterien nicht erfüllt.
- **Karzinogenität** Aufgrund der verfügbaren Daten sind die Einstufungskriterien nicht erfüllt.
- **Reproduktionstoxizität** Aufgrund der verfügbaren Daten sind die Einstufungskriterien nicht erfüllt.
- **Spezifische Zielorgan-Toxizität bei einmaliger Exposition**  
Aufgrund der verfügbaren Daten sind die Einstufungskriterien nicht erfüllt.

(Fortsetzung auf Seite 5)

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## Sicherheitsdatenblatt gemäß 1907/2006/EG, Artikel 31

Druckdatum: 29.05.2019

überarbeitet am: 29.05.2019

**Handelsname: Siliciumdioxid (amorph, chemisch)**

(Fortsetzung von Seite 4)

- **Spezifische Zielorgan-Toxizität bei wiederholter Exposition**  
Aufgrund der verfügbaren Daten sind die Einstufungskriterien nicht erfüllt.
- **Aspirationsgefahr** Aufgrund der verfügbaren Daten sind die Einstufungskriterien nicht erfüllt.

### 12 Umweltbezogene Angaben

- **Toxizität**
- **Aquatische Toxizität:** Keine weiteren relevanten Informationen verfügbar.
- **Persistenz und Abbaubarkeit** Keine weiteren relevanten Informationen verfügbar.
- **Verhalten in Umweltkompartimenten:**
- **Bioakkumulationspotenzial** Keine weiteren relevanten Informationen verfügbar.
- **Mobilität im Boden** Keine weiteren relevanten Informationen verfügbar.
- **Weitere ökologische Hinweise:**
- **Allgemeine Hinweise:** Nicht wassergefährdend.
- **Ergebnisse der PBT- und vPvB-Beurteilung**
- **PBT:** Nicht anwendbar.
- **vPvB:** Nicht anwendbar.
- **Andere schädliche Wirkungen** Keine weiteren relevanten Informationen verfügbar.

### 13 Hinweise zur Entsorgung

- **Verfahren der Abfallbehandlung**
- **Empfehlung:** Kleinere Mengen können gemeinsam mit Hausmüll deponiert werden.
- **Ungereinigte Verpackungen:**
- **Empfehlung:** Entsorgung gemäß den behördlichen Vorschriften.

### 14 Angaben zum Transport

- |  |                  |
|--|------------------|
| · <b>UN-Nummer</b>   |                  |
| · <b>ADR, IMDG, IATA</b>   | entfällt         |
| · <b>Ordnungsgemäße UN-Versandbezeichnung</b>  |                  |
| · <b>ADR, IMDG, IATA</b>   | entfällt         |
| · <b>Transportgefahrenklassen</b>  |                  |
| · <b>ADR, ADN, IMDG, IATA</b>  |                  |
| · <b>Klasse</b>  | entfällt         |
| · <b>Verpackungsgruppe</b>   |                  |
| · <b>ADR, IMDG, IATA</b>   | entfällt         |
| · <b>Umweltgefahren:</b>   | Nicht anwendbar. |
| · <b>Besondere Vorsichtsmaßnahmen für den Verwender</b>                                    | Nicht anwendbar. |
| · <b>Massengutbeförderung gemäß Anhang II des MARPOL-Übereinkommens und gemäß IBC-Code</b> | Nicht anwendbar. |
| · <b>UN "Model Regulation":</b>  | entfällt         |

DE  
(Fortsetzung auf Seite 6)

**Sicherheitsdatenblatt  
gemäß 1907/2006/EG, Artikel 31**

Druckdatum: 29.05.2019

überarbeitet am: 29.05.2019

**Handelsname: Siliciumdioxid (amorph, chemisch)**

(Fortsetzung von Seite 5)

**15 Rechtsvorschriften**

- **Vorschriften zu Sicherheit, Gesundheits- und Umweltschutz/spezifische Rechtsvorschriften für den Stoff oder das Gemisch**
- **Richtlinie 2012/18/EU**
- **Namentlich aufgeführte gefährliche Stoffe - ANHANG I** Der Stoff ist nicht enthalten.
- **Nationale Vorschriften:**
- **Wassergefährdungsklasse:** Im allgemeinen nicht wassergefährdend.
- **Stoffsicherheitsbeurteilung:** Eine Stoffsicherheitsbeurteilung wurde nicht durchgeführt.

**16 Sonstige Angaben**

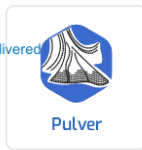
Die Angaben stützen sich auf den heutigen Stand unserer Kenntnisse, sie stellen jedoch keine Zusicherung von Produkteigenschaften dar und begründen kein vertragliches Rechtsverhältnis.

- **Datenblatt ausstellender Bereich:** Abteilung Produktsicherheit
- **Ansprechpartner:** Frank Schütte
- **Abkürzungen und Akronyme:**
  - ADR: Accord européen sur le transport des marchandises dangereuses par Route (European Agreement concerning the International Carriage of Dangerous Goods by Road)
  - IMDG: International Maritime Code for Dangerous Goods
  - IATA: International Air Transport Association
  - GHS: Globally Harmonised System of Classification and Labelling of Chemicals
  - EINECS: European Inventory of Existing Commercial Chemical Substances
  - CAS: Chemical Abstracts Service (division of the American Chemical Society)
  - LC50: Lethal concentration, 50 percent
  - LD50: Lethal dose, 50 percent
  - PBT: Persistent, Bioaccumulative and Toxic
  - vPvB: very Persistent and very Bioaccumulative
- **\* Daten gegenüber der Vorversion geändert**

DE

07.03.24, 11:24

Nylon 6 - Pulver 15-20 µm | Goodfellow



## Nylon 6 - Pulver 15-20 µm PA 6

Produkt-Code: **AM30-PD-000110**  
 Mindestpartikelgröße: **5µm**  
 Mittlere Teilchengröße: **15-20**  
 Maximale Partikelgröße: **50µm**  
 Produktform: **Spheroidal**  
 Particle Size D90:  
 Gewicht: **10g - 10000g**

## Polyamid - Nylon 6

A polyamide is a polymer with repeating units linked by amide bonds. Polyamides occur both naturally and artificially. Examples of naturally occurring polyamides are proteins, such as wool and silk.

## Materialeigenschaften für Polyamid - Nylon 6

### Chemische Beständigkeit

Säuren - konzentriert: **Schlecht**  
 Säuren - verdünnt: **Schlecht**  
 Alkohole: **Good**  
 Laugen: **Good-Fair**

<https://www.goodfellow.com/de/p/am30-pd-000110/nylon-6-powder>

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Nylon 6 - Pulver 15-20 µm | Goodfellow

Aromatische Kohlenwasserstoffe: **Good**  
 Fette und Öle: **Good**  
 Halogenhydrocarbons: **Good-Poor**  
 Halogene: **Schlecht**  
 Ketone: **Good**

### Elektrische Eigenschaften

Auflösungsfaktor bei 1 kHz: **4**  
 dielektrische Widerstandsfähigkeit ( kV mm<sup>-1</sup>): **25.0**  
 Auflösungsfaktor bei 1 kHz: **0.2000**  
 Spezifischer Oberflächenwiderstand ( Ohm/sq ): **5x10<sup>10</sup>**  
 spezifischer Volumenwiderstand ( Ohmcm ): **5x10<sup>12</sup>**

### Mechanische Eigenschaften

Abschleifwiderstand - ASTM D1044 ( mg/1000 cycles ): **5**  
 Coefficient of friction: **0.20 - 0.30**  
 Härte - Rockwell: **M82**  
 Kerbschlagzähigkeit nach Izod ( J m<sup>-1</sup>): **30.0 -- 250.0**  
 Poissonsche Konstante: **0.390**  
 Tensile modulus ( GPa ): **2.60 - 3.00**  
 Tensile strength ( MPa ): **78.00**

### Physikalische Eigenschaften

Density ( g cm<sup>-3</sup>): **1.130**  
 Entzündbarkeit: **HB**  
 Mindestsauerstoffgehalt ( % ): **25**

<https://www.goodfellow.com/de/p/am30-pd-000110/nylon-6-powder>

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Nylon 6 - Pulver 15-20 µm | Goodfellow

Strahlungswiderstand: **Fair**Brechungsindex: **1.530**Widerstand gegen ultraviolettes Licht: **Schlecht**Wasserabsorption - Gleichgewichtsverhältnis ( % ): **> 8.00**Wasserabsorption - über 24 Stunden ( % ): **2.700**

## Thermische Eigenschaften

Linearer Wärmeausdehnungskoeffizient ( $\times 10^{-6} \text{ K}^{-1}$ ): **95.000**Hitzebiegungstemperatur - 0,45 MPa ( C ): **200**Hitzebiegungstemperatur - 1,8 MPa ( C ): **80.0**min. Dauergebrauchstemperatur ( C ): **-40**Spezifische Wärme (  $\text{J K}^{-1} \text{ kg}^{-1}$  ): **1700.0**Wärmeleitfähigkeit (  $\text{W m}^{-1} \text{ K}^{-1}$  ): **0.24 - 0.28 @23°C**max. Dauergebrauchstemperatur ( C ): **80 - 160**

## Daten zu verwandten Produkten

### Form

#### Pulver

Kleine Partikel in einem grob definierten Größenbereich. Die Materialien, die als Vorlegierungen bezeichnet sind, sind keine echten Legierungen; Sie werden durch das Sintern einer Pulvermischung aus Komponentmetallen hergestellt, um per Diffusion eine Legierung zu erzeugen. Der daraus entstehende Kuchen wird so gemahlen und gesiebt, daß man den gewünschten Teilchengroßenbereich bekommt. Wenn nicht anders vermerkt, sind die angegebenen Partikelgrößen nur als Richtwerte zu verstehen. Wir garantieren keine bestimmte Teilchengroßenverteilung zwischen den genannten minimalen und maximalen Größen bzw. keine spezifische Partikelform.


<https://www.goodfellow.com/de/p/am30-pd-000110/nylon-6-powder>

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Nylon 6 - Pulver 15-20 µm | Goodfellow



Mittlere Teilchengroße:

Mittlere Teilchengroße (FSSS):

Mindestpartikelgröße:

## Material

### Polyamid - Nylon 11

A polyamide is a polymer with repeating units linked by amide bonds. Polyamides occur both naturally and artificially. Examples of naturally occurring polyamides are proteins, such as wool and silk.


## Typ

### Polymer

Ein auf Kohlenstoffbasis gefertigtes Material, das aus einer Reihe von kleineren Einheiten (Monomeren) besteht. Die Wahl der Monomere und das endgültige Molekulargewicht (oder Größe) der Polymere bestimmen die mechanischen und physikalischen Eigenschaften des gewonnenen Polymers.


<https://www.goodfellow.com/de/p/am30-pd-000110/nylon-6-powder>

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	<b>Dokumentation Applikation Polyamid PA-6</b>
	<b>Study-No.: 100-22</b>

Datum: 26.05.2020  
Autor: L.Kruckenfellner

### Equipment


1. Uhr
2. Meßzylinder 1000 ml
3. Weißschalen (Kontaminationsschutz)
4. Teichwasser
5. Sonstiges

### Applikationslösungen

Die Applikationslösungen werden bei Mesocosm GmbH am Tag der Applikation hergestellt. Die Abwaage erfolgt im Labor mit der Analysenwaage. Der Ansatz erfolgt zeitnah vor der Applikation auf dem Testgelände in einem Zelt am Teich. Die Anleitung und Dokumentation für den Ansatz wird separat zur Arbeitsanleitung Applikation angelegt.

### Applikation


Die Applikation der Testsubstanz erfolgt mit Hilfe von Scheidetrichtern, welche eine Verlängerung von 30 cm aufweisen. In diese Scheidetrichter werden die Applikationslösungen (1000 mL) aus 2 L Bechergläsern überführt. Anschließend werden die Applikationslösungen, nach Öffnen des Scheidetrichters, in einer Wassertiefe von 15 bis 25 cm unter der Oberfläche gleichmäßig durch zirkulierende Bewegungen eingerührt. Nach der Applikation werden die 2 L-Bechergläser der Applikationslösungen dreimal mit je ca. 500 mL Teichwasser nachgespült. Mit der jeweiligen Spüllösung (500 mL) wird der entsprechende Scheidetrichter ebenso gespült und anschließend wird die Spüllösung im entsprechenden Enclosure appliziert. Jedes Enclosure mit Ausnahme der Kontroll-Enclosures erhält so zusätzlich 3 x 500 mL Spüllösungen. Das Ende der Applikation der 3. Spüllösung wird als Ende der jeweiligen Applikation festgehalten. Die Kontrollen werden nicht appliziert.

	<b>Dokumentation Applikation Polyamid PA-6</b>
	<b>Study-No.: 100-22</b>

**Dokumentation Applikation am 16.04.2020**


 Kontroll-Enclosures MP 1 bis MP 5 umgerührt: ja  nein 

<b>MP6</b>  (0,25*150 mg/l SiO <sub>2</sub> )	Start der Applikation	Uhrzeit:	14:04 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:09 Uhr
	Bemerkungen:		
Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="radio"/>			

	<b>Dokumentation Applikation Polyamid PA-6</b>	
	Study-No.: 100-22	


<b>MP7</b>  (0,25*150 mg/l SiO <sub>2</sub> )	Start der Applikation	Uhrzeit:	14:35 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:41 Uhr
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		

<b>MP8</b>  (0,25*1,50 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:12 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:17 Uhr
	Bemerkungen:		
Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>			

	<b>Dokumentation Applikation Polyamid PA-6</b>	
	Study-No.: 100-22	


<b>MP9</b>  (0,25*1,50 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:44Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:49 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="radio"/>		

<b>MP10</b>  (0,25*1,50 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:16 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:21Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="radio"/>		

	<b>Dokumentation Applikation Polyamid PA-6</b>		
	Study-No.: 100-22		


<b>MP11</b>  (0,25*15,0 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:18 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:23 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		

<b>MP12</b>  (0,25*15,0 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:54 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:59 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		

	<b>Dokumentation Applikation Polyamid PA-6</b>		
	<b>Study-No.: 100-22</b>		


<b>MP13</b>  (0,25*15,0 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:24 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:31 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="radio"/>		

<b>MP14</b>  (0,25*150 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:26 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:33 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="radio"/>		

	<b>Dokumentation Applikation Polyamid PA-6</b>		
	Study-No.: 100-22		

<b>MP15</b>  (0,25*150 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:03 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:09 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="radio"/>		

<b>MP16</b>  (0,25*150 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:34 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:41 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="radio"/>		

	<b>Dokumentation Applikation Polyamid PA-6</b>
	<b>Study-No.: 100-22</b>

Bemerkungen:

Lufttemperatur während des Tages: 18°C


Niederschlag: kein

Bewölkung: leicht

Applikation durchgeführt durch	[Redacted Name]	26.05.2020	[Redacted Signature]
	Name	Datum	Unterschrift
Dokumentation erstellt von	[Redacted Name]	26.05.2020	[Redacted Signature]
	Name	Datum	Unterschrift

26.05.2020  
 Datum

Study Director

	<b>Dokumentation Applikation Polyamid PA-6</b>
	<b>Study-No.: 100-22</b>

Datum: 29.05.2020  
Autor: L.Kruckenfellner

#### **Equipment**


1. Uhr
2. Meßzylinder 1000 ml
3. Weißschalen (Kontaminationsschutz)
4. Teichwasser
5. Sonstiges

#### **Applikationslösungen**

Die Applikationslösungen werden bei Mesocosm GmbH am Tag der Applikation hergestellt. Die Abwaage erfolgt im Labor mit der Analysenwaage. Der Ansatz erfolgt zeitnah vor der Applikation auf dem Testgelände in einem Zelt am Teich. Die Anleitung und Dokumentation für den Ansatz wird separat zur Arbeitsanleitung Applikation angelegt.

#### **Applikation**


Die Applikation der Testsubstanz erfolgt mit Hilfe von Scheidetrichern, welche eine Verlängerung von 30 cm aufweisen. In diese Scheidetricher werden die Applikationslösungen (1000 mL) aus 2 L Bechergläsern überführt. Anschließend werden die Applikationslösungen, nach Öffnen des Scheidetrichers, in einer Wassertiefe von 15 bis 25 cm unter der Oberfläche gleichmäßig durch zirkulierende Bewegungen eingerührt. Nach der Applikation werden die 2 L-Bechergläser der Applikationslösungen dreimal mit je ca. 500 mL Teichwasser nachgespült. Mit der jeweiligen Spüllösung (500 mL) wird der entsprechende Scheidetricher ebenso gespült und anschließend wird die Spüllösung im entsprechenden Enclosure appliziert. Jedes Enclosure mit Ausnahme der Kontroll-Enclosures erhält so zusätzlich 3 x 500 mL Spüllösungen. Das Ende der Applikation der 3. Spüllösung wird als Ende der jeweiligen Applikation festgehalten. Die Kontrollen werden nicht appliziert.

	<b>Dokumentation Applikation Polyamid PA-6</b>
	<b>Study-No.: 100-22</b>

**Dokumentation Applikation am 16.04.2020**


 Kontroll-Enclosures MP 1 bis MP 5 umgerührt: ja  nein 

<b>MP6</b>  (0,25*150 mg/l SiO <sub>2</sub> )	Start der Applikation	Uhrzeit:	14:04 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:09 Uhr
	Bemerkungen:		
Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>			

	<b>Dokumentation Applikation Polyamid PA-6</b>	
	<b>Study-No.: 100-22</b>	


<b>MP7</b>  (0,25*150 mg/l SiO <sub>2</sub> )	Start der Applikation	Uhrzeit:	14:41 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:47 Uhr
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="radio"/>		

<b>MP8</b>  (0,25*1,50 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:11 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:16 Uhr
	Bemerkungen:		
Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="radio"/>			

	<b>Dokumentation Applikation Polyamid PA-6</b>	
	Study-No.: 100-22	


<b>MP9</b>  (0,25*1,50 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:50 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:55 Uhr
	Bemerkungen:		
Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>			

<b>MP10</b>  (0,25*1,50 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:26 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:33 Uhr
	Bemerkungen:		
Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>			

	<b>Dokumentation Applikation Polyamid PA-6</b>	
	<b>Study-No.: 100-22</b>	


<b>MP11</b>  (0,25*15,0 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:18 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:23 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		

<b>MP12</b>  (0,25*15,0 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:59 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:04 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		

	<b>Dokumentation Applikation Polyamid PA-6</b>	
	<b>Study-No.: 100-22</b>	


<b>MP13</b>  (0,25*15,0 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:38 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:44 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		

<b>MP14</b>  (0,25*150 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:29 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:36 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		

	<b>Dokumentation Applikation Polyamid PA-6</b>		
	<b>Study-No.: 100-22</b>		

<b>MP15</b>  (0,25*150 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:14 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:20 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="radio"/>		

<b>MP16</b>  (0,25*150 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:47 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:52 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="radio"/>		

	<b>Dokumentation Applikation Polyamid PA-6</b>
	<b>Study-No.: 100-22</b>

Bemerkungen:

Lufttemperatur während des Tages: 20°C

Niederschlag: kein

Bewölkung: keine


Applikation durchgeführt durch	[Redacted]	<u>29.05.2020</u>	[Redacted]
	Name	Datum	Unterschrift
Dokumentation erstellt von	[Redacted]	<u>29.05.2020</u>	[Redacted]
	Name	Datum	Unterschrift

29.05.2020

Datum

[Redacted]

Study Director

	<b>Dokumentation Applikation Polyamid PA-6</b>
	<b>Study-No.: 100-22</b>

Datum: 02.06.2020  
Autor: L.Kruckenfellner

### **Equipment**


1. Uhr
2. Meßzylinder 1000 ml
3. Weißschalen (Kontaminationsschutz)
4. Teichwasser
5. Sonstiges

### **Applikationslösungen**

Die Applikationslösungen werden bei Mesocosm GmbH am Tag der Applikation hergestellt. Die Abwaage erfolgt im Labor mit der Analysenwaage. Der Ansatz erfolgt zeitnah vor der Applikation auf dem Testgelände in einem Zelt am Teich. Die Anleitung und Dokumentation für den Ansatz wird separat zur Arbeitsanleitung Applikation angelegt.

### **Applikation**


Die Applikation der Testsubstanz erfolgt mit Hilfe von Scheidetrichtern, welche eine Verlängerung von 30 cm aufweisen. In diese Scheidetrichter werden die Applikationslösungen (1000 mL) aus 2 L Bechergläsern überführt. Anschließend werden die Applikationslösungen, nach Öffnen des Scheidetrichters, in einer Wassertiefe von 15 bis 25 cm unter der Oberfläche gleichmäßig durch zirkulierende Bewegungen eingerührt. Nach der Applikation werden die 2 L-Bechergläser der Applikationslösungen dreimal mit je ca. 500 mL Teichwasser nachgespült. Mit der jeweiligen Spüllösung (500 mL) wird der entsprechende Scheidetrichter ebenso gespült und anschließend wird die Spüllösung im entsprechenden Enclosure appliziert. Jedes Enclosure mit Ausnahme der Kontroll-Enclosures erhält so zusätzlich 3 x 500 mL Spüllösungen. Das Ende der Applikation der 3. Spüllösung wird als Ende der jeweiligen Applikation festgehalten. Die Kontrollen werden nicht appliziert.

	<b>Dokumentation Applikation Polyamid PA-6</b>
	<b>Study-No.: 100-22</b>

**Dokumentation Applikation am 16.04.2020**


 Kontroll-Enclosures MP 1 bis MP 5 umgerührt: ja  nein 

<b>MP6</b>  <small>(0,25*150 mg/l SiO<sub>2</sub>)</small>	Start der Applikation	Uhrzeit:	<i>14:19</i> Uhr	
	1000 ml Applikationslösung	Bezeichnung		
	1. Spülung	Menge Teichwasser	<i>500</i> ml	
	2. Spülung	Menge Teichwasser	<i>500</i> ml	
	3. Spülung	Menge Teichwasser	<i>500</i> ml	
	Ende der Applikation	Uhrzeit:	<i>14:23</i> Uhr	
	Bemerkungen:			
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>			

	<b>Dokumentation Applikation Polyamid PA-6</b>	
	<b>Study-No.: 100-22</b>	


<b>MP7</b>  (0,25*150 mg/l SiO <sub>2</sub> )	Start der Applikation	Uhrzeit:	14:48 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:52 Uhr
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="radio"/>		

<b>MP8</b>  (0,25*1,50 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:25 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:29 Uhr
	Bemerkungen:		
Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="radio"/>			

	<b>Dokumentation Applikation Polyamid PA-6</b>	
	<b>Study-No.: 100-22</b>	


<b>MP9</b>  (0,25*1,50 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:55 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:59 Uhr
	Bemerkungen:		
Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>			

<b>MP10</b>  (0,25*1,50 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:18 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:22 Uhr
	Bemerkungen:		
Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>			

	<b>Dokumentation Applikation Polyamid PA-6</b>		
	<b>Study-No.: 100-22</b>		


<b>MP11</b>  (0,25*15,0 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:32 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:37 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		

<b>MP12</b>  (0,25*15,0 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:02 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:06 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		

	<b>Dokumentation Applikation Polyamid PA-6</b>	
	<b>Study-No.: 100-22</b>	


<b>MP13</b>  (0,25*15,0 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:25 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:29 Uhr
	Bemerkungen:		
Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>			

<b>MP14</b>  (0,25*150 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:40 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:45 Uhr
	Bemerkungen:		
Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>			

	<b>Dokumentation Applikation Polyamid PA-6</b>	
	<b>Study-No.: 100-22</b>	

<b>MP15</b>  (0,25*150 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:09 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:14 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		

<b>MP16</b>  (0,25*150 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:32 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:38 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		





	<b>Dokumentation Applikation Polyamid PA-6</b>
	<b>Study-No.: 100-22</b>

Bemerkungen:

Lufttemperatur während des Tages: 26°C

Niederschlag: kein

Bewölkung: leicht


Applikation durchgeführt durch		<u>02.06.2020</u>	
	Name	Datum	Unterschrift
Dokumentation erstellt von		<u>02.06.2020</u>	
	Name	Datum	Unterschrift

02.06.2020

Datum



Study Director

	<b>Dokumentation Applikation Polyamid PA-6</b>
	<b>Study-No.: 100-22</b>

Datum: 05.06.2020  
Autor: L.Kruckenfellner

#### **Equipment**


1. Uhr
2. Meßzylinder 1000 ml
3. Weißschalen (Kontaminationsschutz)
4. Teichwasser
5. Sonstiges

#### **Applikationslösungen**

Die Applikationslösungen werden bei Mesocosm GmbH am Tag der Applikation hergestellt. Die Abwaage erfolgt im Labor mit der Analysenwaage. Der Ansatz erfolgt zeitnah vor der Applikation auf dem Testgelände in einem Zelt am Teich. Die Anleitung und Dokumentation für den Ansatz wird separat zur Arbeitsanleitung Applikation angelegt.

#### **Applikation**


Die Applikation der Testsubstanz erfolgt mit Hilfe von Scheidetrichern, welche eine Verlängerung von 30 cm aufweisen. In diese Scheidetricher werden die Applikationslösungen (1000 mL) aus 2 L Bechergläsern überführt. Anschließend werden die Applikationslösungen, nach Öffnen des Scheidetrichers, in einer Wassertiefe von 15 bis 25 cm unter der Oberfläche gleichmäßig durch zirkulierende Bewegungen eingerührt. Nach der Applikation werden die 2 L-Bechergläser der Applikationslösungen dreimal mit je ca. 500 mL Teichwasser nachgespült. Mit der jeweiligen Spüllösung (500 mL) wird der entsprechende Scheidetricher ebenso gespült und anschließend wird die Spüllösung im entsprechenden Enclosure appliziert. Jedes Enclosure mit Ausnahme der Kontroll-Enclosures erhält so zusätzlich 3 x 500 mL Spüllösungen. Das Ende der Applikation der 3. Spüllösung wird als Ende der jeweiligen Applikation festgehalten. Die Kontrollen werden nicht appliziert.

	<b>Dokumentation Applikation Polyamid PA-6</b>
	<b>Study-No.: 100-22</b>

**Dokumentation Applikation am 16.04.2020**


 Kontroll-Enclosures MP 1 bis MP 5 umgerührt: ja  nein 

<b>MP6</b>  (0,25*150 mg/l SiO <sub>2</sub> )	Start der Applikation	Uhrzeit:	14:02 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:07 Uhr
	Bemerkungen:		
Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="radio"/>			

	<b>Dokumentation Applikation Polyamid PA-6</b>	
	Study-No.: 100-22	


<b>MP7</b>  (0,25*150 mg/l SiO <sub>2</sub> )	Start der Applikation	Uhrzeit:	14:35 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:39 Uhr
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="radio"/>		

<b>MP8</b>  (0,25*1,50 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:10 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:14 Uhr
	Bemerkungen:		
Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="radio"/>			

	<b>Dokumentation Applikation Polyamid PA-6</b>	
	Study-No.: 100-22	


<b>MP9</b>  (0,25*1,50 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:44 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:49 Uhr
	Bemerkungen:		
Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>			

<b>MP10</b>  (0,25*1,50 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:08 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:12 Uhr
	Bemerkungen:		
Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>			

	<b>Dokumentation Applikation Polyamid PA-6</b>		
	Study-No.: 100-22		


<b>MP11</b>  (0,25*15,0 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:18 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:22 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		

<b>MP12</b>  (0,25*15,0 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:51 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:55 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		

	<b>Dokumentation Applikation Polyamid PA-6</b>		
	Study-No.: 100-22		


<b>MP13</b>  (0,25*15,0 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:15 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:19 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		

<b>MP14</b>  (0,25*150 mg/l PA-6)	Start der Applikation	Uhrzeit:	14:26 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	14:31 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		

	<b>Dokumentation Applikation Polyamid PA-6</b>	
	Study-No.: 100-22	

<b>MP15</b>  (0,25*150 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:01 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:05 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		

<b>MP16</b>  (0,25*150 mg/l PA-6)	Start der Applikation	Uhrzeit:	15:22 Uhr
	1000 ml Applikationslösung	Bezeichnung	
	1. Spülung	Menge Teichwasser	500 ml
	2. Spülung	Menge Teichwasser	500 ml
	3. Spülung	Menge Teichwasser	500 ml
	Ende der Applikation	Uhrzeit:	15:26 Uhr
	Bemerkungen:		
	Enclosure nach Applikation umgerührt: ja <input type="radio"/> nein <input checked="" type="checkbox"/>		





	<b>Dokumentation Applikation Polyamid PA-6</b>
	<b>Study-No.: 100-22</b>

Bemerkungen: leichter Wind

Lufttemperatur während des Tages: 13°C

Niederschlag: leichter Regen (anfangs) bis mäßiger Regen

Bewölkung: stark

Applikation durchgeführt durch		<u>05.06.2020</u>	
	Name	Datum	Unterschrift
Dokumentation erstellt von		<u>05.06.2020</u>	
	Name	Datum	Unterschrift

05.06.2020  
Datum

  
Study Director

Date	average daily air temperature [°C]	sum daily precipitation [mm]	sum daily sunshine [min]
05.04.2020	10.1	0.0	764.0
06.04.2020	13.4	0.0	767.0
07.04.2020	13.6	0.0	735.0
08.04.2020	15.3	0.0	751.0
09.04.2020	14.2	0.0	546.0
10.04.2020	12.3	0.0	739.0
11.04.2020	11.1	0.0	774.0
12.04.2020	15.2	0.0	618.0
13.04.2020	7.4	0.0	474.0
14.04.2020	4.1	0.0	411.0
15.04.2020	8.1	0.0	666.0
16.04.2020	14.1	0.0	769.0
17.04.2020	14.1	0.0	776.0
18.04.2020	13.8	4.4	366.0
19.04.2020	10.3	0.0	296.0
20.04.2020	9.6	0.0	824.0
21.04.2020	11.1	0.0	823.0
22.04.2020	12.2	0.0	832.0
23.04.2020	13.5	0.0	811.0
24.04.2020	13.8	0.0	629.0
25.04.2020	9.0	0.0	638.0
26.04.2020	11.6	0.0	837.0
27.04.2020	14.1	0.0	763.0
28.04.2020	11.8	2.1	98.0
29.04.2020	11.8	2.4	348.0
30.04.2020	10.2	5.0	202.0
<b>Mean/sum</b>	<b>10.9</b>	<b>14.0</b>	<b>18439.0</b>
01.05.2020	8.4	2.0	216.0
02.05.2020	7.3	3.6	168.0
03.05.2020	9.6	0.0	515.0
04.05.2020	9.4	0.5	115.0
05.05.2020	7.2	0.0	832.0
06.05.2020	9.5	0.0	881.0
07.05.2020	12.8	0.0	834.0
08.05.2020	15.2	0.0	664.0
09.05.2020	14.9	0.0	445.0
10.05.2020	14.7	2.8	181.0
11.05.2020	4.6	2.1	96.0
12.05.2020	5.8	0.0	630.0
13.05.2020	7.4	0.0	293.0
14.05.2020	7.0	0.0	448.0
15.05.2020	8.7	0.0	875.0
16.05.2020	10.7	0.0	786.0
17.05.2020	12.0	0.0	846.0

Date	average daily air temperature [°C]	sum daily precipitation [mm]	sum daily sunshine [min]
18.05.2020	15.2	0.0	866.0
19.05.2020	16.7	0.0	600.0
20.05.2020	15.6	0.0	319.0
21.05.2020	16.5	0.0	880.0
22.05.2020	17.6	2.6	107.0
23.05.2020	11.8	1.8	178.0
24.05.2020	11.0	0.3	163.0
25.05.2020	13.5	0.0	428.0
26.05.2020	12.6	0.0	539.0
27.05.2020	15.5	0.0	727.0
28.05.2020	13.8	0.0	918.0
29.05.2020	13.8	0.0	920.0
30.05.2020	13.8	0.0	712.0
31.05.2020	13.5	0.0	695.0
<b>Mean/sum</b>	<b>11.8</b>	<b>15.7</b>	<b>16877.0</b>
01.06.2020	16.6	0.0	921.0
02.06.2020	19.0	0.0	850.0
03.06.2020	18.3	1.7	388.0
04.06.2020	12.9	6.7	11.0
05.06.2020	9.3	7.1	182.0
06.06.2020	9.9	7.4	387.0
07.06.2020	12.6	0.0	490.0
08.06.2020	13.3	0.1	539.0
09.06.2020	13.0	1.6	271.0
10.06.2020	13.1	6.8	12.0
11.06.2020	13.9	0.4	105.0
12.06.2020	18.8	0.0	722.0
13.06.2020	21.0	0.0	816.0
14.06.2020	17.5	18.7	51.0
15.06.2020	15.1	13.2	0.0
16.06.2020	15.3	0.8	70.0
17.06.2020	15.4	21.1	89.0
18.06.2020	15.9	0.3	103.0
19.06.2020	16.3	10.7	551.0
20.06.2020	16.9	0.0	595.0
21.06.2020	18.1	0.0	838.0
22.06.2020	18.0	0.0	490.0
23.06.2020	18.7	0.0	909.0
24.06.2020	19.3	0.0	863.0
25.06.2020	19.4	0.0	780.0
26.06.2020	20.3	0.0	883.0
27.06.2020	20.9	0.2	415.0
28.06.2020	17.7	0.0	41.0

Date	average daily air temperature [°C]	sum daily precipitation [mm]	sum daily sunshine [min]
29.06.2020	15.4	2.3	462.0
30.06.2020	16.1	0.0	702.0
<b>Mean/sum</b>	<b>16.3</b>	<b>99.1</b>	<b>13536.1</b>
01.07.2020	18.9	2.3	268.0
02.07.2020	17.2	0.4	429.0
03.07.2020	16.4	0.0	603.0
04.07.2020	18.1	0.0	341.0
05.07.2020	20.3	0.0	235.0
06.07.2020	13.9	2.5	392.0
07.07.2020	14.1	0.4	558.0
08.07.2020	13.1	3.4	0.0
09.07.2020	18.6	0.1	263.0
10.07.2020	17.4	0.0	280.0
11.07.2020	14.1	0.0	382.0
12.07.2020	15.0	0.0	698.0
13.07.2020	17.5	0.0	892.0
14.07.2020	18.6	0.9	505.0
15.07.2020	15.3	0.0	225.0
16.07.2020	13.5	3.2	0.0
17.07.2020	15.9	0.0	186.0
18.07.2020	19.4	0.0	626.0
19.07.2020	20.8	0.0	568.0
20.07.2020	17.7	0.0	352.0
21.07.2020	16.1	0.0	851.0
22.07.2020	16.5	0.0	902.0
23.07.2020	17.7	0.0	870.0
24.07.2020	20.6	0.0	316.0
25.07.2020	20.1	4.7	311.0
26.07.2020	19.0	0.1	429.0
27.07.2020	20.4	0.0	418.0
28.07.2020	20.7	0.0	534.0
29.07.2020	17.3	0.0	794.0
30.07.2020	19.5	0.0	890.0
31.07.2020	23.1	0.0	897.0
<b>mean/sums</b>	<b>17.6</b>	<b>18.0</b>	<b>15015.0</b>
01.08.2020	23.9	2.9	527.0
02.08.2020	19.7	0.1	331.0
03.08.2020	16.8	0.0	218.0
04.08.2020	16.6	0.0	509.0
05.08.2020	19.5	0.0	869.0
06.08.2020	23.0	0.0	867.0
07.08.2020	24.9	0.0	861.0

Date	average daily air temperature [°C]	sum daily precipitation [mm]	sum daily sunshine [min]
08.08.2020	27.3	0.0	765.0
09.08.2020	25.5	1.1	375.0
10.08.2020	22.2	1.2	211.0
11.08.2020	23.8	0.0	601.0
12.08.2020	22.4	4.1	397.0
13.08.2020	21.6	47.5	123.0
14.08.2020	19.6	0.9	98.0
15.08.2020	20.2	0.0	283.0
16.08.2020	21.7	41.3	611.0
17.08.2020	18.3	2.3	112.0
18.08.2020	18.7	0.1	508.0
19.08.2020	18.9	0.1	759.0
20.08.2020	22.1	0.0	319.0
21.08.2020	24.5	0.7	242.0
22.08.2020	21.0	0.1	396.0
23.08.2020	17.3	0.0	341.0
24.08.2020	15.7	0.0	298.0
25.08.2020	17.0	0.0	332.0
26.08.2020	17.1	0.0	43.0
27.08.2020	15.7	2.0	499.0
28.08.2020	16.5	0.0	154.0
29.08.2020	13.9	4.5	104.0
30.08.2020	13.9	3.6	55.0
31.08.2020	13.5	12.1	44.0
<b>mean/sums</b>	<b>19.8</b>	<b>124.6</b>	<b>11852</b>
01.09.2020	12.8	0.1	309.0
02.09.2020	14.6	0.0	639.0
03.09.2020	13.5	5.1	101.0
04.09.2020	18.2	0.0	57.0
05.09.2020	14.7	0.3	112.0
06.09.2020	12.7	0.0	449.0
07.09.2020	13.9	0.0	707.0
08.09.2020	16.7	0.0	758.0
09.09.2020	18.0	0.0	474.0
10.09.2020	15.1	0.0	693.0
11.09.2020	14.7	0.0	717.0
12.09.2020	17.6	0.0	689.0
13.09.2020	17.8	0.0	706.0
14.09.2020	20.7	0.0	707.0
15.09.2020	21.4	0.0	612.0
16.09.2020	20.8	0.0	545.0
17.09.2020	14.2	0.0	517.0
18.09.2020	13.9	0.0	710.0

Date	average daily air temperature [°C]	sum daily precipitation [mm]	sum daily sunshine [min]
19.09.2020	14.8	0.0	685.0
20.09.2020	15.1	0.0	592.0
21.09.2020	16.5	0.0	696.0
22.09.2020	17.4	0.0	681.0
23.09.2020	18.1	1.8	350.0
24.09.2020	14.7	2.9	425.0
25.09.2020	10.1	0.5	66.0
26.09.2020	7.9	14.7	0.0
27.09.2020	8.4	0.0	28.0
28.09.2020	10.1	0.0	246.0
29.09.2020	10.4	6.1	0.0
30.09.2020	11.6	0.0	10.0
<b>mean/sums</b>	<b>14.9</b>	<b>31.5</b>	<b>13281</b>

Mesocosm	1. Measure [cm]	2. Measure [cm]	3. Measure [cm]	Mean [cm]	Distance to top edge [cm]
1	105	104	106	105.0	29.1
2	107	108	108	107.7	28.6
3	105.5	106	106	105.8	30.8
4	106	106	106.5	106.2	31.4
5	105	106	105	105.3	35.6
6	107	107	108	107.3	28.6
7	106	107	106	106.3	32.9
8	106	107	107	106.7	32.2
9	106	106	106	106.0	28.6
10	104	106	104	104.7	28.7
11	107	107	105	106.3	31.1
12	104	106	107	105.7	31.8
13	106	107	106	106.3	31.1
14	107	106	107	106.7	35.2
15	105	106	106.5	105.8	31.8
16	106	107	108	107.0	28.9
	21.04.2020		05.05.2020		
Mesocosm	Distance to top edge [cm]	Water level [cm]	Distance to top edge [cm]	Water level [cm]	
1	28.4	105.7	26.4	107.7	
2	28.4	107.9	26.9	109.4	
3	30.7	105.9	28.7	107.9	
4	29.9	107.7	27.8	109.8	
5	35.2	105.7	32.9	108.0	
6	27.9	108.0	26.2	109.7	
7	32.9	106.3	30.8	108.4	
8	32.1	106.8	29.5	109.4	
9	28.4	106.2	26.3	108.3	
10	28.7	104.7	26.6	106.8	
11	30.7	106.7	28.6	108.8	
12	31.9	105.6	29.6	107.9	
13	30.7	106.7	28.5	108.9	
14	34.9	107.0	32.8	109.1	
15	31.4	106.2	29.4	108.2	
16	28.7	107.2	27	108.9	
	31.7	106.0	29.3	108.4	
	27.5	106.3	25.4	108.4	
	32.6	106.9	30.9	108.6	
	32.2	106.7	30.4	108.5	
	29.2	106.4	27.4	108.2	

	Mean:	106.5	Mean:	108.5
	24.05.2020		29.05.2020	
Mesocosm	Distance to top edge [cm]	Water level [cm]	Distance to top edge [cm]	Water level [cm]
1	26.4	112.5	28.3	110.6
2	26.4	107.0	28.5	104.9
3	28	110.9	30	108.9
4	27.8	111.1	29.4	109.5
5	32	105.7	34.1	103.6
6	26.3	111.3	28.2	109.4
7	30	107.6	32	105.6
8	29	107.6	31	105.6
9	26	114.9	27.7	113.2
10	26.5	108.1	28.3	106.3
11	28.2	107.7	30.2	105.7
12	29.4	109.8	31	108.2
13	28.2	113.7	29.9	112.0
14	32.5	101.3	34.3	99.5
15	29	107.3	30.9	105.4
16	26.3	111.1	28.5	108.9
	Mean:	109.2	Mean:	107.3
	02.06.2020		05.06.2020	
Mesocosm	Distance to top edge [cm]	Water level [cm]	Distance to top edge [cm]	Water level [cm]
1	29.3	109.6	29.5	109.4
2	29.7	103.7	29.5	103.9
3	31.3	107.6	31.1	107.8
4	30.5	108.4	30.9	108.0
5	35	102.7	35.2	102.5
6	29.5	108.1	29.4	108.2
7	33.5	104.1	33.4	104.2
8	32.1	104.5	32.2	104.4
9	29.1	111.8	29	111.9
10	29.7	104.9	29.5	105.1
11	31.7	104.2	31.5	104.4
12	32.7	106.5	32.4	106.8
13	31.6	110.3	31.3	110.6
14	35.9	97.9	35.4	98.4
15	32	104.3	31.8	104.5
16	29.4	108.0	29.5	107.9
	Mean:	106.0	Mean:	106.1

	06.06.2020		08.06.2020	
Mesocosm	Distance to top edge [cm]	Water level [cm]	Distance to top edge [cm]	Water level [cm]
1	28.9	110.0	29	109.9
2	29.2	104.2	28.9	104.5
3	30.7	108.2	30.6	108.3
4	30.5	108.4	30.3	108.6
5	35.2	102.5	34.8	102.9
6	29	108.6	29.2	108.4
7	32.9	104.7	33	104.6
8	31.9	104.7	31.8	104.8
9	28.6	112.3	28.8	112.1
10	29.4	105.2	29.3	105.3
11	31.5	104.4	31.4	104.5
12	32.4	106.8	32	107.2
13	30.9	111.0	30.7	111.2
14	35.3	98.5	34.9	98.9
15	32	104.3	31.8	104.5
16	29.3	108.1	29.2	108.2
	Mean:	106.4	Mean:	106.5
	22.06.2020		07.07.2020	
Mesocosm	Distance to top edge [cm]	Water level [cm]	Distance to top edge [cm]	Water level [cm]
1	23	115.9	27.1	111.8
2	23.3	110.1	27	106.4
3	25.4	113.5	28.9	110.0
4	24.4	114.5	28.4	110.5
5	29.1	108.6	33.1	104.6
6	24	113.6	27.8	109.8
7	27.5	110.1	31.5	106.1
8	26.5	110.1	31.6	105.0
9	23.7	117.2	27.3	113.6
10	24.9	109.7	27.9	106.7
11	26.7	109.2	29.6	106.3
12	27.1	112.1	31	108.2
13	26.1	115.8	29.3	112.6
14	30.5	103.3	33.2	100.6
15	26.6	109.7	30	106.3
16	24.5	112.9	28.1	109.3
	Mean:	111.6	Mean:	108.0
	20.07.2020		03.08.2020	

Mesocosm	Distance to top edge [cm]	Water level [cm]	Distance to top edge [cm]	Water level [cm]
1	29.6	109.3	32.5	106.4
2	29.5	103.9	32.9	100.5
3	31.4	107.5	35	103.9
4	31.5	107.4	34.7	104.2
5	35.6	102.1	39	98.7
6	30.6	107.0	34.8	102.8
7	33.7	103.9	37	100.6
8	32.9	103.7	35.9	100.7
9	29.9	111.0	33.1	107.8
10	30.8	103.8	33.8	100.8
11	32.3	103.6	35.7	100.2
12	33.8	105.4	37.1	102.1
13	31.9	110.0	34.8	107.1
14	35.7	98.1	38.9	94.9
15	32.7	103.6	35.8	100.5
16	31	106.4	34.5	102.9
	Mean:	105.4	Mean:	102.1

17.08.2020		
Mesocosm	Distance to top edge [cm]	Water level [cm]
1	28.5	110.4
2	28.3	105.1
3	30.4	108.5
4	29.9	109.0
5	35	102.7
6	29	108.6
7	32.9	104.7
8	31.5	105.1
9	28.5	112.4
10	29.4	105.2
11	31.1	104.8
12	33	106.2
13	30	111.9
14	34.9	98.9
15	31.5	104.8
16	30.2	107.2
	Mean:	106.6

sampleID	enclosureName	blue	green	diatom	crypto	Gesamtchlorophyll	sampleID	enclosureName	blue	green	diatom	crypto	Gesamtchlorophyll	sampleID	enclosureName	blue	green	diatom	crypto	Gesamtchlorophyll	sampleID	enclosureName	blue	green	diatom	crypto	Gesamtchlorophyll							
08-Mai-20	1	0.42	0.88	0.54	0.28	2.12	01-Jun-20	1	0.64	1.56	2.18	2.09	6.47	26-Jun-20	1	2.26	0	3.58	7.83	13.67	22-Jul-20	1	1.53	0	1.71	4.36	7.6	20-Aug-20	1	2.82	0	0.92	5.14	8.88
08-Mai-20	2	0.76	3.13	1.27	0	5.16	01-Jun-20	2	0.55	0.78	1.37	1.17	3.87	26-Jun-20	2	1.29	0.91	2.64	3.58	8.42	22-Jul-20	2	0.85	0	0.91	2.43	4.19	20-Aug-20	2	3.17	0	1.19	5.76	10.12
08-Mai-20	3	0.35	0.48	0.61	0.72	2.16	01-Jun-20	3	0.22	0.78	0.81	0.52	2.33	26-Jun-20	3	1.02	0.67	2.16	2.62	6.47	22-Jul-20	3	0.88	0.08	0.87	1.8	3.63	20-Aug-20	3	4.16	0	2.04	7.66	13.86
08-Mai-20	4	0.33	1.16	0.73	0.5	2.72	01-Jun-20	4	0.17	1.18	0.62	0.06	2.03	26-Jun-20	4	0.97	0.5	3.08	3.3	7.85	22-Jul-20	4	1.49	0	1.2	2.68	5.37	20-Aug-20	4	2.22	0	0.87	3.95	7.04
08-Mai-20	5	0.25	0.74	0.63	0.48	2.1	01-Jun-20	5	0.21	1.12	0.54	0	1.87	26-Jun-20	5	0.98	0.62	2.13	2.81	6.54	22-Jul-20	5	0.98	0	1.27	2.79	5.04	20-Aug-20	5	1.33	0.1	1.07	2.27	4.77
08-Mai-20	6	0.22	0.7	0.45	0.22	1.59	01-Jun-20	6	0.2	2.82	4.3	2.47	9.79	26-Jun-20	6	0.02	1.17	3.01	1.59	5.79	22-Jul-20	6	1.45	0.31	2.09	3.46	7.31	20-Aug-20	6	0.33	0.59	0.68	0.52	2.12
08-Mai-20	7	0.24	0.54	0.38	0.24	1.4	01-Jun-20	7	0.43	3.78	2.73	1.58	8.52	26-Jun-20	7	0	2.8	6.06	2.85	11.71	22-Jul-20	7	1.49	0.02	1.88	2.98	6.37	20-Aug-20	7	0.83	1.71	3.58	2.04	8.16
08-Mai-20	8	0.17	0.77	0.42	0.07	1.43	01-Jun-20	8	0.53	2.44	2.24	1.37	6.58	26-Jun-20	8	1.17	0.96	1.95	2.55	6.63	22-Jul-20	8	1.41	0	1.31	3.44	6.16	20-Aug-20	8	3.84	0	1.18	6.99	12.01
08-Mai-20	9	0.17	1.51	0.7	0.01	2.39	01-Jun-20	9	0.45	2.78	1.76	0.86	5.85	26-Jun-20	9	1.91	1.4	4.21	4.37	11.89	22-Jul-20	9	1.45	0.01	1.27	2.64	5.37	20-Aug-20	9	2.77	0	1.71	4.96	9.44
08-Mai-20	10	0.29	1.77	0.96	0.45	3.47	01-Jun-20	10	0.33	1.54	1.89	0.76	4.52	26-Jun-20	10	1.46	0.83	3.2	3.8	9.29	22-Jul-20	10	1.27	0	1.77	3.62	6.66	20-Aug-20	10	3.32	0	1.91	6.14	11.37
08-Mai-20	11	0.19	1.44	0.64	0.08	2.35	01-Jun-20	11	0.29	1.73	1.7	0.81	4.53	26-Jun-20	11	1.13	1.46	2.65	2.23	7.47	22-Jul-20	11	1.75	7.59	2.27	0	11.61	20-Aug-20	11	3.12	20.36	4.88	0	28.36
08-Mai-20	12	0.25	1	0.52	0.1	1.87	01-Jun-20	12	0.95	3.53	1.86	0.48	6.82	26-Jun-20	12	1.14	0.11	2.11	3.1	6.46	22-Jul-20	12	1.82	0	1.26	3.93	7.01	20-Aug-20	12	2.28	0	1.34	4.49	8.11
08-Mai-20	13	0.18	1.2	0.74	0.29	2.41	01-Jun-20	13	0.59	1.08	2.08	2.08	5.83	26-Jun-20	13	1.11	1.21	1.55	2.23	6.1	22-Jul-20	13	2.13	14.14	4.1	0	20.37	20-Aug-20	13	3.98	24.66	10.93	0.04	39.61
08-Mai-20	14	0.34	0.85	0.75	0.67	2.61	01-Jun-20	14	1.15	3.34	2	1.31	7.8	26-Jun-20	14	1.77	2.89	4.57	3.27	12.5	22-Jul-20	14	1.2	0	1.16	2.76	5.12	20-Aug-20	14	2.51	0	1.41	4.45	8.37
08-Mai-20	15	0.28	1.58	0.74	0.08	2.68	01-Jun-20	15	0.98	3.05	3.34	2.71	10.08	26-Jun-20	15	1.28	3.1	4.11	2.8	11.29	22-Jul-20	15	2.19	0	1.64	4.72	8.55	20-Aug-20	15	3.25	0	1.86	6.08	11.19
08-Mai-20	16	0.28	1.24	0.77	0.33	2.62	01-Jun-20	16	0.53	1.74	1.56	1.46	5.29	26-Jun-20	16	2.27	1.41	3.3	4.57	11.55	22-Jul-20	16	1.23	0.14	1.03	1.8	4.2	20-Aug-20	16	2.61	0	0.96	4.38	7.95
19-Mai-20	1	0.27	1.91	0.83	0	3.01	04-Jun-20	1	0.91	2.4	3.11	2.92	9.34	02-Jul-20	1	4.03	0	3.01	7.8	14.84	29-Jul-20	1	3.84	0	1.96	7.5	13.3							
19-Mai-20	2	0.38	1.27	1.07	0.67	3.39	04-Jun-20	2	0.45	0.77	1.59	1.61	4.42	02-Jul-20	2	2.16	1.23	3.54	5.12	12.05	29-Jul-20	2	2.22	0	1.18	4.6	8							
19-Mai-20	3	0.15	0.44	0.53	0.4	1.52	04-Jun-20	3	0.67	3.31	0.79	1.4	3.17	02-Jul-20	3	2.1	0	1.8	4.64	8.54	29-Jul-20	3	1.17	0	0.93	2.3	4.4							
19-Mai-20	4	0.26	1.09	0.7	0.37	2.42	04-Jun-20	4	0.33	1.31	0.73	0.18	2.55	02-Jul-20	4	1.85	0	1.49	3.81	7.15	29-Jul-20	4	1.11	0	1.69	3.37	6.17							
19-Mai-20	5	0.51	0.27	0.66	1.07	2.51	04-Jun-20	5	0.62	1.98	1.27	0.69	4.56	02-Jul-20	5	2.36	0.72	3.15	5.23	11.46	29-Jul-20	5	1.44	0.06	1.2	2.66	5.36							
19-Mai-20	6	0.55	2.25	1.47	0.77	5.04	04-Jun-20	6	0	3.84	8.29	3.04	15.17	02-Jul-20	6	1.55	0.97	2.32	3.06	7.9	29-Jul-20	6	1.35	0	1.42	3.29	6.06							
19-Mai-20	7	0.15	1.04	0.92	0.24	2.35	04-Jun-20	7	0.55	4.76	3.37	1.83	10.51	02-Jul-20	7	0.06	0.57	3.51	2.7	6.84	29-Jul-20	7	1.75	0.95	1.96	2.02	6.68							
19-Mai-20	8	0.19	1.05	1.21	0.81	3.26	04-Jun-20	8	0.59	1.6	1.65	1.51	5.35	02-Jul-20	8	0.78	1.73	2.72	2.12	7.35	29-Jul-20	8	1.29	0.09	1.52	2.97	5.87							
19-Mai-20	9	0.13	1.08	0.53	0.04	1.78	04-Jun-20	9	0.79	0.77	1.42	1.94	4.92	02-Jul-20	9	1.59	0.99	3.58	4.01	10.17	29-Jul-20	9	1.78	0	1.07	3.69	6.54							
19-Mai-20	10	0.14	1.15	0.67	0.1	2.06	04-Jun-20	10	0.45	1.61	1.27	0.69	4.02	02-Jul-20	10	3.8	0.33	2.82	6.33	13.28	29-Jul-20	10	2.17	0	2.34	5.1	9.61							
19-Mai-20	11	0.96	5.44	1.42	0	7.82	04-Jun-20	11	0.64	0.81	1.09	0.91	3.45	02-Jul-20	11	1.61	0	1.04	3.17	5.82	29-Jul-20	11	1.82	7.06	1.5	0	10.38							
19-Mai-20	12	0.05	0.95	0.64	0.12	1.76	04-Jun-20	12	0.17	3.53	1.55	0.39	6.18	02-Jul-20	12	2.42	0	2.66	5.86	10.94	29-Jul-20	12	1.29	0	1.2	3.48	5.97							
19-Mai-20	13	0.12	1.28	0.52	0	1.92	04-Jun-20	13	0.52	0.63	1.12	1.37	3.64	02-Jul-20	13	1.37	20.64	5.44	0	27.45	29-Jul-20	13	4.04	35.31	6.58	0	45.93							
19-Mai-20	14	0.54	0.32	0.94	1.6	3.4	04-Jun-20	14	1.66	1.22	2.19	3.71	8.78	02-Jul-20	14	1.79	0.1	2.71	4.67	9.27	29-Jul-20	14	1.78	0.3	1.35	3.19	6.62							
19-Mai-20	15	0.02	0.89	0.93	0.36	2.2	04-Jun-20	15	1.07	2.74	1.98	1.6	7.39	02-Jul-20	15	2	1.81	5.32	5.81	14.94	29-Jul-20	15	1.85	0.12	1.41	3.11	6.49							
19-Mai-20	16	0.18	0.89	0.81	0.52	2.4	04-Jun-20	16	1.07	0.63	2.09	3.28	7.07	02-Jul-20	16	2.61	0	1.54	5.3	9.45	29-Jul-20	16	2.29	0	2.52	5.47	10.28							
26-Mai-20	1	0.11	2.79	1.5	0.22	4.62	08-Jun-20	1	1.21	5.58	3.33	1.93	12.05	09-Jul-20	1	2.53	0	1.42	4.95	8.9	06-Aug-20	1	3.05	0	1.86	6.34	11.25							
26-Mai-20	2	0.25	2.32	1.06	0.08	3.71	08-Jun-20	2	1.06	1.87	1.89	1.46	6.28	09-Jul-20	2	2.46	0	1.78	5.19	9.43	06-Aug-20	2	2.32	0	1.61	5.16	9.09							
26-Mai-20	3	0.19	3.58	1.03	0	4.8	08-Jun-20	3	0.59	2.24	2.35	1.18	6.36	09-Jul-20	3	1.32	0	1.77	3.53	6.62	06-Aug-20	3	1.27	0	0.93	2.43	4.63							
26-Mai-20	4	0.13	1.79	0.79	0	2.71	08-Jun-20	4	0.36	2.12	1.96	1.03	5.47	09-Jul-20	4	1.51	0	0.76	2.87	5.14	06-Aug-20	4	1.45	0	1.36	2.77	5.58							
26-Mai-20	5	0.18	1.41	0.72	0.07	2.38	08-Jun-20	5	0.66	6.23	2.97	0	9.86	09-Jul-20	5	2.04	0	2.23	4.85	9.12	06-Aug-20	5	1.3	0	1.34	2.86	5.5							
26-Mai-20	6	0.49	1.71	1.11	0.56	3.87	08-Jun-20	6	0.09	5.17	7.54	2.97	15.77	09-Jul-20	6	1.75	0.81	3.61	4.63	10.8	06-Aug-20	6	0.5	0	0.49	0.89	1.88							
26-Mai-20	7	0.44	1.52	1.03	0.33	3.32	08-Jun-20	7	1.43	12.51	4	0	17.94	09-Jul-20	7	1.47	0	1.45	3.24	6.16	06-Aug-20	7	2.82	0.24	2.95	5.42	11.43							
26-Mai-20	8	0.35	1.92	1.66	0.67	4.6	08-Jun-20	8	0.85	2.47	2.29	1.89	7.5	09-Jul-20	8	0.81	0	1.05	3.03	4.89	06-Aug-20	8	3.24	0.05	2.21	5.41	10.91							
26-Mai-20	9	0.23	2.71	1.14	0	4.08	08-Jun-20	9	1.25	1.41	2.1	3.19	7.95	09-Jul-20	9	1.71	0	0.88	3.43	6.02	06-Aug-20	9	2.16	0	1.4	3.8	7.36							
26-Mai-20	10	0.25	2.04	0.9	0	3.19	08-Jun-20	10	0.77	1.36	1.93	1.74	5.8	09-Jul-20	10	2.19	0																	

samplingDate	enclosureName	blue	green	diatom	crypto	Gesamtchlorophyll	samplingDate	enclosureName	blue	green	diatom	crypto	Gesamtchlorophyll
26-Mai-20	1	0.02	1.12	1.13	0.36	2.63	22-Jul-20	1	3.21	0	1.77	5.41	10.39
26-Mai-20	2	0.31	0.63	0.56	0.65	2.15	22-Jul-20	2	1.17	0	1.07	2.82	5.06
26-Mai-20	3	0	2.6	2.07	0.23	4.9	22-Jul-20	3	1.19	0	1.13	2.33	4.65
26-Mai-20	4	0.02	0.65	0.53	0.15	1.35	22-Jul-20	4	1.44	0	1.06	3.28	5.78
26-Mai-20	5	0.11	0.79	0.65	0.08	1.63	22-Jul-20	5	0.89	0	1.47	2.95	5.31
26-Mai-20	6	0.25	0.95	0.85	0.59	2.64	22-Jul-20	6	1.6	0.19	1.53	2.93	6.25
26-Mai-20	7	0.29	1.21	0.73	0.28	2.51	22-Jul-20	7	1.13	0	1.39	2.73	5.25
26-Mai-20	8	0.24	1.21	1.32	0.65	3.42	22-Jul-20	8	1.22	0	1.44	2.7	5.36
26-Mai-20	9	0.23	2.01	0.97	0.19	3.4	22-Jul-20	9	1.28	0.12	1.38	2.62	5.4
26-Mai-20	10	0.14	1.06	0.56	0.12	1.88	22-Jul-20	10	1.17	0	1.54	3.47	6.18
26-Mai-20	11	0	1.05	0.61	0.12	1.78	22-Jul-20	11	0.5	0.23	0.6	1.02	2.35
26-Mai-20	12	0.04	1.55	1.12	0	2.71	22-Jul-20	12	1.38	0	1.19	3.45	6.02
26-Mai-20	13	0.18	0.38	0.46	0.4	1.42	22-Jul-20	13	0.11	0.09	0.15	0.16	0.51
26-Mai-20	14	0.32	0.98	0.49	0.14	1.93	22-Jul-20	14	1.18	0	1.24	2.89	5.31
26-Mai-20	15	0.68	1.17	1.61	1.62	5.08	22-Jul-20	15	1.67	0.02	1.62	3.54	6.85
26-Mai-20	16	0.06	0.75	0.51	0.18	1.5	22-Jul-20	16	1.02	0	1	2.44	4.46
26-Jun-20	1	2.34	0	2.28	5.65	10.27	20-Aug-20	1	2.22	0	0.93	4.22	7.37
26-Jun-20	2	2.09	0.73	2.14	3.93	8.89	20-Aug-20	2	4.06	0	1.85	7.93	13.84
26-Jun-20	3	0.91	0.74	1.98	2.43	6.06	20-Aug-20	3	4.02	0	2.18	7.7	13.9
26-Jun-20	4	0.83	0.59	2.92	2.98	7.32	20-Aug-20	4	2.29	0	0.91	4.17	7.37
26-Jun-20	5	0.62	0.83	1.79	1.75	4.99	20-Aug-20	5	1.44	0	0.6	2.35	4.39
26-Jun-20	6	0.07	1.55	3.25	1.86	6.73	20-Aug-20	6	0.3	0.33	0.42	0.47	1.52
26-Jun-20	7	0	2.33	5.78	2.81	10.92	20-Aug-20	7	0.55	0	1.1	1.71	3.36
26-Jun-20	8	2.03	0.7	2.06	3.51	8.3	20-Aug-20	8	3.54	0	1.79	7.39	12.72
26-Jun-20	9	0.59	0.1	0.69	1.21	2.59	20-Aug-20	9	2.81	0	1.54	5.76	10.11
26-Jun-20	10	0.95	0.14	2.06	2.78	5.93	20-Aug-20	10	3.53	0	1.9	7.22	12.65
26-Jun-20	11	0.74	0.71	1.19	1.56	4.2	20-Aug-20	11	0.62	0.35	0.94	1.08	2.99
26-Jun-20	12	1	0.6	2.23	2.67	6.5	20-Aug-20	12	2.87	0	1.11	5.01	8.99
26-Jun-20	13	0.78	0	0.59	1.88	3.25	20-Aug-20	13	2.01	1.01	2.82	4.06	9.9
26-Jun-20	14	0.63	1.3	1.44	1.26	4.63	20-Aug-20	14	3.02	0	1.17	4.84	9.03
26-Jun-20	15	0.99	2.16	2.26	1.62	7.03	20-Aug-20	15	3.4	0	2.13	7.14	12.67
26-Jun-20	16	0.93	0.82	2.23	2.25	6.23	20-Aug-20	16	2.96	0	1.22	4.77	8.95

samplingDate	enclosureName	Gesamtsumme von Coverage%	Ceratophyllum demersum	Chara globularis	Filamental Algae sp_	Myriophyllum spicatum	Potamogeton natans	Zannichéllia palustris	samplingDate	enclosureName	Gesamtsumme von Coverage%	Ceratophyllum demersum	Chara globularis	Filamental Algae sp_	Myriophyllum spicatum	Potamogeton natans	Zannichéllia palustris
24-Mai-20	1	27.2	0	25.1	0	0.9	1.2	0	17-Jul-20	1	69.1	0	42.2	0	11.2	13.7	2
24-Mai-20	2	29.2	0	26.5	0	1.4	1.3	0	17-Jul-20	2	69.1	0	42.2	0	10.6	9.5	6.8
24-Mai-20	3	28.8	0	26.9	0	1.3	0.6	0	17-Jul-20	3	74	0	39.5	0	14.2	11.5	8.8
24-Mai-20	4	28.4	0	25.6	0	1.5	1.3	0	17-Jul-20	4	68.9	0	38.6	0	17.8	4.3	8.2
24-Mai-20	5	32.8	0	29.9	0	1.1	1.8	0	17-Jul-20	5	72.2	0	39.9	0	16.7	11.1	4.5
24-Mai-20	6	26.4	0	22.9	0	1.6	1.9	0	17-Jul-20	6	64.2	0	39	0	9.9	8.9	6.4
24-Mai-20	7	25.5	0	23	0	1.4	1.1	0	17-Jul-20	7	59.4	0	38.9	0	7.8	4.2	8.5
24-Mai-20	8	35.4	0	33.6	0	0.4	1.4	0	17-Jul-20	8	52.1	0	35.2	0	9.8	2.5	4.6
24-Mai-20	9	26.2	0	23	0	1.2	2	0	17-Jul-20	9	64.6	0	42.6	0	14.9	2	5.1
24-Mai-20	10	36.1	0	32.6	0	1.6	1.9	0	17-Jul-20	10	72.4	0	40.9	0	13	13.8	4.7
24-Mai-20	11	40.9	0	37.7	0	1.6	1.6	0	17-Jul-20	11	62.8	0	38.7	0	12	7.5	4.6
24-Mai-20	12	27.9	0	25.8	0	1.3	0.8	0	17-Jul-20	12	66.7	0	36.3	0	14.5	8.5	7.4
24-Mai-20	13	28.6	0	26.3	0	1.1	1.2	0	17-Jul-20	13	54.4	0	33.3	0	11.1	3.7	6.3
24-Mai-20	14	22.7	0	20.2	0	1.3	1.2	0	17-Jul-20	14	71	0	44.7	0	15.2	7.6	3.5
24-Mai-20	15	24.5	0	22	0	1.6	0.9	0	17-Jul-20	15	68.3	0	43.4	0	15.3	7.2	2.4
24-Mai-20	16	26.5	0	23.8	0	1.6	1.1	0	17-Jul-20	16	69.4	0	41.8	0	7.5	7.4	12.7
12-Jun-20	1	33.2	0	20.1	0.3	4.9	1.9	6	22-Aug-20	1	60.3	0.3	50.5	0	0	8.1	1.4
12-Jun-20	2	36.9	0	23.2	0	5.5	3.8	4.4	22-Aug-20	2	90.2	0.5	52.4	0	31.5	5.8	0
12-Jun-20	3	34.6	0	25.5	0	0	3.1	6	22-Aug-20	3	86	0.3	49.6	0	24.1	12	0
12-Jun-20	4	41.5	0	25.2	0	4.6	3.9	7.8	22-Aug-20	4	108.9	0.5	51.5	0	33.5	23.4	0
12-Jun-20	5	30.9	0	19.6	0.5	3.6	3.4	3.8	22-Aug-20	5	87.3	0.7	55.6	0	25.6	5.4	0
12-Jun-20	6	28.5	0	22.6	0	2.7	1.5	1.7	22-Aug-20	6	90.9	0.5	50.4	0	25.2	14.8	0
12-Jun-20	7	39.5	0	24.4	0	2.8	4	8.3	22-Aug-20	7	60.4	0.4	52.1	0	0	4.7	3.2
12-Jun-20	8	38.5	0	29	0	3.7	2.8	3	22-Aug-20	8	99.3	0.7	52.2	0	26.7	14.8	4.9
12-Jun-20	9	42.2	0	28.1	0	4.5	3.6	6	22-Aug-20	9	89	0.5	51.7	0	25.5	9.3	2
12-Jun-20	10	37.9	0	29.7	0	5.8	0	2.4	22-Aug-20	10	80.8	0.4	48.7	0	22.5	9.2	0
12-Jun-20	11	30.3	0	20.8	1	2.6	0	5.9	22-Aug-20	11	76.4	1	51	0	17.6	6.8	0
12-Jun-20	12	39.6	0	23.5	0.4	6.9	3.1	5.7	22-Aug-20	12	93.5	0.2	50.4	0	32.4	8.9	1.6
12-Jun-20	13	41.3	0	25.8	0	5.1	3.5	6.9	22-Aug-20	13	102.6	0.5	61	0	32.2	8.9	0
12-Jun-20	14	32.4	0	21.2	0.4	3.8	3	4	22-Aug-20	14	89.3	1.6	53.9	0	21.9	11.9	0
12-Jun-20	15	46.5	0	27.2	0	6.3	3.5	9.5	22-Aug-20	15	93.8	0	53.8	0	26.2	11.9	1.9
12-Jun-20	16	48.2	0	31.5	0	4.7	4.3	7.7	22-Aug-20	16	113.7	0.7	55.6	0	42.1	15.3	0

samplingdate	endouereName	Gesamtsumme von Individuen/L	Acanthocyclops sp.	Acanthocyclops sp.	Acanthocyclops sp.	Bosmina d.	Brachionus sp.	Cyclopoida sp.	Chaoborus sp.	Chaoborus sp. (oupa)	Chironomidae n. d. sp.	Chydorus sphaericus	Cyclopoida n. d. sp.	Daphnia longiremis	Daphnia magna	Daphnia pulex	Daphnia sp.	Dipteridae n. d. sp.	Echiniscus sp.	Filinia sp.	Graptolobos sp.	Heterosira sp.	Kefersteinia quadrata	Leuciscus sp.	Leptodella sp.	Mytilina sp.	Nauplia n. d. sp.	Onchocercus n. d. sp.	Platyus quadricornis	Polyarthra sp.	Rhynchocera sp.	Scapholobos sp.	Simocyclops sp.	Synchaeta sp.	Tetradinfella sp.	Sumitrella	Sumitrella	Sumitrella	Amount_of_species	shannon	evenness	
18-Mai-20	1	2460.63					0.14	2.16	4.46			1.29	14.96	7.41			0.14		3.17				29.71	1728.92	5.18		24.1	139.06	0.43	461.73	0.72	0.14	1.73	34.32	0.86	2291.01	8.84	154.02	20	0.988	0.33	
18-Mai-20	2	2512.1					0.28	1.57	1.5			0.43	9.4	0.78			0.43		1.71				21.65	2127.21	7.69	0.71	8.12	111.68	1.42	181.84	3.13	0.14	1.71	29.99	0.71	2384.61	1.64	121.08	21	0.663	0.218	
18-Mai-20	3	1429.92						1.73	1.58			0.29	16.95	46.33			3.96		0.29				8.06	1075.61	1.29		7.05	48.13	1.29	104.96	0.29	0.29	0.79	111.98	0.14	1210.93	56.36	64.68	21	0.995	0.327	
18-Mai-20	4	2064.6				1.01	0.29	0.72	0.58		0.29	0.86	20.66	24.03			2.3		0.86				13.96	1311.73	3.74	0.14	14.82	230.22	1.73	0.14	170.5	0.14	0.29	2.73	262.37	1.15	1781.57	26.33	251.08	23	1.224	0.39
18-Mai-20	5	246.2						1.16	0.29			1.16	1.45	1.08					1.16				5.78	1707.07	1.3	0.29	2.75	7.52	1.3	0.29	17.06	0.43	0.29	32.62	0.14	234.05	1.08	8.97	19	1.171	0.398	
18-Mai-20	6	2269.73						1.42	1.35			0.71	16.1	0.5					0.71				5.56	1781.77	4.42	0.14	6.84	210.4	1.71	0.28	194.8	1	4.13	1.21	37.11	0.28	2034.33	0.5	226.5	19	0.809	0.275
18-Mai-20	7	1153.76					0.58	2.45	1.73			0.07	14.53	1.01		0.43	0.5		0.86	0.14			3.6	954.96	5.32	0.58	21.01	30	1.01	93.6	3.31	0.29	0.22	13.53	4.03	1103.97	2.01	44.53	23	0.775	0.247	
18-Mai-20	8	788.11						1.29	3.94			0.47	7.73	1.43			0.57		2	0.43			4.01	482.89	12.03	0.72	10.02	94.85	2.72	144.88	0.57	0.27	0.21	14.03	3.15	676.02	2.07	102.58	21	1.264	0.415	
18-Mai-20	9	1149.38					0.15	0.29	3.65			1.61	6.86	5.04		0.44	3.07		1.61				7.74	870.44	2.77	0.44	4.82	70.88	0.73	158.39	3.8	2.12	0.15	5.55	0.44	1056.44	8.55	77.74	21	0.899	0.295	
18-Mai-20	10	1944.18						2.96	2.81			0.89	13.33	3.19			1.63		1.48				22.3	1515.41	1.19	0.3	8.3	175.56	0.89	159.41	1.04	0.3	4.67	27.04	1.48	1740.91	5.71	188.89	20	0.864	0.288	
18-Mai-20	11	2589.89		0.14				0.29	1.74			0.29	17.35	6.29			1.16		3.04				11.14	2101.52	1.16		5.21	144.83	2.6	0.14	168.76	0.29	6.94	0.22	118.66	1.45	2408.76	7.45	162.18	19	0.777	0.264
18-Mai-20	12	2265						8.82	2.17			0.82	15.91	9.4			1.01		3.04				23.79	1753.43	6.07	0.14	91.4	67.61	1.74	265.31	3.47	0.29	2.6	6.65	0.29	2163.84	10.84	83.52	21	0.893	0.293	
18-Mai-20	13	3649.24						1.02	0.22		0.15	0.07	86.71	5.16				1.16		1.16			29.41	2669.43	2.03	0.58	10.46	293.97	1.74	0.87	328.4	0.29	1.31	2.9	212.49	0.87	3257.01	5.23	380.68	21	0.936	0.327
18-Mai-20	14	1000.85					0.14	2.01	1.37			0.46	4.66	6.69					1.16				7.34	789.64	1.29		13.81	36.33	0.43	0.14	93.6	0.29	0.14	0.22	42.66	0.29	951.21	6.69	40.79	18	0.86	0.298
18-Mai-20	15	2140.44						0.43	2			0.47	12.88	1.15			0.43		1.43				4.44	1728.06	14.6	0.43	4.44	182.75	0.72	167.07	0	3.72	0.43	14.03	1.29	1936.22	1.72	195.63	20	0.746	0.249	
18-Mai-20	16	2241.72						3.42	2.99	0.07		0.07	10.83	6.7			1.21		2.85				37.39	1984.4	2.42		1.42	41.95	1.14	106.48	1.85	0.28	35.97	0.28	2176.48	7.98	52.78	18	0.56	0.194		
23-Jun-20	1	914.6					0.56	1.98	3.95			0.85	34.79	12.21				0.07				352.58	147.99		0.14	2.68	147.99	0.71	28.86	0.14	0.56	2.96	128.65		710.51	13.06	182.85	18	1.656	0.573		
23-Jun-20	2	2500.91					0.14	0.71	1.13		0.14	0.21	53.69	8.58			3.55						1057.8	501.77	0.28		7.52	587.09	2.84	0.14	269.01	0.07	0.99	5.25	1842.62	12.34	640.78	19	1.428	0.485		
23-Jun-20	3	2508.6		0.14				0.14	1.13		0.28	13.83	5.08				5.41						630.49	952.22			3.25	386.03	0.56	0.28	458.08			56.53	0.56	2101.69	5.08	399.86	15	1.459	0.539	
23-Jun-20	4	1203.35						0.57	1.57	0.14		0.28	0.57	29.54	8.73		0.14					443.74	365.43	0.29		4.01	263.64	1.43	79	1.14	0.14	1.43	2.96	896	9.44	29.2	16	1.438	0.519			
23-Jun-20	5	1811.1						1.29			0.14	0.43	21.55	3.22				0.29				365.43	657.84			2.72	336.72	0.86	0.43	40.2	0.14	0.29	17.75	1446.31	3.65	358.56	16	1.474	0.532			
23-Jun-20	6	1262.73	0.28					1.83	0.71			0.42	9.2	1.41			0.14		0.14				90.05	362.88	0.28		0.28	388.57	0.99	0.56	29.15	13.55	0.92	275.37	774.09	2.25	483.77	19	1.618	0.549		
23-Jun-20	7	1115.79					0.14	0.57	2.07			0.28	25.28	0.07									1.42	131.84			5.98	480.48		0.14	138.96	3.42	0.78	324.64	607.11	0.07	505.76	14	1.397	0.529		
23-Jun-20	8	367.28	0.28					0.42	1.75			0.07	25.73	1.54			2.1						59.72	73.15			0.98	67.83	0.14	30.56	0.28	0.7	102.03	267.14	3.99	93.56	16	1.807	0.652			
23-Jun-20	9	1712.39						0.14	1.85			0.14	8.78	9.12			4.34						446.72	264.96			2.42	345.44	0.71	270.16	0.43	0.5	337.68	0.14	1322.65	13.46	373.21	15	1.709	0.631		
23-Jun-20	10	2227.68						0.14	3.88		0.29	1.01	80.29	8.63			2.95						944.46	493.24			9.06	314.82	0.72	0.14	149.57	0.86	1.22	216.4	1813.87	12.59	395.11	17	1.581	0.558		
23-Jun-20	11	3735.95						0.93				0.71	71.79	15.81			5.41						1940.24	1176.57	0.14		9.12	477.92	5.41	0.28	25.43	0.43	0.14	3.77	1.71	0.14	3154.06	21.22	549.71	18	1.151	0.398
23-Jun-20	12	1564					0.14	1.42	1.99			0.28	37.78	10.04			0.14						402.56	664.89	0.28		13.96	410.4	0.57	0.14	0.43	0.43	1112.52	18.32	438.18	18	1.313	0.454				
23-Jun-20	13	2111.7						0.28				0.14	124.11	35.39									900	628.44	0.14		0.43	398.3	2.7	0.14	14.47	1.56	3.83	1547.45	35.53	522.41	15	1.344	0.496			
23-Jun-20	14	1593.14						1.5			0.28	0.28	41.38	2.21			0.57						277.92	1023.36	0.14		1.71	192.24	1.42	0.85	43.09	0.14	0.07	0.71	5.27	1352.48	3.06	233.62	18	1.1	0.381	
23-Jun-20	15	1016.99	0.14					4.33	0.14			0.14	0.14	29.57	2.34		0.14					111.91	351.7	0.14		4.68	121.77	0.28	0.14	72.77	1.13	0.14	315.53	858	2.76	151.34	18	1.599	0.553			
23-Jun-20	16	1947.95					0.71	0.43	2.84		0.57	0.64	33.26	12.48			4.75	0.14					569.01	1052.62			2.7	223.9	5.67	34.4	0.07	0.07	8.79	1668.66	17.87	257.3	18	1.185	0.41			
06-Jul-20	1	973.08						0.44	6.71	0.15	0.29	13.12	102.8	28.52			1.03	0.15				0.44	136.4	39.57		0.44	239.57	7.66	239.57	0.56	26.12	21.22	69.34	485.32	43.11	382.46	17	1.967	0.694			
06-Jul-20	2	974.75	0.14					3.9			0.14	29.86	31.31									0.29	203.69	108.6		295.3	1.74	228.27	1.3	1.3	68.91	609.47	31.88	325.16	14	1.734	0.657					
06-Jul-20	3	1034.27						0.74				2.51	38.1	9.73			0.15						162.86	167.72			400.44	1.47	234.93	0.15	0.44	15.18	580.69	12.24	438.54	12	1.551	0.624				
06-Jul-20	4	801.22						1.02				12.26	73.28	20.36			0.15					0.29	151.02	14.31		0.15	423.21	9.34	77.59	3.5	2.77	11.97	255.04	33.06	496.49	15	1.498	0.553				
06-Jul-20	5	1044.83		0.15				0.67	0.07			16.53	114.07	5.06			0.15					0.89	239.69	76.25		0.3																













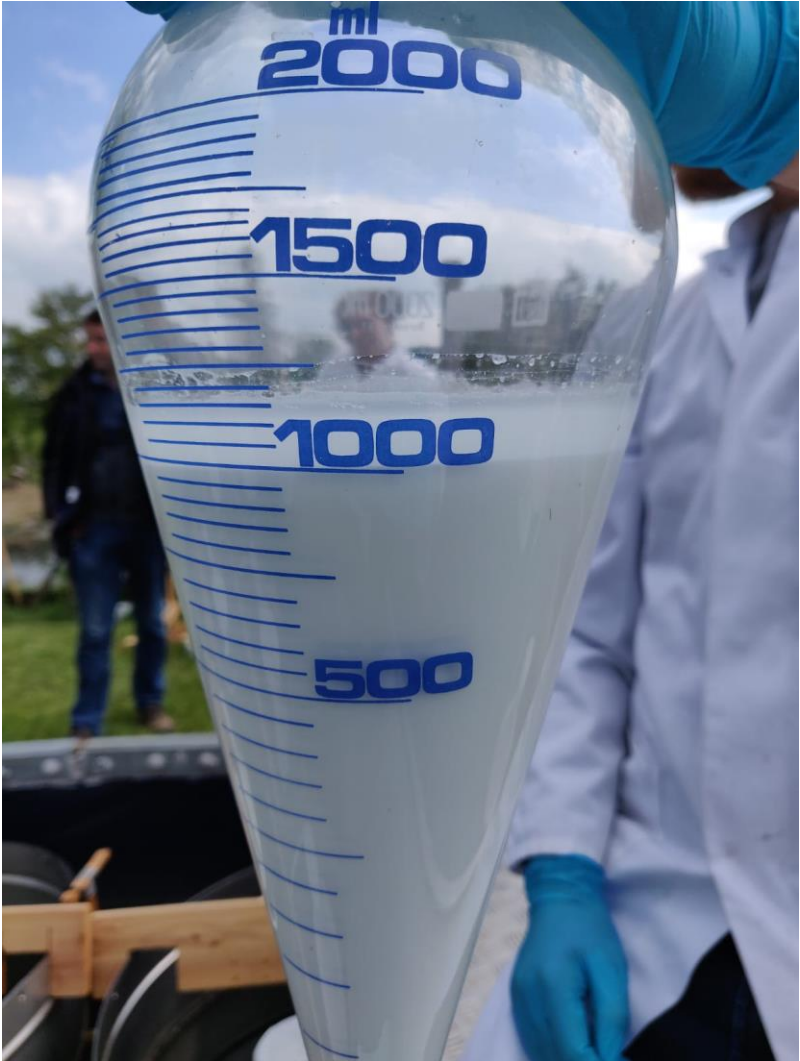


SamplingDate	enclosureName	Gesamtsumme von Individuals/Sample	Anisoptera n_d_sp_	Ceratopogonidae n_d_sp_	Chaoborus sp_	Chironomidae n_d_sp_	Chironomidae n_d_<2mm sp_	Chironominae n_d_sp_	Chironomus sp_	Cloen dipterum	Coenagrionidae n_d_sp_	Coleoptera n_d_sp_	Culicidae n_d_sp_	Curculionidae n_d_sp_	Dixidae sp_	Dytiscidae n_d_sp_	Helophorus sp_	Hydrophilidae n_d_sp_	Myrmecidae n_d_sp_	Nematocera n_d_sp_	Orthocladinae n_d_sp_	Podonominae n_d_sp_	Psychodidae n_d_sp_	Tanyptodinae n_d_sp_	Thysanoptera n_d_sp_	Trichoptera n_d_sp_	SumCironomidae	Sum Odonata	Cloen male	Cloen female	Cloen unident	Chaoborus male	Chaoborus female	Chaoborus unident	Amount_of_species	shannon	evenness		
18-Aug-20	1	74				43				2																72	0	1		1				0	3	0.78	0.71		
18-Aug-20	2	26			3	11				4																	17	0	1		2			2	6	1.49	0.832		
18-Aug-20	3	31			1	6				5																	8	0	1	3	1		1	0	7	1.473	0.757		
18-Aug-20	4	43			7	15		1		1			15														14	0	1		2	1	2	4	7	1.532	0.787		
18-Aug-20	5	34			14	15				1						2											4	0	1	0	5	2	7	4	1.082	0.78			
18-Aug-20	6	35			5	14				9																	6	0	5	2	2		1	4	5	1.398	0.868		
18-Aug-20	7	106			5	75		3		5																	18	0	3	1	1	3	1	1	5	0.935	0.581		
18-Aug-20	8	102			2	76		2		3		1															17	0	2	1	0	1	1	0	7	0.866	0.445		
18-Aug-20	9	24			3	3		1		1	1																18	1	1		0			0	5	0.873	0.542		
18-Aug-20	10	45				23		1			1			1													19				0			0	5	0.961	0.597		
18-Aug-20	11	66			11	24				1						1			4									43	1			1	1	2	8	6	1.331	0.743	
18-Aug-20	12	51			5	29		1		1		1															25	0			1	1	0	2	3	6	1.135	0.633	
18-Aug-20	13	14			8	3				1																	14	0		1	0		4	0	4	1.116	0.805		
18-Aug-20	14	11			4	4				1							1										2				1		4	4	5	1.39	0.863		
18-Aug-20	15	28			6	1				9																	1	0			1	1			4	5	1.3	0.808	
18-Aug-20	16	82			1	61				1									1									11	0	4	4	1	1		5	5	0.714	0.444	
																												18	0			1		1	1	5			

Mesocosm	EMT20	EMT50	EMT80	spec	Mesocosm	EMT20	EMT50	EMT80	spec	Mesocosm	EMT20	EMT50	EMT80	spec	Mesocosm	EMT20	EMT50	EMT80	spec
1	28.3	40.2	52.65	Chaoborus_gesamt	1	13.3	22.7	32.7	Cloeon dipterum_gesamt	1	4.6	17.5	72.2	Chironomidae_gesamt	1	3.75	9.25	23.05	Tanypodinae
2	24.4	36.15	48.8	Chaoborus_gesamt	2	1.5	22.15	32.8	Cloeon dipterum_gesamt	2	5.6	16.05	48.65	Chironomidae_gesamt	2	5.05	13	41.95	Tanypodinae
3	26.85	37.1	48.3	Chaoborus_gesamt	3	8.5	21.2	38.25	Cloeon dipterum_gesamt	3	3.5	12.85	49.8	Chironomidae_gesamt	3	0.5	9.4	43.9	Tanypodinae
4	22.5	30.55	41.05	Chaoborus_gesamt	4	1.6	23.5	34.75	Cloeon dipterum_gesamt	4	4.1	16.95	53.4	Chironomidae_gesamt	4	2.95	9.8	42.1	Tanypodinae
5	28.2	40.85	55.2	Chaoborus_gesamt	5	10.55	20.45	32.7	Cloeon dipterum_gesamt	5	5.15	15.35	56.35	Chironomidae_gesamt	5	2.55	10.25	42.4	Tanypodinae
6	29.05	41	51.95	Chaoborus_gesamt	6	12.85	24.45	41.2	Cloeon dipterum_gesamt	6	5.4	14.4	58.15	Chironomidae_gesamt	6	4.1	10	48.2	Tanypodinae
7	8.25	43.15	54.65	Chaoborus_gesamt	7	5.65	17.4	36.1	Cloeon dipterum_gesamt	7	9	55.2	75.65	Chironomidae_gesamt	7	5.05	13.35	60.15	Tanypodinae
8	26.8	40.75	51.25	Chaoborus_gesamt	8	12.1	23.8	37.65	Cloeon dipterum_gesamt	8	5	11.55	74.7	Chironomidae_gesamt	8	3.05	7.55	17.3	Tanypodinae
9	2.75	33.55	46.35	Chaoborus_gesamt	9	12.8	23.55	36	Cloeon dipterum_gesamt	9	5.25	11.6	32.05	Chironomidae_gesamt	9	4	12.45	64.05	Tanypodinae
10	27.75	38.75	49.9	Chaoborus_gesamt	10	16.15	25.85	36.65	Cloeon dipterum_gesamt	10	7.6	24.5	70.05	Chironomidae_gesamt	10	0	15.55	34.3	Tanypodinae
11	23	31.65	42.95	Chaoborus_gesamt	11	12.85	22.4	33.6	Cloeon dipterum_gesamt	11	4.4	13.4	47.3	Chironomidae_gesamt	11	4.4	11	39.65	Tanypodinae
12	24.65	34.2	44.35	Chaoborus_gesamt	12	8.75	22.75	34.75	Cloeon dipterum_gesamt	12	6.8	32.4	61.75	Chironomidae_gesamt	12	3.65	11.75	43.2	Tanypodinae
13	18	24.85	32.95	Chaoborus_gesamt	13	11.45	22.35	35.95	Cloeon dipterum_gesamt	13	1.95	7.7	22.4	Chironomidae_gesamt	13	2.1	6.15	14	Tanypodinae
14	23.95	31.75	41.8	Chaoborus_gesamt	14	5.85	21.85	33	Cloeon dipterum_gesamt	14	4.2	11.35	31.9	Chironomidae_gesamt	14	0	8.35	24.55	Tanypodinae
15	28.85	39.2	49.5	Chaoborus_gesamt	15	11.2	23.35	36.9	Cloeon dipterum_gesamt	15	3.85	9.65	28.35	Chironomidae_gesamt	15	3.55	8.1	19.1	Tanypodinae
16	3.75	33.45	43.55	Chaoborus_gesamt	16	9.25	20.45	32.9	Cloeon dipterum_gesamt	16	17.95	61.1	75.35	Chironomidae_gesamt	16	0	15.75	50	Tanypodinae
1	28	39.85	51.85	Chaoborus_male	1	11.65	21.5	32.1	Cloeon dipterum_male	1	14.25	35.85	68.55	Chironominae.n_d_sp	1	18.3	31.75	45.05	Coenagrionidae.n_d_sp
2	25.6	36.6	48.45	Chaoborus_male	2	9.65	19.45	29.95	Cloeon dipterum_male	2	0	14.2	55.45	Chironominae.n_d_sp	2	19.95	32.1	46.55	Coenagrionidae.n_d_sp
3	25.2	34.75	46.1	Chaoborus_male	3	8.55	19.75	34.15	Cloeon dipterum_male	3	4.85	10.6	22.4	Chironominae.n_d_sp	3	23.35	36.85	55.05	Coenagrionidae.n_d_sp
4	21.4	1.75	36.95	Chaoborus_male	4	2.25	22.45	32.45	Cloeon dipterum_male	4	9.5	33	59.35	Chironominae.n_d_sp	4	2.55	33.95	44.75	Coenagrionidae.n_d_sp
5	25.1	37.5	52.7	Chaoborus_male	5	10.65	19.75	30.6	Cloeon dipterum_male	5	6.85	12.2	21.1	Chironominae.n_d_sp	5	9.3	29.3	47.8	Coenagrionidae.n_d_sp
6	26.5	39.95	52.3	Chaoborus_male	6	11.7	21.7	35.7	Cloeon dipterum_male	6	10.7	24.35	59.5	Chironominae.n_d_sp	6	17.55	31.55	45	Coenagrionidae.n_d_sp
7	18.3	44.3	53.9	Chaoborus_male	7	4.75	14.05	34.75	Cloeon dipterum_male	7	0.95	6.45	58.6	Chironominae.n_d_sp	7	14.7	22.05	30.05	Coenagrionidae.n_d_sp
8	28.15	40.45	50.65	Chaoborus_male	8	9.4	22.15	36.35	Cloeon dipterum_male	8	0	54.2	73.85	Chironominae.n_d_sp	8	6.05	21.4	33.45	Coenagrionidae.n_d_sp
9	16.2	29.35	44.3	Chaoborus_male	9	9.2	20.85	32.85	Cloeon dipterum_male	9	2.75	7.4	17.6	Chironominae.n_d_sp	9	12	39.1	53.65	Coenagrionidae.n_d_sp
10	27.7	37.7	48.75	Chaoborus_male	10	4.1	26.05	35.85	Cloeon dipterum_male	10	13.6	51.35	71.85	Chironominae.n_d_sp	10	34.55	45.5	56.45	Coenagrionidae.n_d_sp
11	22.6	30.45	40.6	Chaoborus_male	11	13.05	22.1	32.15	Cloeon dipterum_male	11	3.2	8.85	22.05	Chironominae.n_d_sp	11	13.6	29.5	45.4	Coenagrionidae.n_d_sp
12	23.75	32.15	41.55	Chaoborus_male	12	10.15	22.95	35.25	Cloeon dipterum_male	12	0	19.65	49.95	Chironominae.n_d_sp	12	20.4	0.45	37.5	Coenagrionidae.n_d_sp
13	17.4	23.6	30.7	Chaoborus_male	13	11.1	22	35.35	Cloeon dipterum_male	13	3.75	3.75	23.9	Chironominae.n_d_sp	13	21.4	33	43.8	Coenagrionidae.n_d_sp
14	22.3	29.1	0.25	Chaoborus_male	14	8.35	20.95	31	Cloeon dipterum_male	14	11.5	55.2	68.75	Chironominae.n_d_sp	14	22.15	29.6	46.35	Coenagrionidae.n_d_sp
15	26.6	36.55	47.2	Chaoborus_male	15	12	25.1	37.4	Cloeon dipterum_male	15	5.85	18.8	36.7	Chironominae.n_d_sp	15	29	38.95	47.9	Coenagrionidae.n_d_sp
16	23.75	31.75	41.4	Chaoborus_male	16	9.55	19.9	30.95	Cloeon dipterum_male	16	2.65	39.05	67.45	Chironominae.n_d_sp	16	12.7	31.65	46.6	Coenagrionidae.n_d_sp
1	26.85	39.45	51.5	Chaoborus_female	1	12.85	21.75	31.1	Cloeon dipterum_female	1	5.35	5.35	36.95	Orthocladiinae.n_d_sp					
2	21.65	37.25	50.25	Chaoborus_female	2	12.95	23.3	34.65	Cloeon dipterum_female	2	0	0	24.85	Orthocladiinae.n_d_sp					
3	29	39.9	51.35	Chaoborus_female	3	8.45	22.3	40.75	Cloeon dipterum_female	3	3.7	19.7	58.5	Orthocladiinae.n_d_sp					
4	4.05	32.5	43.55	Chaoborus_female	4	13.9	24.55	36.9	Cloeon dipterum_female	4	0	15.9	36.35	Orthocladiinae.n_d_sp					
5	32.05	43.35	54.35	Chaoborus_female	5	9.65	20.3	34.6	Cloeon dipterum_female	5	0	4.35	32.15	Orthocladiinae.n_d_sp					
6	31.7	44.1	54.7	Chaoborus_female	6	12.95	25.5	44.7	Cloeon dipterum_female	6	0	13.45	51.35	Orthocladiinae.n_d_sp					
7	6.3	37.6	54.45	Chaoborus_female	7	6.1	18.1	37.65	Cloeon dipterum_female	7	0.75	12.65	50.85	Orthocladiinae.n_d_sp					
8	19.9	37.15	48.9	Chaoborus_female	8	13.75	25.75	39.55	Cloeon dipterum_female	8	0	8.1	20.55	Orthocladiinae.n_d_sp					
9	5.5	34.2	51	Chaoborus_female	9	13.75	25	40.4	Cloeon dipterum_female	9	0.85	9.3	19.55	Orthocladiinae.n_d_sp					
10	28.95	39.6	49.9	Chaoborus_female	10	17.65	28.4	40.3	Cloeon dipterum_female	10	0.45	10.2	24.5	Orthocladiinae.n_d_sp					
11	2.55	33.1	45.95	Chaoborus_female	11	9.75	21.15	37.5	Cloeon dipterum_female	11	1.15	1.15	11.15	Orthocladiinae.n_d_sp					
12	25.3	35.8	45.35	Chaoborus_female	12	7.1	22.4	34.6	Cloeon dipterum_female	12	0	3.6	21.9	Orthocladiinae.n_d_sp					
13	18.75	26.25	35.45	Chaoborus_female	13	9.7	20.35	36.8	Cloeon dipterum_female	13	0	1.7	22.55	Orthocladiinae.n_d_sp					
14	25.6	34.6	45.55	Chaoborus_female	14	7.45	20.95	33	Cloeon dipterum_female	14	1.15	1.15	17.15	Orthocladiinae.n_d_sp					
15	32.45	42.45	51.75	Chaoborus_female	15	10.6	21.85	37.45	Cloeon dipterum_female	15	0.75	3.8	18.85	Orthocladiinae.n_d_sp					
16	25.2	34.6	45	Chaoborus_female	16	7.75	20.6	34.25	Cloeon dipterum_female	16	0.25	7.45	20.8	Orthocladiinae.n_d_sp					

samplingDate	Mesoscom	temperature	oxygen	ph	conductivity	samplingDate	Mesoscom	temperature	oxygen	ph	conductivity	samplingDate	Mesoscom	temperature	oxygen	ph	conductivity	samplingDate	Mesoscom	temperature	oxygen	ph	conductivity	
26-Mai-20	1	14.9	8.51	7.8	159	16-Jun-20	1	18.3	8.63	8.1	154	22-Jul-20	1	18.4	11.41	10.1	140	18-Sep-20	1	14.5	6.64	7.9	146	
26-Mai-20	2	14.9	7.66	7.9	177	16-Jun-20	2	18.3	8.33	7.9	185	22-Jul-20	2	18.2	13.07	9.85	148	18-Sep-20	2	14.5	8.31	9.1	152	
26-Mai-20	3	14.9	7.62	7.7	172	16-Jun-20	3	18.2	8.81	8.2	165	22-Jul-20	3	18.3	12.91	10.1	142	18-Sep-20	3	14.3	8.92	9.3	146	
26-Mai-20	4	14.9	7.9	7.6	160	16-Jun-20	4	18.3	9.65	8.1	150	22-Jul-20	4	18.2	12.09	9.9	138	18-Sep-20	4	14.4	8	8.6	144	
26-Mai-20	5	15	6.52	7.5	186	16-Jun-20	5	18.2	9.69	8	174	22-Jul-20	5	18.3	13.57	9.94	145	18-Sep-20	5	14.3	8.95	9.4	145	
26-Mai-20	6	15	6.85	7.5	165	16-Jun-20	6	18.3	9.03	7.8	164	22-Jul-20	6	18.2	11.12	8.98	140	18-Sep-20	6	14.3	8.43	8.5	172	
26-Mai-20	7	14.9	7.92	7.7	160	16-Jun-20	7	18.2	9.71	8.1	157	22-Jul-20	7	18.3	13.31	9.55	136	18-Sep-20	7	14.3	9.1	8.9	150	
26-Mai-20	8	14.9	8.41	7.8	156	16-Jun-20	8	18.2	8.02	7.7	160	22-Jul-20	8	18.2	12.72	9.47	138	18-Sep-20	8	14.3	9.25	9.1	140	
26-Mai-20	9	14.9	9.25	7.9	150	16-Jun-20	9	18.3	8.99	7.8	142	22-Jul-20	9	18.2	13.07	9.73	133	18-Sep-20	9	14.3	9	9.1	150	
26-Mai-20	10	14.9	9.2	8.4	162	16-Jun-20	10	18.2	8.62	7.9	168	22-Jul-20	10	17.9	12.92	9.92	146	18-Sep-20	10	14.4	8.72	9	155	
26-Mai-20	11	14.9	8.83	7.8	158	16-Jun-20	11	18.2	8.48	7.7	151	22-Jul-20	11	18.1	12.2	9.29	136	18-Sep-20	11	14.4	9.71	9.1	162	
26-Mai-20	12	15	9.51	8.1	160	16-Jun-20	12	18.3	9.61	8.2	158	22-Jul-20	12	18.1	11.93	9.82	144	18-Sep-20	12	14.5	8	8.4	164	
26-Mai-20	13	15	7.1	7.5	156	16-Jun-20	13	18.3	8.8	7.8	151	22-Jul-20	13	18.6	13.28	9.49	148	18-Sep-20	13	14.3	9.87	9.1	142	
26-Mai-20	14	15	7.35	7.6	178	16-Jun-20	14	18.2	8.73	7.9	173	22-Jul-20	14	18.4	13.38	9.73	143	18-Sep-20	14	14.3	8.67	9.1	162	
26-Mai-20	15	14.9	7.27	7.5	161	16-Jun-20	15	18.1	9.3	7.9	152	22-Jul-20	15	18.5	11.77	9.52	137	18-Sep-20	15	14.3	8.56	8.4	162	
26-Mai-20	16	14.9	7.4	7.7	175	16-Jun-20	16	18.2	8.89	8.2	173	22-Jul-20	16	18.1	13.43	10	158	18-Sep-20	16	14.3	8.55	8.9	177	
29-Mai-20	1	16.9	8.75	8	162	25-Jun-20	1	21	11.92	9.2	137	29-Jul-20	1	18.2	11.54	10.3	142							
29-Mai-20	2	17	7.92	7.9	182	25-Jun-20	2	21.2	10.12	8.5	168	29-Jul-20	2	18.7	11.4	10.1	149							
29-Mai-20	3	16.8	8.38	7.8	175	25-Jun-20	3	21.2	11.42	9	145	29-Jul-20	3	18.7	11.26	10.2	142							
29-Mai-20	4	16.9	8.8	7.8	162	25-Jun-20	4	21.1	11.26	8.9	138	29-Jul-20	4	18.8	10.82	9.92	138							
29-Mai-20	5	16.8	7.64	7.7	188	25-Jun-20	5	21	11.25	8.6	148	29-Jul-20	5	18.7	12.01	10.1	145							
29-Mai-20	6	16.8	6.5	7.6	169	25-Jun-20	6	21.3	11.92	8.5	148	29-Jul-20	6	18.8	9.97	9.05	144							
29-Mai-20	7	16.7	8.31	7.7	162	25-Jun-20	7	21.1	11.8	8.8	146	29-Jul-20	7	18.9	12.16	9.62	138							
29-Mai-20	8	16.7	7.22	7.6	161	25-Jun-20	8	21	11.93	8.1	145	29-Jul-20	8	18.7	11.63	9.85	149							
29-Mai-20	9	16.7	9.42	7.9	152	25-Jun-20	9	21.1	12.02	8.8	130	29-Jul-20	9	18.6	12.12	9.81	132							
29-Mai-20	10	17	9.4	8.3	170	25-Jun-20	10	21.1	10.58	8.7	155	29-Jul-20	10	18.6	11.74	10.2	150							
29-Mai-20	11	16.9	9.22	7.9	159	25-Jun-20	11	21	11.11	8.2	138	29-Jul-20	11	18.7	11.95	9.56	139							
29-Mai-20	12	17	9.56	8.1	162	25-Jun-20	12	21	10.97	8.9	145	29-Jul-20	12	18.5	11.14	9.76	151							
29-Mai-20	13	16.9	7.72	7.6	158	25-Jun-20	13	21	9.59	7.9	144	29-Jul-20	13	18.7	11.01	9.58	149							
29-Mai-20	14	16.8	7.95	7.7	182	25-Jun-20	14	21	11.24	8.6	156	29-Jul-20	14	18.6	12.02	9.91	145							
29-Mai-20	15	17	7.88	7.6	163	25-Jun-20	15	20.9	10.64	8.2	140	29-Jul-20	15	18.7	11.07	9.49	140							
29-Mai-20	16	17.1	7.86	7.8	178	25-Jun-20	16	21.1	10.21	9.2	157	29-Jul-20	16	18.2	11.92	10.3	142							
02-Jun-20	1	18.2	9.7	8.5	164	02-Jul-20	1	20	10.88	9.5	137	06-Aug-20	1	19	10.08	9.87	145							
02-Jun-20	2	18.4	8.52	8	189	02-Jul-20	2	19.8	10.44	8.9	162	06-Aug-20	2	19	10.31	10	151							
02-Jun-20	3	18.2	8.77	7.9	180	02-Jul-20	3	19.9	11.42	9.4	143	06-Aug-20	3	18.6	9.89	10	141							
02-Jun-20	4	18.4	9.14	8	164	02-Jul-20	4	19.9	11.51	9.4	154	06-Aug-20	4	18.9	11.27	9.84	140							
02-Jun-20	5	18.3	8.4	7.8	192	02-Jul-20	5	19.7	11.68	9	173	06-Aug-20	5	18.7	11.54	10	146							
02-Jun-20	6	18.4	8.04	7.7	175	02-Jul-20	6	19.9	10.85	8.7	160	06-Aug-20	6	18.8	8.76	8.52	153							
02-Jun-20	7	18.2	8.92	7.9	166	02-Jul-20	7	19.9	10.99	7.9	162	06-Aug-20	7	18.8	9.59	9.45	142							
02-Jun-20	8	18.2	8.01	7.7	166	02-Jul-20	8	19.8	10.82	8.4	161	06-Aug-20	8	18.8	10.17	9.53	138							
02-Jun-20	9	18.2	9.51	8.1	153	02-Jul-20	9	19.8	10.6	9.9	146	06-Aug-20	9	18.7	10.27	9.56	134							
02-Jun-20	10	18.4	9.48	8.3	175	02-Jul-20	10	19.9	10.79	9.2	169	06-Aug-20	10	18.9	11.29	9.9	149							
02-Jun-20	11	18.2	9.43	8	161	02-Jul-20	11	19.8	11.08	8.8	153	06-Aug-20	11	18.8	9.96	9.28	143							
02-Jun-20	12	18.3	9.74	8.4	167	02-Jul-20	12	19.9	11.02	9.3	163	06-Aug-20	12	18.8	10.35	9.68	148							
02-Jun-20	13	18.4	8.48	7.7	161	02-Jul-20	13	19.9	11.43	8.5	165	06-Aug-20	13	18.8	11.53	9.54	152							
02-Jun-20	14	18.5	7.96	7.7	185	02-Jul-20	14	20	10.66	8.8	173	06-Aug-20	14	18.9	11.12	9.89	147							
02-Jun-20	15	18.2	8.16	7.7	166	02-Jul-20	15	19.8	11.24	8.9	156	06-Aug-20	15	18.9	10.16	9.48	144							
02-Jun-20	16	18.3	8.59	7.9	185	02-Jul-20	16	20	10.67	9.4	178	06-Aug-20	16	18.7	9.8	9.87	162							
05-Jun-20	1	16.3	8.05	7.9	159	06-Jul-20	1	18.5	11.37	9.7	133	12-Aug-20	1	21	8.3	9.83	144							
05-Jun-20	2	16.3	6.47	7.7	185	06-Jul-20	2	18.6	11.34	9.2	153	12-Aug-20	2	21.4	8.88	10	148							
05-Jun-20	3	16.3	7.32	7.7	173	06-Jul-20	3	18.3	12	9.6	138	12-Aug-20	3	21.3	8.43	10.1	137							
05-Jun-20	4	16.3	7.31	7.6	158	06-Jul-20	4	18.5	11.9	9.5	134	12-Aug-20	4	21	8.9	9.86	137							
05-Jun-20	5	16.2	6.99	7.6	183	06-Jul-20	5	18.4	11.71	9.3	147	12-Aug-20	5	21.4	10.3	10.1	141							
05-Jun-20	6	16.3	6.08	7.4	169	06-Jul-20	6	18.4	10.21	9.3	141	12-Aug-20	6	21.4	9.93	9.09	146							
05-Jun-20	7	16.2	8.																					





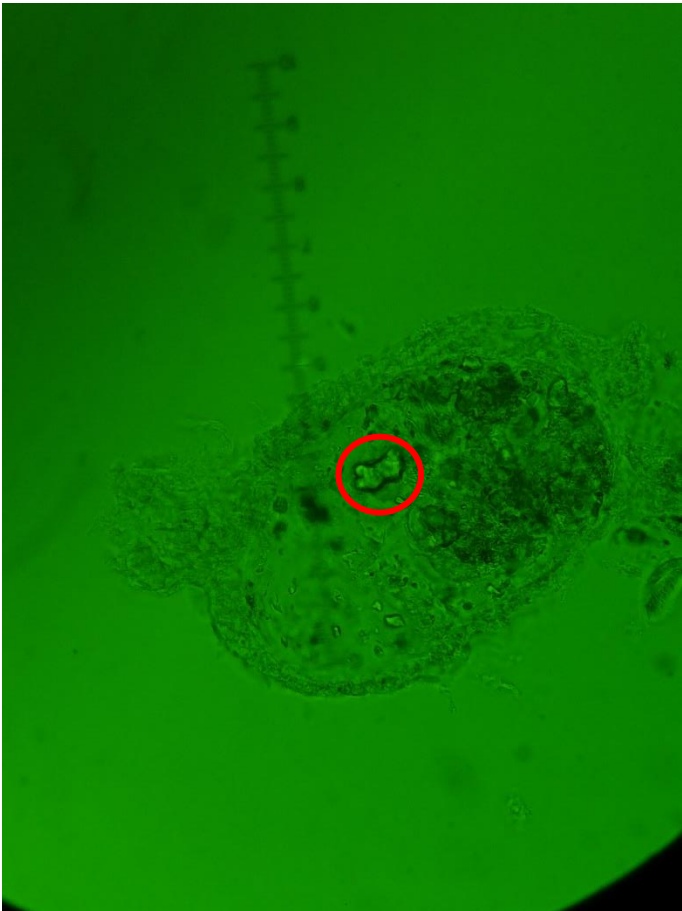
Application solution (HighPA)



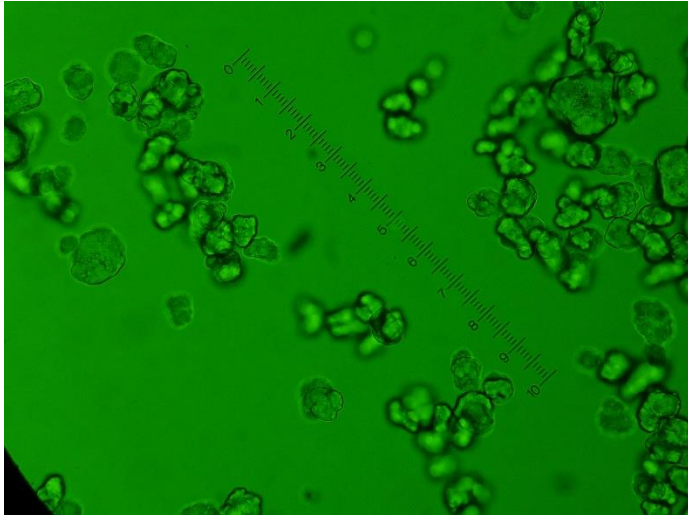
Application solutions (left to right: HighPA/MedPA/LowPA)



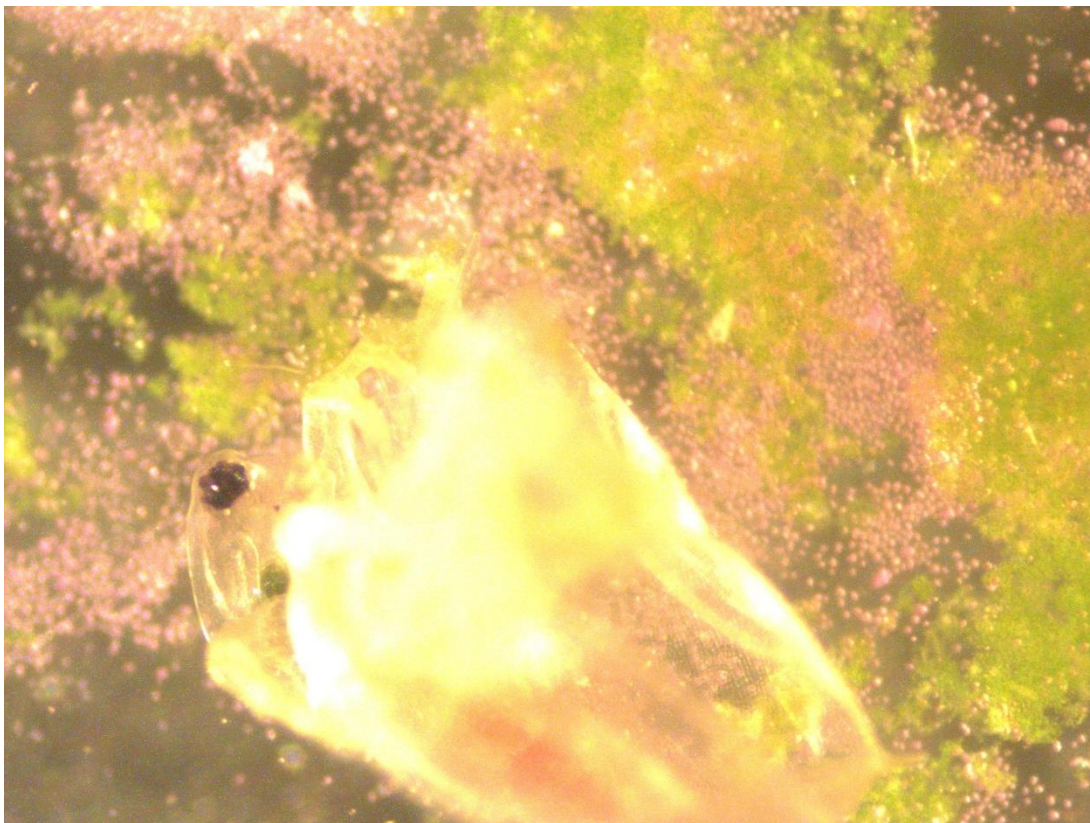
*Keratella quadrata* next to microplastic particles



Ostracoda with microplastic particles and pollen under microscope



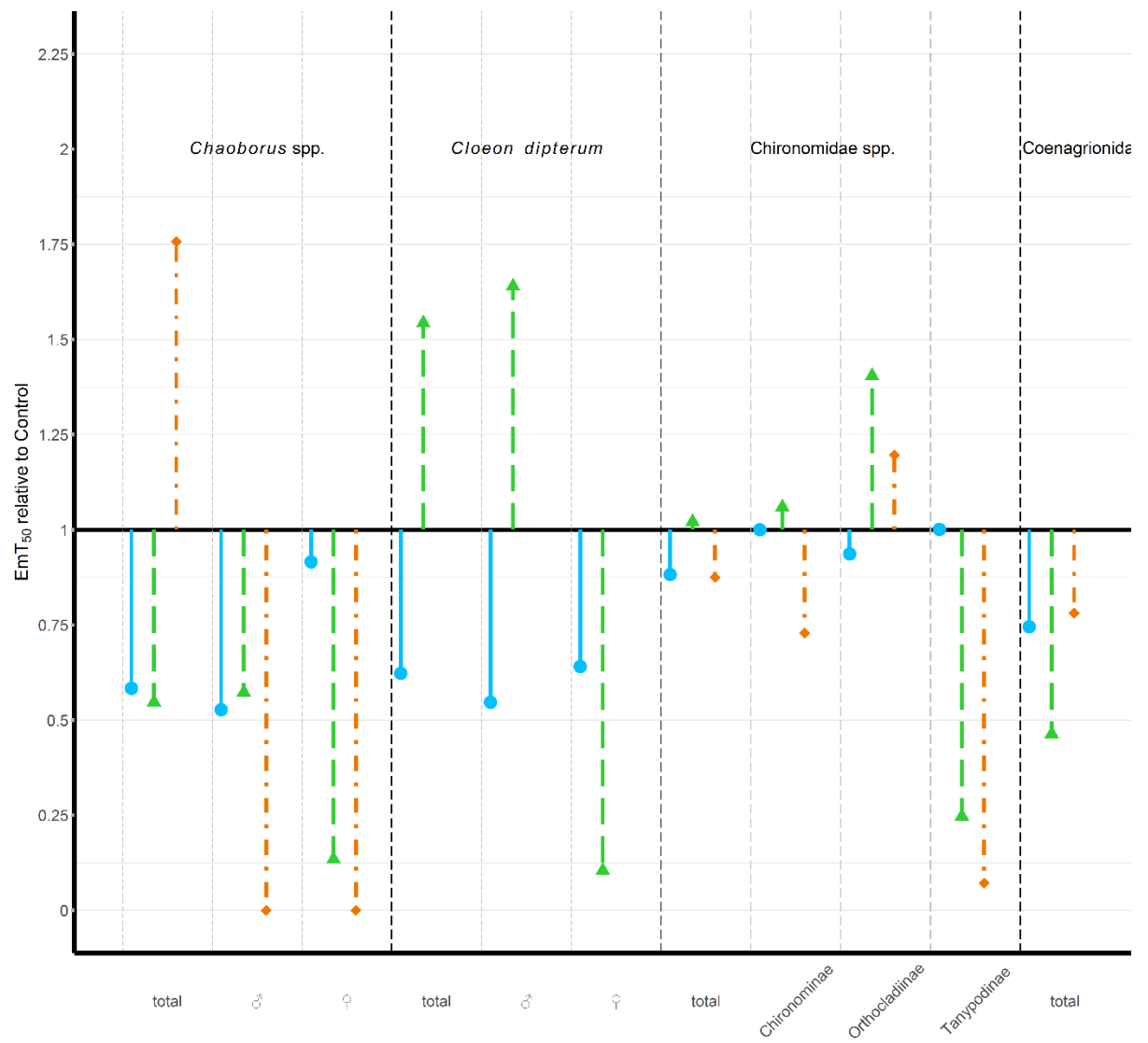
Microplastic particles under microscope



*Daphnia magna* with algae (green) and colored (pink) microplastic particles



*Potamopyrgus antipodarum* bio-assay



Dr. Graner & Partner GmbH, Im Steingrund 2, 63303 Dreieich

Institut für Gewässerschutz  
MESOCOSM GmbH  
Neu-Ulrichstein 5

35315 Homberg (Ohm)

Dreieich, 31.03.2020

## Prüfbericht 2014516\_2

Auftraggeber:	Institut für Gewässerschutz MESOCOSM GmbH
Projektleiter:	Frau Stegger
Auftrags-Nr.:	
Auftraggeberprojekt:	Wasserproben Versuchsteiche
Probenahmedatum:	
Probenahmeort:	Homberg
Probenahme durch:	Auftraggeber
Probengefäße:	Braunglas
Eingang am:	04.03.20
Beginn/Ende Prüfung:	04.03.2020 / 13.03.2020

Die Prüfergebnisse beziehen sich ausschließlich auf den Prüfgegenstand. Eine auszugsweise Vervielfältigung des Prüfberichtes ist nur mit schriftlicher Genehmigung der Prüflaborleitung erlaubt. Die in den zitierten Normen und Richtlinien angegebenen Meßunsicherheiten werden eingehalten. Die aktuellen Ausgabestände der verwendeten Prüfverfahren können auf unserer Homepage (<http://www.labor-graner.de/qualitaetsicherung.html>) eingesehen werden. Unsachgemäße Probengefäße können zu Verfälschungen der Messwerte führen. Prüfergebnisse von Mischproben die unterhalb des Grenzwertes liegen, können trotzdem zu Grenzwertüberschreitungen von einer oder mehreren Teilproben führen. Um die Überprüfung des Grenzwertes sicher zu gewährleisten, wird angeraten, gemäß Prüfvorschrift die Einzelproben zu untersuchen. Mikrobiologisches Untersuchungsmaterial wird nach der Auswertung sofort vernichtet.

**Akkreditiertes Prüflabor nach DIN EN ISO 17025 - D-PL-18601-01-00**  
Arzneimittel, Lebensmittel, Kosmetika, Bedarfsgegenstände, Wasser, Boden, Luft, Medizinprodukte  
Analytik, Entwicklung, Qualitätskontrolle, Beratung, Sachverständigengutachten, amtliche Gegenproben, Mikrobiologie, Arzneimittelzulassung  
Abgrenzungsfragen AMG/LFGB  
Amtsgericht München Nr. 84402, Geschäftsführer: Alexander Hartmann, Dr. Manfred Holz  
Bankverbindung: Genossenschaftsbank Aubing eG (BLZ 70169464) Kr.: 69922  
BIC: GENODEFIM07; IBAN: DE30 7016 9464 0000 0699 22

Prüfbericht: 2014516\_2

31.03.2020

Auftraggeberprojekt: Wasserproben Versuchsteiche

<b>Probenbezeichnung:</b>	<b>Teich 1</b>			
<b>Probenahmedatum:</b>				
<b>Labornummer:</b>	<b>2014516-001</b>			
<b>Material:</b>	<b>Wasser</b>			
<b>Bemerkung:</b>				
	Gehalt	Einheit	Best.gr.	Verfahren
Natrium	8,0	mg/l	1	DIN EN ISO 11885
Kalium	6,5	mg/l	1	DIN EN ISO 11885
Blei	u.d.B.	mg/l	0,0025	DIN EN ISO 17294-2
Cadmium	u.d.B.	mg/l	0,0005	DIN EN ISO 11885
Chrom	u.d.B.	mg/l	0,005	DIN EN ISO 11885
Eisen	0,18	mg/l	0,03	DIN EN ISO 11885
Kupfer	u.d.B.	mg/l	0,01	DIN EN ISO 11885
Mangan	0,021	mg/l	0,01	DIN EN ISO 11885
Nickel	u.d.B.	mg/l	0,01	DIN EN ISO 11885
Quecksilber	u.d.B.	mg/l	0,00005	DIN EN ISO 12846
Zink	u.d.B.	mg/l	0,01	DIN EN ISO 11885
Schwefel gesamt	0,44	mg/l	0,1	DIN EN ISO 11885
PCB Nr. 28	u.d.B.	µg/l	0,005	DIN EN ISO 6468
PCB Nr. 52	u.d.B.	µg/l	0,005	
PCB Nr. 101	u.d.B.	µg/l	0,005	
PCB Nr. 153	u.d.B.	µg/l	0,005	
PCB Nr. 138	u.d.B.	µg/l	0,005	
PCB Nr. 180	u.d.B.	µg/l	0,005	
Summe der bestimmten PCB	0,00	µg/l		



Prüfbericht: 2014516\_2  
 Auftraggeberprojekt: Wasserproben Versuchsteiche

31.03.2020

<b>Probenbezeichnung:</b>	<b>Teich 1</b>			
<b>Probenahmedatum:</b>				
<b>Labornummer:</b>	<b>2014516-001</b>			
<b>Material:</b>	<b>Wasser</b>			
<b>Bemerkung:</b>				
	Gehalt	Einheit	Best.gr.	Verfahren
Atrazin	u.d.B.	mg/l	0,00001	DIN 38407-35 / DIN 38407-36 / DIN 38407-2
Desethylatrazin	u.d.B.	mg/l	0,00001	
Desisopropylatrazin	u.d.B.	mg/l	0,00003	
Sebuthylazin	u.d.B.	mg/l	0,00001	
Simazin	u.d.B.	mg/l	0,00001	
Terbuthylazin	u.d.B.	mg/l	0,00001	
Hexazinon	u.d.B.	mg/l	0,00001	
Chlortoluron	0,000036	mg/l	0,00002	
Diuron	u.d.B.	mg/l	0,00001	
Isoproturon	u.d.B.	mg/l	0,00001	
Methabenzthiazuron	u.d.B.	mg/l	0,00001	
Metobromuron	u.d.B.	mg/l	0,00003	
Metazachlor	u.d.B.	mg/l	0,00001	
Mecoprop	u.d.B.	mg/l	0,00003	
MCPA	u.d.B.	mg/l	0,00003	
Dichlorprop (2,4-DP)	u.d.B.	mg/l	0,00003	
Bentazon	u.d.B.	mg/l	0,00003	
Monuron	u.d.B.	mg/l	0,00002	
Carbofuran	u.d.B.	mg/l	0,00005	
Propazin	u.d.B.	mg/l	0,00002	
Bromacil	u.d.B.	mg/l	0,00001	
Parathion-ethyl	u.d.B.	mg/l	0,00005	
Lindan	u.d.B.	mg/l	0,00005	
Heptachlor	u.d.B.	mg/l	0,00002	
Heptachlorepoxyd	u.d.B.	mg/l	0,00002	
Aldrin	u.d.B.	mg/l	0,00002	
Dieldrin	u.d.B.	mg/l	0,00002	
Summe PSM nach TrinkwV	0,000036	mg/l		

**Akkreditiertes Prüflabor nach  
DIN EN ISO 17025-D-PL-18601-01-00**  
Lochhausener Str. 205  
81249 München  
Internet: [www.labor-graner.de](http://www.labor-graner.de)

**Niederlassung Rhein-Main**  
Telefon +49(0)6103/48 56 98-0  
E-Mail: [info.rm@labor-graner.de](mailto:info.rm@labor-graner.de)

Dr. Graner & Partner GmbH, Im Steingrund 2, 63303 Dreieich

Institut für Gewässerschutz  
MESOCOSM GmbH  
Neu-Ulrichstein 5

35315 Homberg (Ohm)

Dreieich, 16.03.2020

## Prüfbericht 2014517

Auftraggeber: Institut für Gewässerschutz  
MESOCOSM GmbH  
Projektleiter: Frau Stegger  
Auftrags-Nr.:  
Auftraggeberprojekt: Sedimentproben aus Teichen  
Probenahmedatum:  
Probenahmeort: Homberg  
Probenahme durch: Auftraggeber  
Probengefäße: Glasflasche + Glasgefäß  
Eingang am: 04.03.20  
Beginn/Ende Prüfung: 04.03.2020 / 13.03.2020

Die Prüfergebnisse beziehen sich ausschließlich auf den Prüfgegenstand. Eine auszugsweise Vervielfältigung des Prüfberichtes ist nur mit schriftlicher Genehmigung der Prüflaborleitung erlaubt. Die in den zitierten Normen und Richtlinien angegebenen Meßunsicherheiten werden eingehalten. Die aktuellen Ausgabestände der verwendeten Prüfverfahren können auf unserer Homepage (<http://www.labor-graner.de/qualitaetsicherung.html>) eingesehen werden. Unsachgemäße Probengefäße können zu Verfälschungen der Messwerte führen. Prüfergebnisse von Mischproben die unterhalb des Grenzwertes liegen, können trotzdem zu Grenzwertüberschreitungen von einer oder mehreren Teilproben führen. Um die Überprüfung des Grenzwertes sicher zu gewährleisten, wird angeraten, gemäß Prüfvorschrift die Einzelproben zu untersuchen. Mikrobiologisches Untersuchungsmaterial wird nach der Auswertung sofort vernichtet.

**Akkreditiertes Prüflabor nach DIN EN ISO 17025 · D-PL-18601-01-00**  
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Analytik, Entwicklung, Qualitätskontrolle, Beratung, Sachverständigen Gutachten, amtliche Gegenproben, Mikrobiologie, Arzneimittelzulassung  
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Amtsgericht München Nr. 84402, Geschäftsführer: Alexander Hartmann, Dr. Manfred Holz  
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BIC: GENODEFIM07; IBAN: DE30 7016 9464 0000 0699 22

Prüfbericht: 2014517

16.03.2020

Auftraggeberprojekt: Sedimentproben aus Teichen

<b>Probenbezeichnung:</b>	<b>Teich 1</b>			
<b>Probenahmedatum:</b>				
<b>Labornummer:</b>	<b>2014517-001</b>			
<b>Material:</b>	<b>Feststoff</b>			
<b>Bemerkung:</b>				
	Gehalt	Einheit	Best.gr.	Verfahren
Trockenrückstand	42	%		DIN EN 14346
Blei	12	mg/kg TS	0,2	DIN EN ISO 11885
Cadmium	0,14	mg/kg TS	0,1	DIN EN ISO 11885
Chrom	18	mg/kg TS	0,2	DIN EN ISO 11885
Eisen	21000	mg/kg TS	2	DIN EN ISO 11885
Kupfer	14	mg/kg TS	0,2	DIN EN ISO 11885
Mangan	510	mg/kg TS	0,5	DIN EN ISO 11885
Nickel	18	mg/kg TS	0,5	DIN EN ISO 11885
Quecksilber	u.d.B.	mg/kg TS	0,1	DIN EN ISO 12846
Zink	89	mg/kg TS	0,2	DIN EN ISO 11885
TOC	2,5	% TS	0,1	DIN EN 13137
PCB Nr. 28	u.d.B.	mg/kg TS	0,005	DIN EN 15308
PCB Nr. 52	u.d.B.	mg/kg TS	0,005	
PCB Nr. 101	u.d.B.	mg/kg TS	0,005	
PCB Nr. 153	u.d.B.	mg/kg TS	0,005	
PCB Nr. 138	u.d.B.	mg/kg TS	0,005	
PCB Nr. 180	u.d.B.	mg/kg TS	0,005	
Summe der bestimmten PCB	0,00	mg/kg TS		
Atrazin	u.d.B.	mg/kg TS	0,001	DIN 38407-36 / DIN 38407-2
Desethylatrazin	u.d.B.	mg/kg TS	0,001	
Sebuthylazin	u.d.B.	mg/kg TS	0,001	
Simazin	u.d.B.	mg/kg TS	0,001	
Terbutylazin	u.d.B.	mg/kg TS	0,001	
Hexazinon	u.d.B.	mg/kg TS	0,001	
Chlortoluron	u.d.B.	mg/kg TS	0,001	
Diuron	u.d.B.	mg/kg TS	0,001	
Isoproturon	u.d.B.	mg/kg TS	0,001	
Methabenzthiazuron	u.d.B.	mg/kg TS	0,001	
Metobromuron	u.d.B.	mg/kg TS	0,001	
Metazachlor	u.d.B.	mg/kg TS	0,001	
Mecoprop	u.d.B.	mg/kg TS	0,001	
MCPA	u.d.B.	mg/kg TS	0,001	
Dichlorprop (2,4-DP)	u.d.B.	mg/kg TS	0,001	
Bentazon	u.d.B.	mg/kg TS	0,001	
Monuron	u.d.B.	mg/kg TS	0,001	
Carbofuran	u.d.B.	mg/kg TS	0,001	
Desisopropylatrazin	u.d.B.	mg/kg TS	0,001	
Propazin	u.d.B.	mg/kg TS	0,001	
Bromacil	u.d.B.	mg/kg TS	0,001	

Prüfbericht: 2014517

16.03.2020

Auftraggeberprojekt: Sedimentproben aus Teichen

<b>Probenbezeichnung:</b>	<b>Teich 1</b>			
<b>Probenahmedatum:</b>				
<b>Labornummer:</b>	<b>2014517-001</b>			
<b>Material:</b>	<b>Feststoff</b>			
<b>Bemerkung:</b>				
	Gehalt	Einheit	Best.gr.	Verfahren
Parathion-ethyl	u.d.B.	mg/kg TS	0,001	
Lindan	u.d.B.	mg/kg TS	0,01	
Heptachlor	u.d.B.	mg/kg TS	0,01	
Heptachlorepoxyd	u.d.B.	mg/kg TS	0,01	
Aldrin	u.d.B.	mg/kg TS	0,01	
Dieldrin	u.d.B.	mg/kg TS	0,01	
Summe PSM nach TrinkwV	0,0	mg/kg TS		



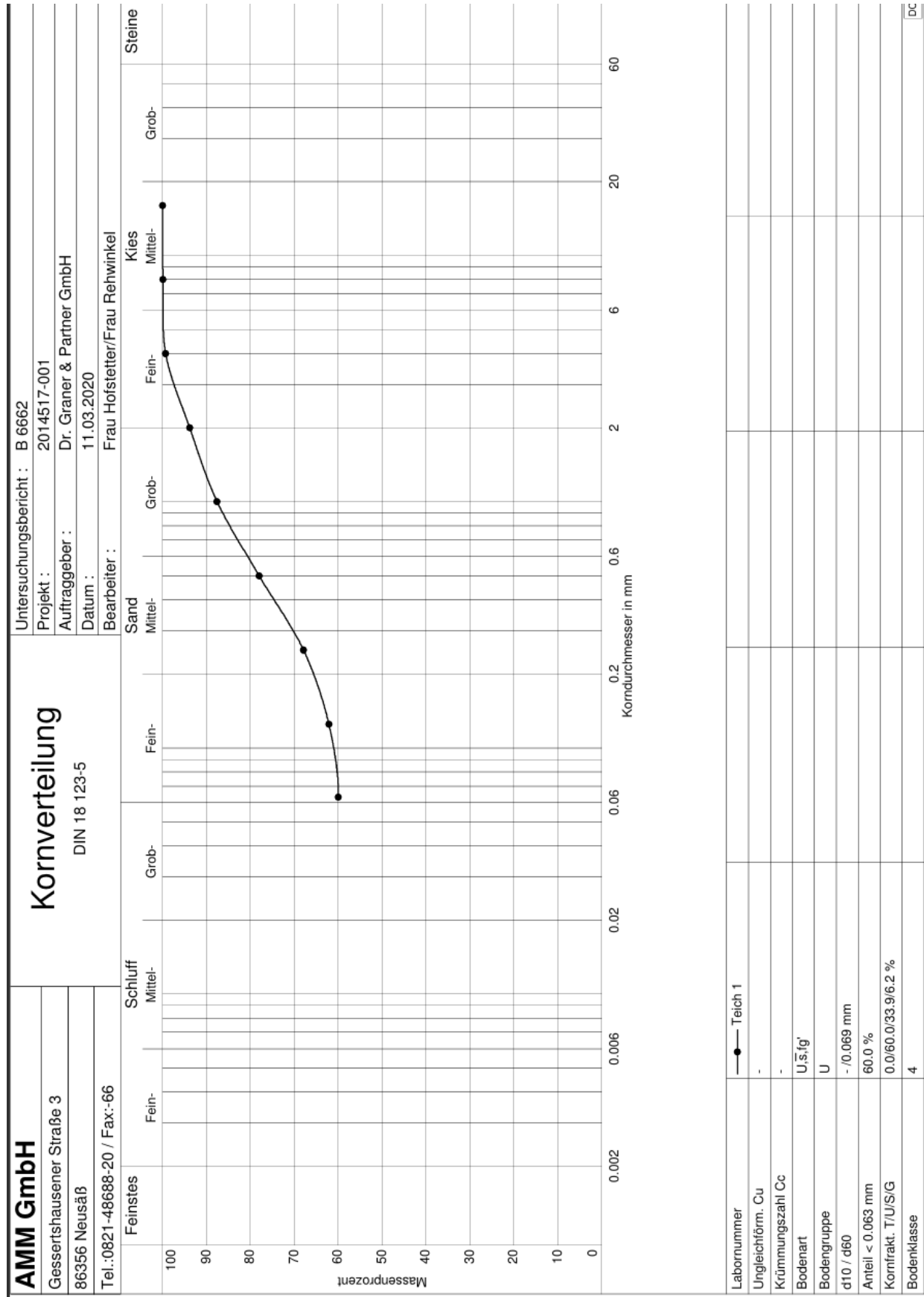
**AMM GmbH**

Gesellschaft für Altlastenmanagement, Mineralstoffverwertung und Materialprüfung mbH  
Gessertshausener Straße 3, 86356 Neusäß

Tel.: 0821 – 48 688-0  
Fax.: 0821 – 48 688-66  
e-mail: info@ammgmbh.com  
web: www.ammgmbh.com

**Untersuchungsbericht B 6662**

Auftraggeber:	Dr. Graner & Partner GmbH
Auftragsnummer:	1
Projektnummer:	2014517-001
Probenahmedatum:	nicht bekannt
Probenort:	nicht bekannt
Probengefäß:	PE-Behälter
Zu untersuchende Parameter:	Korngrößenverteilung
Zeitraum der Prüfung:	10.03. – 11.03.2020



<b>AMM GmbH</b>	U-Bericht: B 6662
Gessertshausener Straße 3	BV / Projektnr.: 2014517-001
86356 Neusäß	Auftraggeber: Dr. Graner & Partner GmbH
Tel.: 0821-48688-20 / Fax: -66	Datum: 11.03.2020
	Bearbeiter: Frau Hofstetter/Frau Rehwinkel

## KORNVERTEILUNG

Teich 1

### SIEBUNG

Durchmesser [mm]	Siebrückstand [g]	Siebdurchgang [%]	Durchmesser [mm]	Siebrückstand [g]	Siebdurchgang [%]
0.000	610.74	0.0	2.000	55.70	93.8
0.063	22.29	60.0	4.000	6.20	99.3
0.125	58.74	62.1	8.000	0.94	99.9
0.250	102.61	67.9	16.0	0.00	100.0
0.500	97.53	78.0	31.5	0.00	100.0
1.000	63.83	87.6	63.0	0.00	100.0

Gesamtgewicht: 1018.58 g

Durchmesser Teich ~~Ø~~ 7.68 m      Ø 9.39 m

Teich genutzt für Studie: \_\_\_\_\_

Befüllung mit Lehm in folgender Schichtdicke: 10 cm 26.10.18 See

Befüllung mit Sediment aus Referenzteich 2      Sediment von 60 cm  
 unterhalb der Wasseroberfläche

Schichtdicke Sediment 8-10 cm      entspricht 112 Wannen à 55 Liter 23.10.18  
See

Befüllung mit Wasser aus      Wasserstand: ca. 80 cm

Referenzteich 2 23.10.18 See

Befüllung mit Sand am 01.11.2018 See

Wasserstand bei Beginn des Vormonitorings: \_\_\_\_\_ cm

Probenahme für Vorab-Rückstandsanalytik in ~~Ø~~ Wasser ~~Ø~~ Sediment am: 04.03.20

Einsetzen von \_\_\_\_\_ Enclosures    Ø 143 cm ø      Ø \_\_\_\_\_ cm ø

Dichtigkeitsprüfung am: \_\_\_\_\_

Grundbepflanzung mit Chara am 28.2.19 TB

Entfernen der Grundbepflanzung am 12.03.2020 LM

Bepflanzung am \_\_\_\_\_ mit:

*Chara* \_\_\_\_\_ in \_\_\_\_\_-Ausrichtung

*Myriophyllum* \_\_\_\_\_ in \_\_\_\_\_-Ausrichtung

*Potamogeton natans* \_\_\_\_\_ in \_\_\_\_\_-Ausrichtung

*Ceratophyllum* \_\_\_\_\_ in \_\_\_\_\_-Ausrichtung

Laubblätter \_\_\_\_\_ in Gesamt -Ausrichtung 18.12.19 LM

date	day	Treatment	Mesocosm	DOC [mg/l]	TOC [mg/l]
06.06.2020	11	1	1	9.2	11
06.06.2020	11	1	2	8.2	9.1
06.06.2020	11	1	3	8.3	8.6
06.06.2020	11	1	4	9.2	10
06.06.2020	11	1	5	8.3	8.4
06.06.2020	11	2	6	8.5	8.6
06.06.2020	11	2	7	9.1	9.3
06.06.2020	11	3	8	8.9	8.9
06.06.2020	11	3	9	8.6	10
06.06.2020	11	3	10	8.5	9
06.06.2020	11	4	11	8.9	9
06.06.2020	11	4	12	8.9	8.8
06.06.2020	11	4	13	8.2	9.5
06.06.2020	11	5	14	8.3	9.1
06.06.2020	11	5	15	8.6	9.6
06.06.2020	11	5	16	8.4	9.4