Microplastics in surface waters and the water column of limnic ecosystems – A case study in the model catchment of Lake Tollense, Germany, with special regard to sampling concepts

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Abstract

Plastics have become an environmental contaminant of raising concern. Their ubiquity in all aspects of human life has led to globally increasing production rates. Plastics may subsequently enter natural ecosystems through various pathways. As most plastic polymers are optimized for durability, plastic waste accumulates in the environment. The fragmentation of larger plastic items as well as the introduction of plastic particles that are produced at a microscopic size leads to the enrichment of such microplastics in all environmental compartments. While the world's oceans represent the final sink for microplastics in the environment, freshwaters are major pathways for microplastics from their predominantly terrestrial sources to the marine environment. Organisms of all trophic levels encounter microplastics in their habitats and may accidentally or intentionally ingest them. Besides adverse effects on the receiving organism, microplastics may be enriched within the food chain and may thus be present in human food.

However, it is not fully clear to which degree microplastics pose a risk to ecosystem function or human health, which is partly due to a lack of consistent data on the abundance of microplastics in the environment. The comparability of data among studies is hampered due to a severe deficiency of methodical harmonization across working groups and an overall insufficient amount of data. Especially freshwaters are underrepresented in microplastic research, despite comprising major sources for microplastics due to the proximity to human activities. Moreover, data on microplastics in freshwaters is mostly limited to comparably large particles (>300 µm) sampled at the surface of the respective water bodies. Thus, only a rather small share of the overall amount of microplastics in freshwaters has been characterized yet.

This dissertation is embedded into the framework of the DFG-funded project MICROLIM (project number 411261467), which aims to budget microplastics across environmental compartments applying harmonized methods. This is necessary for understanding the mechanisms that determine the abundance and distribution of microplastics in freshwater as well as their flow paths. Lake Tollense in Mecklenburg-Western Pomerania was chosen as a model lake in this context. The present dissertation analyzes microplastics in the aqueous phase of the study area, namely in Lake Tollense and its tributaries. Besides enlarging the database on microplastics in freshwater in general, this dissertation specifically aimed to (1) develop and evaluate an *in situ* pump filtration system for consistently sampling small microplastics (<300 µm), (2) evaluate the representativeness of different sampling concepts for microplastics in freshwater, (3) obtain data on microplastic concentrations in the water column of Lake Tollense and connected driving factors, and (4) determine microplastic concentrations and fluxes in the tributaries of the Tollense catchment.

These specific aims were addressed in three consecutive publications that are the foundation of this cumulative dissertation. In the first publication, microplastic concentrations obtained by manta trawling and by sampling with a self-developed *in situ* pump filtration system were compared. Moreover, the influence of sample volume (amount of filtered water) and number of samples on the representativeness of calculated microplastic concentrations was assessed. In the second publication, the vertical concentration profile of microplastics in Lake Tollense was analyzed and distinct vertical gradients were found. These gradients were foremost influenced by particle properties of the sampled microplastics as well as by wind-induced vertical mixing processes. In the third and final publication of this cumulative dissertation, microplastic concentrations and fluxes in tributaries of the Tollense catchment were evaluated. Local hydrodynamics and land cover were found to be of relevance for the obtained microplastic concentrations. Additionally, two in *in situ* pump filtration systems with deviating cut-off sizes were compared.

Microplastic concentrations in the Tollense catchment were generally at an intermediate level compared to previous reports, although it has to be stated that this comparison is hampered due to methodical differences between studies. Identified driving factors for the abundance and distribution of microplastics in the Tollense catchment comprise wind forcing and local hydrodynamics, lateral inputs, anthropogenic activities, and properties of the microplastic particles. The newly developed *in situ* pump filtration system used in all three original publications demonstrated its capability to balance between collecting sufficiently small microplastics (>63 µm) and filtrating sufficiently large amounts of water (1,000 l). It also facilitated the analyses of vertical concentration gradients on a sound number of samples.

Synthesizing the findings of all three original publications, this dissertation emphasizes the importance of sampling concepts for the derived microplastic concentrations. The variability of microplastic concentrations between the sampling systems exceeded the environmental variability (spatial and temporal) determined with a single sampling system by far. Therefore, derived pollution patterns largely depend on the respective sampling concept. Moreover, this dissertation shows that representativeness in microplastic research depends on a complex network of pollution characteristics, such as particle concentrations or shapes, and that representative sampling can unlikely be achieved by a single sampling device.

In the context of MICROLIM, this dissertation could not verify a potential storage function of Lake Tollense based on the microplastics fluxes in its tributaries. The inclusion of cross-compartment data, especially atmospheric deposition, will help to clarify this aspect and allow for estimating a microplastic budget for an entire freshwater catchment for the first time.

Zusammenfassung

Plastik ist ein Umweltkontaminant mit zunehmender Bedeutung. Seine Allgegenwärtigkeit in allen Aspekten des menschlichen Lebens hat zu einer weltweit steigenden Plastikproduktion geführt. In der Folge kann Plastik über verschiedene Wege in natürliche Ökosysteme gelangen. Da die meisten Kunststoffpolymere im Hinblick auf ihre Langlebigkeit optimiert sind, reichert sich Kunststoffabfall in der Umwelt an. Die Fragmentierung größerer Plastikpartikel sowie der Eintrag von Plastikpartikeln, die bereits mikroskopisch klein hergestellt werden, führt zur Anreicherung von solchem Mikroplastik in allen Umweltkompartimenten. Während die Weltmeere die finale Senke für Mikroplastik in der Umwelt darstellen, sind Süßgewässer wichtige Transportwege für Mikroplastik von ihren überwiegend terrestrischen Quellen in die marine Umwelt. Organismen aller trophischer Ebenen treffen in ihren Lebensräumen auf Mikroplastik und können es versehentlich oder absichtlich aufnehmen. Neben schädlichen Auswirkungen auf den aufnehmenden Organismus, kann Mikroplastik in der Nahrungskette angereichert werden und somit auch in der menschlichen Nahrung vorhanden sein.

Es ist nicht vollständig klar, in welchem Ausmaß Mikroplastik ein Risiko für die Funktion von Ökosystemen oder die menschliche Gesundheit darstellt, was zum Teil auf einen Mangel an konsistenten Daten über das Vorkommen von Mikroplastik in der Umwelt zurückzuführen ist. Die Vergleichbarkeit der Daten zwischen den Studien wird durch mangelnde methodische Harmonisierung und eine insgesamt unzureichende Datenlage erschwert. Insbesondere Süßgewässer sind in der Mikroplastikforschung unterrepräsentiert, obwohl sie aufgrund der Nähe zu menschlichen Aktivitäten wichtige Quellen für Mikroplastik zusammenführen. Zudem beschränken sich Daten zu Mikroplastik in Süßgewässern meist auf vergleichsweise große Partikel (>300 µm), die an der Oberfläche der jeweiligen Gewässer gesammelt wurden. Somit ist bisher nur ein relativ kleiner Teil der Gesamtmenge an Mikroplastik in Süßgewässern charakterisiert worden.

Vorliegende Dissertation ist in das von der DFG geförderte Projekt MICROLIM (Projekt-Nr. 411261467) eingebettet, welches darauf abzielt, Mikroplastik über Umweltkompartimente hinweg mittels harmonisierter Methoden zu bilanzieren. Dies ist notwendig, um die Mechanismen zu verstehen, die das Vorkommen und die Verteilung sowie die Flüsse von Mikroplastik in Süßgewässern bestimmen. Der Tollensesee in Mecklenburg-Vorpommern wurde in diesem Zusammenhang als Modellsee ausgewählt. In der vorliegenden Dissertation wird Mikroplastik in der wässrigen Phase des Untersuchungsgebietes, nämlich im Tollensesee und seinen Zuflüssen, analysiert. Neben der Erweiterung der Datenbasis zu Mikroplastik in Süßgewässern im Allgemeinen, hat diese Dissertation folgende spezifische Ziele: (1) Entwicklung und Evaluierung eines *in situ* Pumpenfiltrationssystems zur konsistenten Beprobung von kleinem Mikroplastik (<300 µm), (2) Evaluierung der Repräsentativität verschiedener Probenahmekonzepte für Mikroplastik in Süßwasser, (3) Gewinnung von Daten zu Mikroplastikkonzentrationen in der Wassersäule des Tollensesees sowie den damit verbundenen Steuergrößen und (4) Bestimmung von

Mikroplastikkonzentrationen und -flüssen in den Zuflüssen und dem Abfluss des Tollense-Einzugsgebietes.

Diese spezifischen Ziele wurden in drei aufeinanderfolgenden Publikationen verfolgt, die die Grundlage dieser kumulativen Dissertation bilden. In der ersten Publikation wurden Mikroplastikkonzentrationen verglichen, die mittels Manta Trawl und durch Beprobung mit einem selbstentwickelten *in situ* Pumpenfiltrationssystem gewonnen wurden. Außerdem wurde der Einfluss des Probenvolumens (Volumen des gefilterten Wassers) und der Probenanzahl auf die Repräsentativität der abgeleiteten Mikroplastikkonzentrationen untersucht. In der zweiten Veröffentlichung wurde das vertikale Konzentrationsprofil von Mikroplastik im Tollensesee analysiert und dabei deutliche vertikale Gradienten gefunden. Diese Gradienten wurden in erster Linie durch Partikeleigenschaften des beprobten Mikroplastiks sowie durch windinduzierte vertikale Mischungsprozesse im See beeinflusst. In der dritten und letzten Veröffentlichung dieser kumulativen Dissertation wurden Mikroplastikkonzentrationen und -flüsse in Fließgewässern des Tollense-Einzugsgebietes ausgewertet. Dabei wurde festgestellt, dass die lokale Hydrodynamik und die Landbedeckung für die abgeleiteten Mikroplastikkonzentrationen von Bedeutung sind. Zusätzlich wurden zwei *in situ* Pumpenfiltrationssysteme mit unterschiedlichen unteren Erfassungsgrenzen verglichen.

Im Allgemeinen lagen die Mikroplastikkonzentrationen im Tollense-Einzugsgebiet im Vergleich zu früheren Studien auf einem mittleren Niveau, obwohl darauf hingewiesen werden muss, dass dieser Vergleich durch methodische Unterschiede zwischen den Studien erschwert wird. Steuergrößen, die sichtbaren Einfluss auf das Vorkommen und die Verteilung von Mikroplastik im Tollense-Einzugsgebiet haben, umfassen das lokale Windfeld und die lokale Hydrodynamik, laterale Einträge, anthropogene Aktivitäten und die Partikeleigenschaften des Mikroplastiks. Mittels des hier entwickelten *in situ* Pumpenfiltrationssystem, welches in allen drei Originalpublikationen verwendet wurde, konnte eine Balance zwischen der Erfassung von hinreichend kleinem Mikroplastik (>63 µm) und der Filtration ausreichend großer Wassermengen (1.000 l) hergestellt werden. Das System ermöglichte darüber hinaus die Analyse vertikaler Konzentrationsgradienten auf Grundlage einer soliden Probenanzahl.

Durch die Synthese der Ergebnisse aller drei Originalpublikationen unterstreicht diese Dissertation die Bedeutung der Probenahmekonzepte für die abgeleiteten Mikroplastikkonzentrationen. Die Variabilität der Mikroplastikkonzentrationen zwischen den Probenahmesystemen überstieg die mit einem einzelnen Probenahmesystem ermittelte Umweltvariabilität (räumlich und zeitlich) bei weitem. Daher hängen die abgeleiteten Verschmutzungsmuster stark vom jeweiligen Probenahmekonzept ab. Darüber hinaus zeigt diese Arbeit, dass die Repräsentativität in der Mikroplastikforschung von einem komplexen Geflecht von Verschmutzungsmerkmalen, wie z.B. Partikelkonzentrationen oder -formen, abhängt und eine repräsentative Beprobung mit einem einzelnen Probenahmegerät wahrscheinlich nicht erreicht werden kann.

Im Rahmen von MICROLIM konnte in dieser Dissertation eine mögliche Speicherfunktion des Tollensesees anhand der Mikroplastikflüsse in seinen Zuflüssen nicht verifiziert werden. Die Einbeziehung von kompartimentübergreifenden Daten, insbesondere der atmosphärischen Deposition, wird zur Klärung dieses Aspekts beitragen und erstmals die Abschätzung eines Mikroplastikbudgets für ein ganzes Süßwassereinzugsgebiet ermöglichen.

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1 Introduction

Plastics are omnipresent in the life of most, if not all, people. The term plastics comprises a group of artificial organic polymers, most of which are made of petrochemicals. Thereby, the production of most plastics relies on a non-renewable resource, which is related to various environmental threads (Zalasiewicz et al. 2016, Ivleva et al. 2017). The mentioned plastic polymers constitute a heterogeneous compilation of materials that differ in their properties e.g., in terms of density or durability. Many plastic materials have advantages over traditional resources (e.g., wood or metal), such as being lightweight, cheap, durable, flexible, and adaptable to various applications. These advantages led to the introduction of plastics into almost every aspect of human existence, ranging from everyday products (e.g., food packaging) to highly specialized applications in research or medicine (Brandsch and Piringer 2008, Ryan 2015, Rhodes 2018, Jones et al. 2020). In order to meet the specific application requirements a variety of polymers has been developed, including polyethylene (PE), polypropylene (PP), polyvinylchloride (PVC), polyethylene terephthalate (PET), polyurethane (PU), and polystyrene (PS) as the most common varieties. These polymers account for more than 80 % of the world plastic market (PlasticsEurope 2020). The broad and comparably easy adaptability of plastic materials promoted a drastic increase in global plastic production. Since the first widespread use of plastics in the early 1950s, annual global production rose from 1.5 million tons in 1950 to 368 million tons in 2019 (PlasticsEurope 2008, 2020).

Some of its most advantageous properties, such as its inertness and durability, make plastic a potentially persistent pollutant, when introduced into the environment. The combination of increasing production rates, material longevity, inappropriate (single) use of plastic products, and insufficient or non-existent waste management lead to the introduction of plastic waste into the environment (Barnes et al. 2009, GESAMP 2015, Ivleva et al. 2017, Lambert and Wagner 2018). Several studies showed that plastics are widespread in all environmental spheres including the atmosphere (Cai et al. 2017, Allen et al. 2019, Klein and Fischer 2019), biosphere (Leslie et al. 2017, Bessa et al. 2018, Philipp et al. 2020), cryosphere (Kanhai et al. 2020), hydrosphere (Enders et al. 2015, Faure et al. 2015, Setälä et al. 2016, Kanhai et al. 2018, Bordós et al. 2019) and pedosphere (Woodall et al. 2014, Vaughan et al. 2017, Corradini et al. 2019). Thus, it has been suggested to establish plastics as a stratigraphic indicator of the so-called Anthropocene, the epoch in which humans dominated surface-geological processes on the earth (Crutzen and Stoermer 2000, Zalasiewicz et al. 2016).

Plastic particles in the environment are exposed to environmental conditions that promote their degradation. This degradation leads to the continued fragmentation of plastics into smaller units predominantly due to UV-photooxidation and mechanical abrasion (Barnes et al. 2009, Cole et al. 2011). When plastic particles reach a small size (generally <5 mm in their longest dimension), they are referred to

as microplastics (Thompson et al. 2004, Arthur et al. 2009). The ongoing fragmentation of microplastics may further generate nanoplastics (<1 µm in their longest dimension; Koelmans et al. 2015). Besides their generation by fragmentation, both micro- and nanoplastics are also purposely produced in the respective size class for different uses, such as abrasive scrubs in cosmetics (Fendall and Sewall 2009, Cole et al. 2011, GESAMP 2015) and carrier particles for drugs (Stenzel 2021). Plastic particles contained in such products often end up in the drainage system and may subsequently enter the environment.

The world's oceans, specifically deep ocean basins, serve as final sinks for all microplastics entering the environment (Woodall et al. 2014, Kanhai et al. 2019). Microplastics may be introduced into the ocean by so-called direct or marine inputs on the ocean itself, or may be transported into the oceans from land-based sources (indirect or terrestrial input; GESAMP 2015, Magnusson et al. 2016). Typical marine sources comprise shipping and fishing, while terrestrial sources are more divers. Commonly considered terrestrial sources are, among others, microplastic emissions by individual households (e.g., microbeads from cosmetics or washing of synthetic clothes), plastic-processing industries, agricultural applications, and the abrasion of tire and break wear particles (GESAMP 2015, Magnusson et al. 2016, Lambert and Wagner 2018). Although wastewater from human settlements is usually purified in wastewater treatment plants (WWTPs), even the most sophisticated treating technologies fail to extract all plastics before the water is released into natural (fresh-) water bodies, especially when plastic particles are microscopically small (Mintenig et al. 2017, Edo et al. 2020). Moreover, storm water overflows may cause an uncontrolled release of microplastics into the environment (Horton et al. 2017, Liu et al. 2019, Piñon-Colin et al. 2020). While lakes may function as (temporal) sinks for microplastics, rivers are major vectors for their transport into the oceans. Using modeling approaches it has been estimated that between 0.4 and 4.0 million tons of plastic is annually transported into the oceans by rivers (Lebreton et al. 2017, Schmidt et al. 2017). Additionally, aeolian transport has been demonstrated, especially for microplastics (e.g., Allen et al. 2019).

Once in the environment organisms encounter these plastics, which imposes various potentially hazardous consequences. Animals may suffer from the entanglement with larger plastic debris (e.g., fishing nets) and subsequent immobility (Votier et al. 2011, Ryan 2018). Moreover, it has been shown that especially microplastics can be ingested by various species (Leslie et al. 2017, Bessa et al. 2018, Philipp et al. 2020). The ingestion can happen accidently (e.g., filter feeders) or intentionally, when microplastics are mistaken for food. It is plausible to assume that nanoplastics are likewise ingested, but to the present, no reliable analytical protocols for nanoplastics in environmental samples exist (Koelmans et al. 2015, Sobhani et al. 2020). When microplastics are ingested, the receiving organisms' vitality and thus their capability to survive and reproduce may be negatively affected (Foley et al. 2018, de Ruijter

et al. 2020). Negative effects may occur due to the microplastic particles themselves (food dilution; e.g., Wright et al. 2013, Foley et al. 2018), associated additives, sorbed hazardous substances, such as polychlorinated biphenyls (PCBs, e.g. Wardrop et al. 2016, Mohamed Nor and Koelmans 2019), and pathogens on the particles surface (e.g., Rummel et al. 2017, Murphy et al. 2020). Moreover, microplastics may enrich within the food chain towards higher trophic levels (Carbery et al. 2018, Diepens and Koelmans 2018). Humans are at the endpoint of many food chains. Thus, it has to be considered that microplastics can enter the human body through food, among other pathways, such as inhalation (Cox et al. 2019, van Raamsdonk et al. 2020). Taking into account that adverse effects have been demonstrated for animals due to the uptake of microplastics, it could be assumed that microplastics may pose a potential risk for human health as well (Prata et al. 2020, van Raamsdonk et al. 2020).

To assess the probability of potential risks towards the ecosystem in general and humans in particular, it is necessary to acquire data on the environmental abundance of microplastics. At the current stage, reports on microplastics in the environment suffer from a general incomparability of generated data due to lack of harmonization in sampling and analytics (see chapter 2). Additionally, it is not fully clear to which degree microplastic concentrations depend on the respective sampling concept and whether reported data is reliable (chapter 2.1). Available data is further limited to comparably large microplastics (>300 µm) in many cases, although smaller microplastics are far more abundant and are assumed to be of greater toxicological relevance. Despite representing major pathways for microplastics, freshwaters are generally underrepresented in microplastic research and the potential retention function lakes may have is poorly understood (chapter 2.4).

The concept for this cumulative dissertation was developed targeting the addressed research gaps. The dissertation is embedded into the framework of the DFG-funded project MICROLIM, which aims to model a budget for microplastics in freshwater lakes based on cross-compartment data obtained by applying harmonized methods (chapter 3.1). In this context, this dissertation intends to obtain multi-temporal and multi-spatial, thus reliable, data on microplastics in the aqueous phase of a freshwater lake and its catchment (chapter 3.2). Moreover, a special focus is on evaluating different sampling concepts with respect to the representativeness of retrieved data.

2 State of Research

2.1 Terms and definitions

First reports of microscopic plastic debris in the environment date back to the early 1970s (Carpenter and Smith 1972, Carpenter et al. 1972). Since then, little scientific attention has been paid to plastics as environmental pollutants, until Thompson et al. (2004) introduced the term microplastics and raised concerns about the fate of the million tons of plastics produced each year. Although the term microscopic would imply particles <1 mm in size, microplastics were subsequently defined as plastic particles being <5 mm in their largest dimension (Arthur et al. 2009). For ensuring a comparable nomenclature across studies, it has been argued that a more holistic definition is needed, which is not limited to particle size alone (Hartmann et al. 2019). Hartmann et al. (2019) suggested defining microplastics according to their chemical composition, solid state, solubility, size, shape, structure, color, and origin. Although this definition may describe the nature of microplastics more concise, the majority of literature (GESAMP 2019) applies the original definition according to Arthur et al. (2009).

Microplastics are usually separated into primary and secondary microplastics (Fendall and Sewell 2009, Cole et al. 2011). Primary microplastics are originally produced as microscopic plastic particles and used in a wide range of applications such as cosmetics or industrial applications (e.g., abrasive scrubs). Secondary microplastics arise from the fragmentation of larger plastic items due to environmental conditions. Photooxidative UV-degradation and mechanical fragmentation, e.g., by waves, are considered important processes in this regard (Barnes et al. 2009, Cole et al. 2011).

2.2 Sampling techniques for microplastics in freshwaters

Microplastics in water can generally be sampled implementing either volume-reduced or bulk water approaches (Hidalgo-Ruz et al. 2012). The first refers to enriching particles on filters or in nets, as concentrations in the aqueous phase are often comparably low (compared, e.g., to sediments) (Klein et al. 2018). This enrichment is usually achieved by skimming large water volumes with plankton nets, a technique that was adopted from studies in the marine environment (Eerkes-Medrano et al. 2015, Campanale et al. 2020, Li et al. 2018, Li et al. 2020). Contrastingly, bulk water approaches collect the entire water volume of a sample without reducing it by nets or sieves (Hidalgo-Ruz et al. 2012). Bulk water sampling is usually carried out using either bottles, jars or buckets (Li et al. 2018, Koelmans et al. 2019, Campanale et al. 2020).

Volume-reduced approaches have the advantage that large volumes (several tens m^3) of water can be sampled. To do this, applied nets and sieves are often relatively coarse, typically having a mesh widths of 300-333 µm, which are commonly applied in plankton research (Klein et al. 2018, Li et al. 2018). While finer meshes would be more prone to clogging, coarse meshes are not suitable for quantifying

microplastics smaller than their mesh size. Some authors refer to this fact as non-quantitative or semi-quantitative sampling (Klein et al. 2018, LfU 2019). On the other hand, bulk water samples are often limited to a few liters of water due to logistical or transportation constraints. Yet, they are theoretically able to provide information on very small microplastics, as the lower size limit is then defined by laboratory analytical procedures (Li et al. 2018, Campanale et al. 2020). Considering that the abundance of microplastics increases exponentially with decreasing particle size (Kooi and Koelmans 2019), both volume-reduced and bulk water techniques neglect a certain share of the overall microplastic continuum. For bulk samples, large microplastics are not representatively collected due to their low abundance, which is often below ten particles per m^3 (e.g., Baldwin et al. 2016, Mani et al. 2016, Scherer et al. 2020). For volume-reduced approaches, small particles below the cut-off size (e.g., 300 µm) of the respective system are not quantifiable, although being most abundant in terms of particle numbers (Dris et al. 2015, Heß et al. 2018, Li et al. 2018, LfU 2019).

Recently, *in situ* pump filtration approaches added to methodical diversity in the field of aquatic microplastic research (Setälä et al. 2016, Mintenig et al. 2017, Talvitie et al. 2017, Di and Wang 2018). *In situ* pump filtration approaches allow for targeting smaller microplastics than most net-based approaches, while reaching considerably higher sample volumes than bulk water techniques (Li et al. 2018, Campanale et al. 2020). In some cases, a cascade of sieves with different mesh sizes has been applied to prevent clogging of the finest sieve (Talvitie et al. 2017, Mintenig et al. 2020). A challenging aspect of *in situ* pump filtration systems is that they require a power supply and produce bulk-like samples that can be challenging to analyze (Campanale et al. 2020).

2.3 Sample processing and analysis

Microplastic samples from the aqueous phase are usually a concentrate of suspended particulate matter in residual water. This complex mixture consists of biogenic organic matter, sediments (especially in running freshwaters), and the targeted microplastics (Campanale et al. 2020). Thus, it is necessary to purify the samples by destroying or extracting all natural debris, as the effectivity and speed of the subsequent analysis will largely depend on the samples' purification state.

For freshwater samples, this purification is normally carried out by digesting biogenic organic matter (Ivleva et al. 2017). The extraction of sedimentary debris by density separation is less frequently applied for the treatment of water samples, except for high-energy running waters (Campanale et al. 2020), and is therefore not described here. The digestion of biogenic organic matter is usually conducted by applying different chemical agents or enzymes. Oxidizing agents, specifically hydrogen peroxide, were most frequently applied for investigating microplastics in freshwaters (Li et al. 2018, Koelmans et al. 2019). Moreover, the use of acids (e.g., Eriksen et al. 2013, Cole et al. 2015) and bases

(e.g., Mintenig et al. 2020, Scherer et al. 2020) has been reported. For enzymatic protocols, combinations of different specific enzymes are applied, among which lipase, amylase, proteinase, chitinase, and cellulase have been reported (Cole et al. 2015, Löder et al. 2017). Besides applying different reaction agents to digest biogenic organic matter, reaction temperatures vary considerably between studies (Li et al. 2018, Koelmans et al. 2019). Sometimes samples were opposed to high temperatures (e.g., Bordós et al. 2019, Mao et al. 2020a), which was shown to affect the integrity of various artificial polymers (Munno et al. 2018).

Various methods exist to quantify and subsequently qualify microplastics in environmental samples. Commonly, a visual pre-selection of suspected microplastics is carried out by using a microscope or the naked eye (e.g., Bordós et al. 2019, Stanton et al. 2020, Uurasjärvi et al. 2020). In case of chemical mapping, such as focal plane array Fourier-transform infrared spectroscopy (FPA-FTIR) (Primpke et al. 2017), only subsets of sample filters are usually analyzed. Sub-setting filter areas and pre-selecting particles is necessary to reduce the number of particles to be analyzed, as most chemical analysis techniques are time-consuming and laborious (Koelmans et al. 2019). Chemical analysis techniques in microplastic research comprise µFTIR-spectroscopy (e.g., Bordós et al. 2019, Stanton et al. 2020, Uurasjärvi et al. 2020), µRaman-spectroscopy (e.g., Di and Wang 2018, González-Pleiter et al. 2020, Pan et al. 2020), and pyrolysis-gaschromatography-massspectrometry (e.g., Hendrickson et al. 2018, Becker et al. 2020, Funck et al. 2020).

Nile Red staining has been suggested an alternative technique for quantifying microplastics (Shim et al. 2016, Maes et al. 2017, Tamminga et al. 2017). Nile Red adsorbs to the surface of various artificial polymers based on their surface polarity. Stained particles can be afterwards detected through the emitted fluorescence when excited by an energy source. Various combinations of different wavelengths for excitation and emission have been tested in this regard. However, Nile Red staining is not capable of qualifying microplastics, as no information on their chemical composition can be retrieved. Maes et al. (2017) suggested combining Nile Red staining with existing analytical techniques, such as µRaman-spectroscopy, to speed up the analytical process.

Assuring and controlling the quality of analyses is of particular importance in microplastic research, where analytical procedures are comparably new and under constant development (Hidalgo-Ruz et al. 2012, Torre et al. 2016, Koelmans et al. 2019). Prata et al. (2021) analyzed 50 recent publications dealing with microplastics in various environmental compartments regarding ten contamination control parameters. Contamination control is an essential aspect of quality assurance and control due to the omnipresent potential of laboratory background contamination. In conclusion, they formulated seven vital aspects of contamination control that should be followed to ensure a reliable analysis. These aspects comprise to equip all personnel with cotton laboratory coats, to ensure clean room conditions,

to use cleaned non-plastic equipment, to filter all working solutions, to use high-quality decontaminated filters, to cover all materials and samples to prevent airborne contamination, and to conduct field blanks, procedural blanks, and open filter samples to control for remaining contamination.

2.4 Microplastics in global freshwaters

Microplastic pollution is a global phenomenon. Likewise, the presence of microplastics has been demonstrated in freshwater bodies around the entire world. Microplastics are abundant in freshwater environments of Africa (e.g., Naidoo et al. 2015, Biginagwa et al. 2016, Dahms et al. 2020), North America (e.g., Eriksen et al. 2013, Baldwin et al. 2016, Lenaker et al. 2019) and South America (e.g., Castro et al. 2016, Alfonso et al. 2020, Bertoldi et al. 2021), Asia (e.g., Su et al. 2016, Di and Wang 2018, Peng et al. 2018), Australia (e.g., Nel et al. 2018, He et al. 2020, Nan et al. 2020) and Europe (e.g. Bordós et al. 2019, Mintenig et al 2020, Stanton et al. 2020). Freshwater microplastic pollution was documented in highly urbanized environments (e.g., Dris et al. 2018a, b, Luo et al. 2019) and at some of the remotest places on earth (e.g., Feng et al. 2020, González-Pleiter et al. 2020).

It could be assumed that a large amount of data is available based on the above compiled reports. In fact, the number of studies dealing with microplastics in freshwaters is increasing and this increase accelerated in recent years (Fig. 1). However, freshwaters are still underrepresented in microplastic research when compared to the marine environment.

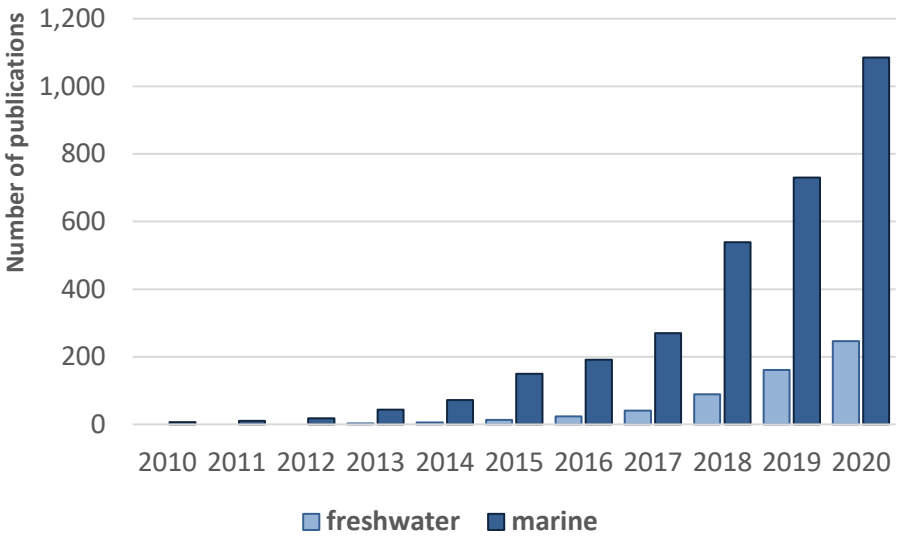

Fig. 1: Number of yearly publications on microplastics in freshwaters and the marine environment based on studies listed in the web of Science.

Moreover, data sets are often incomparable or limitedly comparable amongst studies. The reported concentrations of microplastics per water volume span several orders of magnitude in freshwaters ranging from less than one particle per m^3 to dozens of particles per liter (Koelmans et al. 2019, Scherer et al. 2020). The reasons for this high variability are numerous. Above-described methodical diversity

(chapter 2.1-2.3) is one important aspect leading to hampered comparisons between studies. In this sense, Koelmans et al. (2019) questioned the quality of the available data stating "[…] there is a significant need to improve quality assurance of microplastic sampling and analysis in water samples" (Koelmans et al. 2019).

It should be further noted that the term freshwater summarizes different types of water bodies. For example, rivers and lakes are commonly in the focus of freshwater microplastic research, but provide utterly different conditions (e.g., hydrodynamics). Therefore, comparisons between studies should consider the type of the investigated water body, although this specification reduces the amount of data.

It is known from the marine environment that the concentration of microplastics varies within the water column (Lattin et al. 2004, Kukulka et al. 2012, Kooi et al. 2016, Song et al. 2018, Egger et al. 2020). Nevertheless, the vast majority of studies investigating microplastic pollution in freshwaters are focusing on surface sampling only (e.g., Baldwin et al. 2016, Di and Wang 2018, Scherer et al. 2020). The vertical distribution of microplastics within a water body was rarely examined and most of such investigations were carried out in rivers (Dris et al. 2018a, Liedermann et al. 2018, Eo et al. 2019, Lenaker et al. 2019, LfU 2019). Information on microplastics in the water column of lakes is even scarcer, as only two studies carried out samplings below the water surface (Lenaker et al. 2019, LfU 2019). Lenaker et al. (2019) investigated the water column of Lake Michigan and Milwaukee River using a 333 µm-net and did not find significant differences across the sampled depth levels. Another study investigated microplastics in four different Bavarian lakes (LfU 2019). In this study, microplastics were sampled using vertical net tows (mesh width 300 µm), which do not provide depth-differentiated information. Both studies used relatively coarse nets for sampling and thus no information on microplastics smaller than 300 µm within limnic sub-surface water is available. The scarcity of data on microplastics below common trawling mesh sizes (300-333 µm) is not specific to water column samplings, but is a general limitation in freshwater microplastic research (Horton et al. 2017, Ivleva et al. 2017, Li et al. 2018).

Despite the above-described restrictions, several driving factors could be identified that have considerable influence on the abundance and distribution of microplastics within freshwaters. These driving factors include, but are not limited to, meteorological conditions (foremost rain and wind), the hydrodynamics of the respective water body, and the land cover and anthropogenic activity of the respective catchment.

Rain and associated lateral microplastic input into lakes or streams via surface run-off are generally considered major driving factors for the abundance and distribution of microplastics within freshwaters. Hitchcock (2020) found increased microplastic concentrations in the Cooks River estuary, Australia, after a storm event and stated that microplastic abundance was positively related to antecedent rainfall. Likewise, Xia et al. (2020) observed a strong relationship between microplastic concentrations in surface water and rainfall investigating an urban lake in China.

Wind may influence the abundance and distribution of microplastics within freshwaters in several ways. The surface currents of lakes depend on prevailing wind patterns among other factors and microplastics can be transported by these currents. Imhof et al. (2016) found a distinct spatial gradient of microplastics at the shoreline of Lake Garda, Italy, and related this gradient to strong surface currents induced by local wind patterns. Investigating microplastics in surface waters of Lake Bolsena, Italy, Fischer et al. (2016) found noticeably higher microplastic concentrations after a heavy wind event. They hypothesized that wind-induced vertical water circulation may have led to elevated surface concentrations of microplastics.

Further important aspects are the hydrodynamics of the investigated water body. This is of relevance for lakes (Eriksen et al. 2013), but more prominent for rivers and streams. Mintenig et al. (2020) analyzed the abundance of microplastics in two rivers in the Netherlands and Belgium and found that reduced surface concentrations for microplastics were associated with lower flow velocities. Watkins et al. (2019) reported a relation of seasonal discharge regimes with microplastic abundance and observed a dilution of microplastic concentrations by high stream discharges.

The above-described metrohydrological factors often relate to the mobilization of microplastics from the surrounding land surface. The land cover and associated anthropogenic activities within the respective study area are thereby of severe importance. In this concern, Mani et al. (2016) emphasized that microplastic pollution in the River Rhine was higher in proximity to urban and industrial clusters. This relation has been supported by several other studies (e.g., Baldwin et al. 2016, Kataoka et al. 2019, Mintenig et al. 2020).

Various potential input pathways for microplastics into freshwater environments have been reported in literature. Some of these pathways were already mentioned above (lateral inputs via surface run-off, tributaries and storm waters). Besides, wastewater treatment plants are an important entry point of microplastics into natural water bodies (Mintenig et al. 2017, Talvitie et al. 2017, Simon et al. 2018). WWTPs were occasionally referred to as sources of microplastics in terrestrial environments, but it has to be pointed out that the actual sources of microplastics passing WWTPs are the emitters of wastewater (e.g., household, industry). In fact, WWTPs reduce the amount of microplastics entering

the environment by the treatment process. As some microplastics are not retained in these processes, large amounts of microplastics enter freshwaters through WWTP outlets (Mintenig et al. 2017, Edo et al. 2020). In addition, to the described pathways, direct inputs caused by human activities on or in the specific water body, such as sports and other recreational activities (e.g., kayaking), (professional) fishing, and ship traffic, have to be considered (GESAMP 2019).

Recently, the atmospheric transport of microplastics gained increasing interest (Cai et al. 2017, Allen et al. 2019, Klein and Fischer 2019). Atmospheric deposition of microplastics has also been demonstrated in remote areas, thus suggesting that microplastics may be transported long distances through the air (Allen et al. 2019, Brahney et al. 2020, Zhang et al. 2021). So far, little is known about the rate and variability of this deposition and only few studies have actually assessed the importance of this pathway towards freshwaters (Stanton et al. 2020).

3 Project context and project aims

3.1 The MICROLIM project

The present dissertation integrates into the framework of the DFG-funded project 'Matter budget of microplastics in limnic ecosystems: sources, flow paths and sinks of microplastic particles in the model catchment area of Lake Tollense, Mecklenburg-Western Pomerania' (MICROLIM). The project was granted to Dr. Elke Fischer (Institute of Geography at Universität Hamburg) and was started in 2018. The planned project duration is 36 months.

The main project goals reflect the state of research at the time of application (cf. chapter 2). At that time, many studies addressing microplastic pollution in limnic or terrestrial ecosystems had a rather spotlight-like character. The term spotlight refers to the fact that often single (temporal and spatial) samplings or measurements were conducted to demonstrate the presence of microplastics in an area or a specific environmental compartment. Fewer studies tried to inductively understand the mechanisms that control the abundance and distribution of microplastics and often conclusions were drawn based on little available data. The quality of this data was further limited by the methodical heterogeneity with respect to all analysis steps including sampling, the digestion of biogenic matter, density separation, and the quantification and qualification of sampled microplastics. To address these challenges MICROLIM aims

(1) to develop and evaluate sampling and sample processing procedures that are able to generate representative data on microplastics in multiple environmental compartments,
(2) to sample each of these compartments repeatedly over the entire project duration to ensure that sufficient data is available for drawing solid conclusions on sources, flow paths and sinks of microplastics,
(3) and to model a catchment-scale microplastic budget for limnic ecosystems.

The environmental compartments included in this strategy comprise the surface water and water column of Lake Tollense, the water of its tributaries and the lake's shoreline and lakebed sediments. Moreover, atmospheric deposition is sampled at four locations around Lake Tollense (Fig. 2). All samples are processed based on a modular system of working steps (see the respective original publications for a detailed description of the applied methods). The individual steps are identical for all environmental compartments so that analysis results are comparable across matrices.

The study area and model lake for the project was selected considering the catchment area size, the lake surface area, sufficient water depth, density gradients and mixing cycles, intensity of anthropogenic use, form factors, stream morphology, and access to riparian sediments of relevant exposures.

Lake Tollense, Mecklenburg-Western Pomerania, provides several favorable conditions in this regard. The lake has a comparable simple structure and is sufficiently large and deep to develop a thermal stratification. Moreover, most of the lake's tributaries (Nonnenbach, Gaetenbach, Lieps Channel, Wustrower Bach) are located at its southern shore and only one single outlet (Tollense) drains Lake Tollense at its northern end.

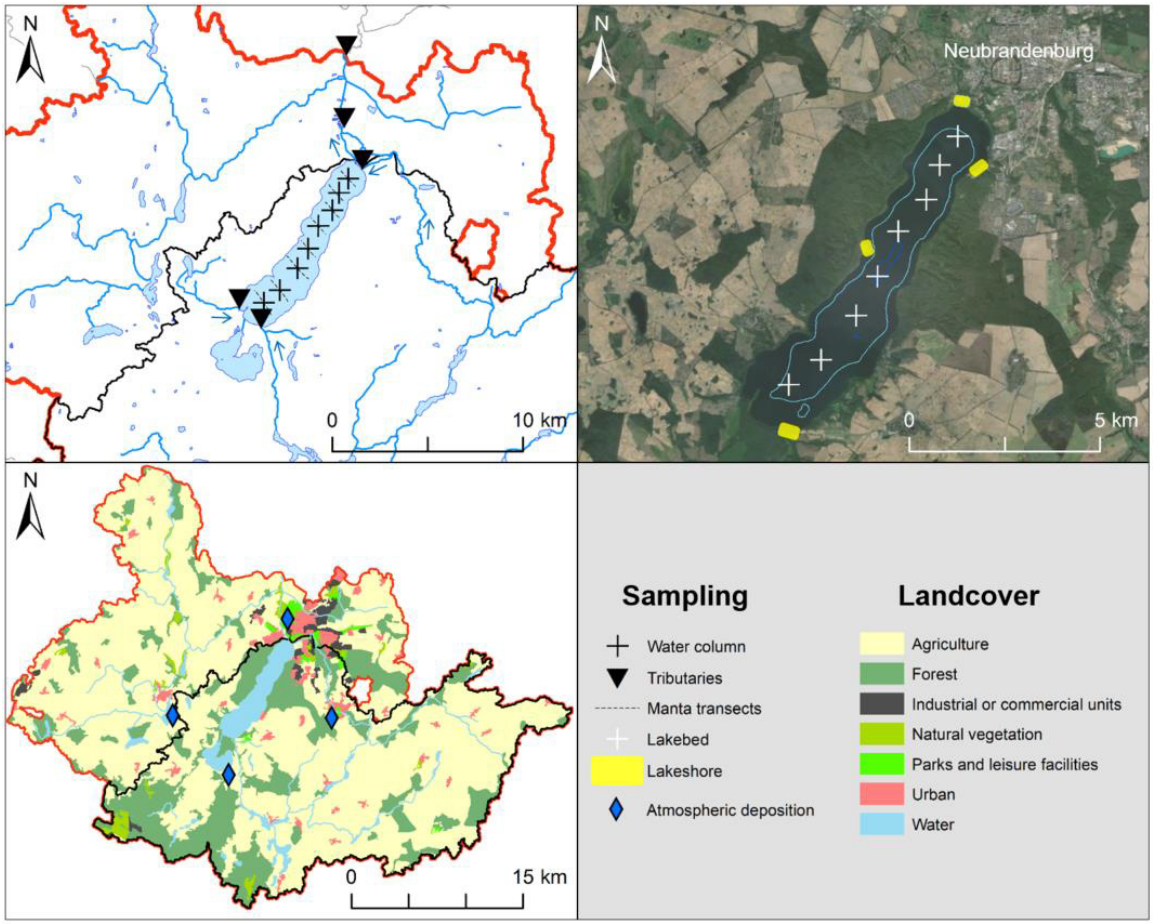

Fig. 2: Lake Tollense, its catchment, tributaries and sampling locations comprising water samples (top left), sediment samples (top right) and atmospheric samples (bottom left). Blue arrows indicate flow directions of tributaries. Coordinate System: ETRS 1989 UTM Zone 33N; Lake shapes and catchment: MLUMV 2017; River network: LUNG 2021; Satellite imagery: ESRI 2021; Land cover data (CORINE): EEA 2021.

3.2 Aims of this dissertation project

Within MICROLIM, this dissertation project investigates microplastics in the water phase of the Lake Tollense catchment. A general aim is to enlarge the database on microplastic pollution and its characteristics (e.g., concentrations, distribution, polymer composition) in freshwater ecosystems. Furthermore, the following specific aims (A-1 to A-4) were formulated based on the research gaps reflected in the literature (see chapter 2):

A-1 Development and evaluation of an *in situ* pump filtration system that is capable of filtrating 1,000 l of freshwater to collect microplastic particles smaller than common manta meshes.

Background: Current sampling approaches targeting microplastics that are smaller in size than common manta meshes often rely on small sample volumes (see chapter 2.2). A common reporting unit for microplastics in (fresh)water is particles per m^3. Developing a sampling device that is capable of capturing small microplastics, while maintaining a large sample volume may help to reduce the uncertainty induced by extrapolating concentrations towards large water volumes.

A-2 Evaluation of the representativeness of different sampling strategies for microplastics in Lake Tollense.

Background: Current sampling techniques cannot be considered representative for the entire microplastic continuum, especially in terms of particle sizes and shapes (see chapter 2.2). It has not yet been shown whether commonly applied manta trawling data is 'translatable' towards other sampling techniques. A comparison of different sampling techniques is necessary to understand the differences that are related to the selected sampling approach.

A-3 Investigation of microplastics in the water column of Lake Tollense.

Background: Currently, only few studies provide information on the vertical distribution of microplastics in the water column of freshwaters and especially lakes (see chapter 2.4). No data on the vertical concentration profile of small microplastics (smaller than common manta mesh width) is currently available. Nevertheless, this data is required for assessing the microplastic population of the entire Lake Tollense (budget approach of MICROLIM) and to evaluate driving factors for the abundance and distribution of microplastics in lakes.

A-4 Assessment of microplastic concentrations and fluxes in the tributaries of the Tollense catchment.

Background: This dissertation projects integrates into the framework of the MICROLIM project (see chapter 3.1). Data on the in- and outputs of microplastics at Lake Tollense is crucial for being able to model a cross-compartment budget for its catchment. Assessing the fluxes of microplastics in Lake Tollense tributaries can further help to evaluate whether the lake may serve as (temporal) sink for microplastics.

To generate a solid database on microplastics in the Lake Tollense catchment, sampling was generally carried out biannually in March (mixed phase) and September (stratified phase). Moreover, multiple stations were sampled at each campaign to account for the spatial variability (see Fig. 2).

3.3 Study area

Lake Tollense is located in northeastern Germany approximately 100 km north of Berlin. The lake is part of the Mecklenburg Lake Plateau in Mecklenburg-Western Pomerania. The landscape of Mecklenburg-Western Pomerania was formed throughout the entire Pleistocene, but most of its present geomorphology developed within the last glacial Weichselian period and by more recent processes (LUNG 2015). It is assumed that Lake Tollense was formed as a tunnel valley by melt water flows under the ice cover during the last glaciation (StALU MS 2017). As a result of its genesis, the lake has a rather uniform depth profile (u-shape) and its shape is comparably simple with a little fragmented shoreline. Due to its depth and the climatic conditions, Lake Tollense is a dimictic lake with mixing phases in March/April and towards the end of October (Nixdorf 2004). Table 1 provides an overview of the morphometric, bathymetric, hydrologic and land cover characteristics of Lake Tollense and its catchment.

Most of Lake Tollenses tributaries enter the lake at its southern shore, while its only discharge, the Tollense (mean discharge: 2.6 m^3), drains the lake at its northernmost point. Main tributaries are the Nonnenbach (0.57 m^3/s), Gaetenbach (0.55 m^3/s), Lieps Channel (0.49 m^3/s) and Wustrower Bach (0.10 m^3/s). Besides, the lake is fed by direct surface run-off from areas at its western and eastern shore (Nixdorf 2004). The catchment of Lake Tollense is characterized by a temperate climate with an annual mean temperature of 9.1 °C and a mean annual precipitation sum of 580 mm (climate station 'Trollenhagen' 1991-2020, DWD 2021). A climate diagram for the study area can be accessed within the supporting material of publication II (see chapter 4.2 and appendix).

Tab. 1: Characteristics of Lake Tollense and its catchment (MLUMV 2017).

Lake	Surface area	17.8 km^2
	Mean depth	17.8 m
	Max. depth	31.3 m
	Volume	315 million m^3
	Shore length	26.7 km
	Retention time	4.1 years
Catchment	Area	522.5 km^2*
	Land cover	64% agriculture, 25% forest, 6% water, 3% urban, 1% natural vegetation, 1% other

*Values computed as described in the original publication III of this dissertation

Present soils of the study were formed on the sandy and loamy substrate that has been deposited glacially and periglacially. Dominant soils are cambisols and luvisols as well as cumulic anthrosol, as most of the area was anthropogenically modified (LUNG 2015). Likewise, the potential natural vegetation of beech and alluvial forests was transformed into a landscape that is dominated by agriculture and forestry (cumulative share 89%, see Tab. 1). Neubrandenburg, which is the only larger town in the area, is partly situated within the lake's catchment (2019: 64,086 inhabitants, StatA MV 2019). The

town is the main gateway for recreational and touristic activities at and on the lake, such as fishing, sailing, kayaking, and surfing. Additionally, regular ferry connections from Neubrandenburg towards the south of Lake Tollense and into Lake Lieps are offered during summer.

4 Overview of original publications

The original publications and connected supporting material of this cumulative dissertation can be retrieved within the appendix. In this chapter, an overview of all three publications is given and the personal contribution by the author is described.

4.1 Publication I

Title	**On the representativeness of pump water samples versus manta sampling in microplastic analysis**
Authors	Matthias Tamminga, Sarah-Christin Stöwer, and Elke K. Fischer
Published	November 2019
Journal and volume	Environmental Pollution 254
DOI	https://doi.org/10.1016/j.envpol.2019.112970
Personal contribution	I developed the concept and most methods of this study (especially regarding the *in situ* pump filtration system). Further, I conducted the sampling, laboratory analysis, data examination, and wrote the original draft of the manuscript. Sarah-Christin Stöwer supported the field sampling and the laboratory analysis and revised the manuscript draft. Elke K. Fischer supervised the study, supplied necessary resources, acquired the funding, and revised the manuscript draft. Moreover, Elke K. Fischer participated in the study's conceptualization.
Abstract	To broaden the understanding of sources, pathways and sinks for microplastic pollution in the environment, the exact and representative determination of pollution levels is crucial. Still, sampling techniques differ greatly between studies and the influence of these differences is not fully understood. Thus, we evaluate the representativeness of manta trawling and pump sampling for microplastics in a freshwater lake. While large microplastics are not captured by most pump sampling approaches due to their low abundance, small and fibrous microplastics pass the relatively coarse nets of volume-reduced techniques. Testing different water volumes for pump samples, we show that sample volumes should be large enough to minimize overestimation induced by scaling up results. Moreover, we discuss the influence of sample numbers for microplastic analysis. Finally, we argue that manta trawling and pump sampling are complementary techniques, as they cover different parts of the overall microplastic pollution.

4.2 Publication II

Title **Microplastics in a deep, dimictic lake of the North German Plain with special regard to vertical distribution patterns**

Authors Matthias Tamminga and Elke K. Fischer

Published December 2020

Journal and volume Environmental Pollution 267

DOI https://doi.org/10.1016/j.envpol.2020.115507

Personal contribution I developed the concept and most methods of this study. Further, I conducted the sampling, laboratory analysis, data examination, and wrote the original draft of the manuscript.
Elke K. Fischer supervised the study, supplied necessary resources, acquired the funding, and revised the manuscript draft. Moreover, Elke K. Fischer participated in the study's conceptualization.

Abstract The investigation of microplastics (MPs) in freshwater has received increased attention within the last decade. To date, sampling is mainly conducted at the surface of both rivers and lakes and only a few studies assessed the vertical distribution of MPs in the water column of freshwater bodies. To contribute to the understanding of MP pollution in the water column of freshwater lakes, this study evaluated the vertical profile of MPs in Lake Tollense considering particles between 63 and 5000 µm in size. Sampling was conducted on three occasions at three depths (surface, 7 m and 10 m) along a transect including eight sampling stations. The retrieved samples were digested with hydrogen peroxide and sodium hypochlorite and investigated via Nile Red staining and fluorescence microscopy. Subsequently, a sub-sample of stained particles was verified by µRaman-spectroscopy. The vertical distribution of MPs in Lake Tollense differed considerably between particle shapes (irregular particles (IPs) and fibers). Fibers did not show a noticeable pattern with depth and ranged between 22 fibers m^{-3} at 0 m to 19 fibers m^{-3} at 10 m. In contrast, IPs were distinctly less abundant in sub-surface samples with concentrations between 50 IPs m^{-3} at 0 m to 29 IPs m^{-3} at 10 m. Concerning IPs, buoyant polymers (mainly PE and PP) and concerning fibers PET and PP dominated the polymeric composition. Besides particle inherent properties, wind-induced mixing is likely affecting the intensity of vertical concentration gradients. This study highlights the need for depth-integrated sampling approaches in order to achieve representative data without over- or underestimating the overall abundances.

4.3 Publication III

Title **Microplastic concentrations, characteristics, and fluxes in water bodies of the Tollense catchment, Germany, with regard to different sampling systems**

Authors Matthias Tamminga, Elena Hengstmann, Ann-Kristin Deuke, and Elke K. Fischer

Published September 2021

Journal Environmental Science and Pollution Research

DOI https://doi.org/10.1007/s11356-021-16106-4

Personal contribution I developed the concept and most methods of this study. Further, I conducted the sampling, laboratory analysis, data examination, and wrote the original draft of the manuscript.
Elena Hengstmann and Ann-Kristin Deuke supported the field sampling and the laboratory analysis and revised the manuscript draft. Elke K. Fischer supervised the study, supplied necessary resources, acquired the funding and, revised the manuscript draft. Moreover, Elke K. Fischer participated in the study's conceptualization.

Abstract The widespread presence of microplastics in multiple environmental compartments has largely been demonstrated. Assessing the ecological risk that microplastics pose is, at the present stage, hindered due to methodical differences. Moreover, different methods hamper meaningful comparisons between studies and data on small microplastics (<300 µm) is scarce. Therefore, we focused on small microplastics (>20 µm) in freshwater and sampling-related aspects in this concern. Sampling was conducted between 2018 and 2020 in the Tollense catchment in northeastern Germany and was carried out by *in situ* pump filtration. Two different sampling systems (cut-off sizes 20 µm and 63 µm) were applied to filter water volumes of 0.075-1.836 m^3. Retained particles were analyzed by a combination of Nile Red staining and µRaman-spectroscopy. Thereby, we found microplastic concentrations between 123 and 1,728 particles m^{-3} using the 63 µm cut-off size and between 1,357 and 2,146 particles m^{-3} using the 20 µm cut-off size. Local hydrodynamics (discharge and flow velocity) and land cover are likely influencing the observed microplastic concentrations and fluxes. The variability between both sampling systems cannot fully be explained by the different mesh sizes used. We argue that a differentiation between a theoretical cut-off size (finest mesh) and a factual cut-off size (reliable quantification) can help to understand sampling related differences between studies.

5 Summarizing discussion

In the following, a synthesis of the original publications of this dissertation is carried out. The respective publication will be referred to according to the publication numbering in chapter 4. A focus is set on those aspects that are reflected with importance in at least two of the three publications. Moreover, the relevance of the presented results with respect to this dissertation's aims and for the MICROLIM project is highlighted. In this regard, the aims A-1 and A-2 are addressed in chapter 5.1 and the aims A-3 and A-4 are addressed in chapter 5.3.

5.1 Relevance of sampling techniques

The impact of methodical differences on data comparability across microplastic studies is widely discussed in literature (see chapter 2.1-2.4). However, few studies analyzed uncertainty related to and representativeness of different sampling concepts (Hildebrandt et al. 2021). In this regard, the present dissertation evaluates three different sampling concepts for microplastics in the aqueous phase. Key characteristics of the sampling systems used as well as the related microplastic concentration are shown in table 2.

Tab. 2: Key characteristics of applied sampling systems and compilation of microplastic pollution data obtained with the respective sampling system. RSD: Relative standard deviation, IP: Irregular particle (cf. Hartmann et al. 2019).

Sampling device	**Manta trawl**	**63 µm pump system**	**20 µm pump system**
Applied in publication	I	I, II, and III	III
Sample number (sum)	8	130	12
Sampling volume (m^3)	129-187	0.075-3	0.7-1
Lower cut-off size (µm)	300	63	20
Absolute number of counted particles	231	10,511	11,083
Mean (median) concentration ($\#*m^{-3}$)*	0.18 (0.13)	61 (58)	489 (496)
Concentration Range ($\#*m^{-3}$)*	0.05-0.49	17-134	234-625
Standard deviation*	0.14	32	118
Coefficient of variation (or RSD)*	80%	52%	24%
Particle shapes (Mean Fibers/IPs %)*	20/80	36/64	23/77
Polymers	PE>PET>PP>PS>PA	PET>PE>PP>PA>PVC	PET>PP>PE>PA>PVC

*only lake surface samples considered

Microplastic concentrations are utterly different comparing the three applied sampling systems. The variability of microplastic concentrations between the sampling systems exceeds the systems internal variability (spatial and temporal variability) by far. The choice for one system used for sampling may thus be of greater relevance than the season in which the sampling is conducted. In accordance with these findings, Hildebrandt et al. (2021) assessed the uncertainty of two centrifugal separators for microplastic sampling in the Elbe estuary (Germany) by calculating an expanded uncertainty model (cf. JCGM 2008). Within the Elbe estuary, sampling contributed the highest share of uncertainty (~70%)

followed by the analytical procedure (~16%), thus, the authors conclude that many observed patterns in literature might be insignificant (Hildebrandt et al. 2021).

The most striking difference between the different systems is their lower cut-off size. The lower cut-off size does not equal the size limit for the reliable quantification of microplastics. This is due to the fact that size is commonly reported as length in microplastic research, although length is an inaccurate descriptor for size fractions obtained by sieving/filtering (cf. publication III, Hung et al. 2021). Different fractions of the overall microplastic abundance are targeted, when applying different cut-off sizes. Soundly estimating numerical concentrations for microplastics requires the collection of small particles, taking into account that the microplastic abundance increases exponentially with decreasing size (publications I-III). Collecting small microplastics and ensuring large sample volumes are conflicting goals due to several reasons (cf. chapter 2.2). The 63 µm pump filtration system developed here can be seen as compromise in this regard (publication I and Tab. 2). However, this system is less suitable for sampling large microplastics, which abundance is well below one particle per m^3 in Lake Tollense. In this regard, the results of publication I suggest that manta trawling and pump filtration can be seen as complementary rather than competing techniques. Similarly, Hung et al. (2021) compared different sampling methods investigating microplastics in the Bay of San Francisco (USA) and concluded "Multiple methods may be used to provide complementary data on a wider range of sizes […]" (Hung et al. 2021).

Sampling different size fractions due to the respective system's cut-off size has further implications on the derived pollution characteristics, such as particle shape. The share of fibers is higher for the 63 µm pump samples compared to manta samples but lower compared to the 20 µm pump samples (see Tab. 2). This is well explained by the size histograms of microplastics from the Tollense catchment, which consistently show that fibers do not follow the general exponential increase of microplastic abundance with decreasing size (publication I and II). While the relatively thin fibers (diameters often <50 µm) may slip through the coarse manta net (300 µm), they are more reliably retained in the 63 µm sieve of the pump filtration system (cf. Karlsson et al. 2020). When further reducing the cut-off size to 20 µm the relative increase in the abundance of fibers is below that of irregular particles. Therefore, irregular particles then represent a higher share of all particles. As the polymeric composition of microplastics in Lake Tollense differs significantly by particle shape, the relative abundance of polymer types also varies due to the relative frequency of particle shapes. Accordingly, Hung et al. (2021) emphasized that the array of microplastic particle characteristics is highly dependent on the collection method employed.

Beyond these general aspects of sampling representativeness, this dissertation evaluated the influence of sample volume and the number of samples on the robustness of calculated concentration data. The

63 µm pump filtration system was used exemplarily (publication I). The results suggested that scaling up concentrations obtained for some hundred liters to concentrations per m³ leads to extensive variation, which is generally supported by former studies (Rios Mendoza and Balcer 2019, Skalska et al. 2020). Moreover, based on a resampling experiment (cf. Bancin et al. 2019) the importance of repeated samplings is highlighted (publication I). Various studies have emphasized that sampling in replicates is essential for obtaining reliable microplastic concentrations (Bruge et al. 2020, Karlsson et al. 2020, Hildebrandt et al. 2021).

5.2 General comparison with previous studies

Reported microplastic concentrations in global freshwaters usually span several orders of magnitude across studies (see chapter 2.4). To enable a meaningful comparison of microplastic concentrations obtained in this project with former reports, a selection of previous studies was compiled based on either methodical or geographical comparability (Tab. 3). These pre-conditions severely restrict the number of available studies. The comparison is further limited to samples from surface waters of Lake Tollense that were retrieved using the 63 µm pump filtration system, as this system was used in all three original publications (see chapter 4). Moreover, the implications associated to sampling concepts are addressed above (see chapter 5.1). Finally, microplastic concentrations of the present studies were corrected with respect to the deviating lower cut-off sizes in literature following Koelmans et al. (2020). A detailed description of the correction procedure and a comparison of microplastic concentrations in the tributaries of Lake Tollense with former studies are accessible in publication III.

Compared to previous studies, microplastic pollution in Lake Tollense is at an intermediate level. Despite the applied correction, methodical differences still seem to have a prominent influence on the reported microplastic concentrations. Studies showing similar microplastic pollution levels as the studies within this dissertation are characterized by approaches that target small microplastics (<300 µm) while maintaining comparably large water volumes (Bordós et al. 2019, Uurasjärvi et al. 2019). Bordós et al. (2018) investigated natural freshwaters and fishponds within the Carpathian basin using a similar *in situ* filtration system as applied here. They reported a mean concentration of 13.8 particles per m³, which is at the lower end of the compiled range of this project. This might be due to considerably high digestion temperatures applied in Bordós et al. (2018), which may cause a degradation of plastics and thereby lead to an underestimation of the actual microplastic abundance (cf. Munno et al. 2018; Pfeiffer and Fischer 2020). Similarly, microplastic concentrations in this dissertation are comparable to values of Uurasjärvi et al. (2019), who sampled microplastics down to 20 µm in size in Lake Kallavesi bordering the city of Kuopio in Finland. The actual microplastic concentrations in Lake Kallavesi are slightly lower than in Lake Tollense. In this concern, the authors state that their actual cut-off size might

be higher than 20 µm due to the manual selection of particles, which might explain the observed difference.

Tab. 3: Comparison of microplastic abundance in freshwaters. Temperature (Temp.) refers to the highest temperature applied. Concentrations in this dissertation were corrected following Koelmans et al. (2020). N.a.: not applied, n.r.: not reported.

		Sampling			Sample processing		Conc. (MP/m³)	
Reference	**Country**	**Method**	**Volume (l)**	**Cut-off (µm)**	**Digestion Temp.**	**Identification**	**Mean/Median Min-Max**	**This study, corrected (Min-Max)**
Felismino et al. 2021*	CAN	Grab samples	4	125	n.a.	Visual, FTIR, Raman	40/n.r. 0-n.r.	8-67
Mao et al. 2020b*	CHN	Steel buckets	20	75	H_2O_2 (30%) 60 °C	Visual, FTIR	n.r./n.r. 3,120-11,250	14-112
Wang et al. 2018	CHN	Pump, filtration	20	50	H_2O_2 (30%)	Visual, Raman	1,737/n.r. 900-4,650	21-169
Xia et al. 2020*	CHN	Pump, filtration	20	50	H_2O_2 (30%)	Visual, Raman	14,010/n.r. n.r.	21-169
Uurasjärvi et al. 2019	FIN	Pump, filtration	6-468**	20	n.a.	Visual, FTIR	168.8/n.r. n.r.	54-426
LfU 2019	GER	Manta Trawl	n.r.	300	Enzymes	Visual, SWIR, FTIR	n.r./4 <1-42	3-27
Present studies	*GER*	*Pump, filtration*	*1,000*	*63*	*H_2O_2 (30%), NaClO (6-14%)*	*Nile Red, Raman*	*61/58 17-134*	-
Bordós et al. 2018	HUN	Pump, filtration	1,492**	100	H_2O_2 (30%) 80 °C	Visual, FTIR	13.8/n.r. 3.5 - 32.1	11-84
Egessa et al. 2020	UGA	Manta Trawl	n.r.	300	WPO 90 °C	Visual, FTIR	0.73/n.r. 0.02-2.19	3-27

*originally reported in particles per liters, **Mean of sampling volumes

Two studies investigating microplastics in Bavarian lakes and Lake Simcoe (Canada), respectively, reported comparable concentrations as in this dissertation, despite of severe methodical differences (LfU 2019, Felismino et al. 2021). In case of the Bavarian lakes, it has to be considered that microplastics below the cut-off size of the sampling system were included in reported concentrations. Thereby, these concentrations may not be fully comparable to this project (LfU 2019). In case of Lake Simcoe, only two polymer types could be identified using either Raman- or FTIR-spectroscopy consequently questioning the reliability of the overall analytical procedure.

One of the very scarce studies on microplastics in African freshwaters found microplastic concentrations that are considerably below those of the present dissertation (Egessa et al. 2020). The authors conducted manta trawling in the Ugandan part of Lake Victoria and reported a concentration range of microplastics that is rather typical for manta trawling in freshwaters (cf. Baldwin et al. 2016, Mani et al. 2016, Scherer et al. 2020). The observed difference towards microplastic concentrations at Lake Tollense might be due to the intensive digestion procedure, as discussed above for Bordós et al. (2019).

Moreover, it must be considered that the difference concerning the cut-off sizes used is too large to be compensated by the applied correction.

Among the considered studies, those reporting higher microplastic concentrations than in this dissertation investigated microplastics in Chinese lakes (Wang et al. 2018, Mao et al. 2020b, Xia et al. 2020). On the one hand, this may be induced by the low sample volumes (20 l), which might not be representative of larger water volumes (cf. publication I). On the other hand, these three studies state a severe impact of human activities within the catchments of the respective lakes. Moreover, a high degree of mismanaged waste and insufficiently treated wastewater may contribute to the comparably high abundance (Wang et al. 2018, Xia et al. 2020).

5.3 Driving factors for abundance and distribution of microplastics

The abundance and distribution of microplastics in the Lake Tollense catchment is characterized by a high degree of both temporal (publications I and II) and spatial (publications I-III) variability. Vertical concentration gradients of microplastics in Lake Tollense varied considerably between two consecutive sampling days and might even show a diurnal distribution pattern, as described in publication II. Likewise, the raster sampling experiment conducted in publication I revealed a high spatial heterogeneity of microplastic abundance even when sampled on the same day. The observed factors influencing these variation patterns are manifold and complex. They comprise, but are not limited to, wind forcing and local hydrodynamics, lateral inputs through tributaries, anthropogenic activities in the catchment and on the lake as well as properties of the microplastic particles such as particle shape.

In accordance with previous studies, wind forcing and related surface currents did have a noticeable influence on the distribution of microplastics in Lake Tollense (cf. Fischer et al. 2016, Imhof et al. 2016). The influence of wind forcing can be observed by the fact that surface microplastic concentrations increased from SW to NE, when coinciding with southwesterly winds. This is evident in publications I and II and the influence of wind is thereby visible across different sampling systems (manta trawl and 63 µm pump system). Moreover, wind speeds correlated with the intensity of vertical distribution gradients (publication II). These gradients were less pronounced when higher wind speeds occurred that induced vertical mixing within the water column. Although no information on the influence of wind on the vertical distribution of microplastics in freshwaters is available, findings of studies in marine environment support this relation (Lattin et al. 2004, Kukulka et al. 2012, Kooi et al. 2016, Song et al. 2018, Egger et al. 2020). In contrast to conditions on the open oceans, the local wind fields nearby freshwaters are influenced by the roughness of the surrounding landscape. Thus, the described relation is not constant (publication II).

Microplastic concentrations in the tributaries of Lake Tollense are up to one order of magnitude higher than in the lake's surface water (publication I-III). Thus, these tributaries can be considered an important pathway for microplastics into Lake Tollense, which is generally in line with previous reports (e.g., Baldwin et al. 2016). However, it could not be verified that Lake Tollense may serve as a (temporal) sink for microplastics based on the sampled tributaries, as calculated output fluxes were mostly higher than inputs (publication III). Although the major tributaries of Lake Tollense were included in this calculation, further lateral inputs (smaller tributaries and direct surface runoff), direct inputs (such as shipping and water sports), and atmospheric inputs may lead to an underestimation of the actual microplastic fluxes into Lake Tollense (cf. Allen et al. 2019, Klein and Fischer 2019).

Microplastic pollution in the Lake Tollense catchment reflected anthropogenic pressure in all three publications of this dissertation. The observed microplastic concentrations were often higher in proximity to the town of Neubrandenburg, which is the economic and touristic center of the region. While for lake surface samples this may partly be attributed to wind forcing (publication I and II), higher microplastic concentrations were observed in tributaries that are characterized by higher shares of urban land cover (publication III). Likewise, the importance of human activities and settlements for microplastic pollution in freshwaters has been emphasized in several former studies (e.g., Baldwin et al. 2016, Mani et al. 2016, Kataoka et al. 2019).

Besides these environmental or external drivers, particle properties of the sampled microplastics, such as particle shape or size, were strongly related to distribution patterns. Fibers, for example, did not show vertical concentration gradients, but were rather uniformly distributed across Lake Tollense's water column (publication II). Waldschläger and Schüttrumpf (2019) found that the terminal settling is distinctly lower for fibers when compared to fragments, cylinders or spheres in freshwater, thus supporting the idea that the particle shape influences the vertical distribution of microplastics (see also Khatmullina and Isachenko 2017). In publication III, microplastics down to 20 µm in size were targeted. The inclusion of particles >20-63 µm (compared to the 63 µm pump filtration system) did severely reduce the spatial heterogeneity of microplastic concentrations, thereby emphasizing that very small microplastics may behave differently within the environment compared to larger particles (cf. Song et al. 2018).

5.4 Polymeric composition of microplastics in the study area

In this dissertation, a comprehensive data set on polymer types of microplastics within the Lake Tollense catchment was generated comprising a total of 777 particles analyzed via µRaman-spectroscopy (see publication I-III). Figure 3 displays the relative abundance of recorded polymer types by particle

shape, as irregular particles (cf. Hartmann et al. 2019) and fibers showed significantly different compositions. The data of all original publications was combined to allow for the evaluation of potential sources of microplastics in the study area regardless of the sampling system used or the specific location sampled.

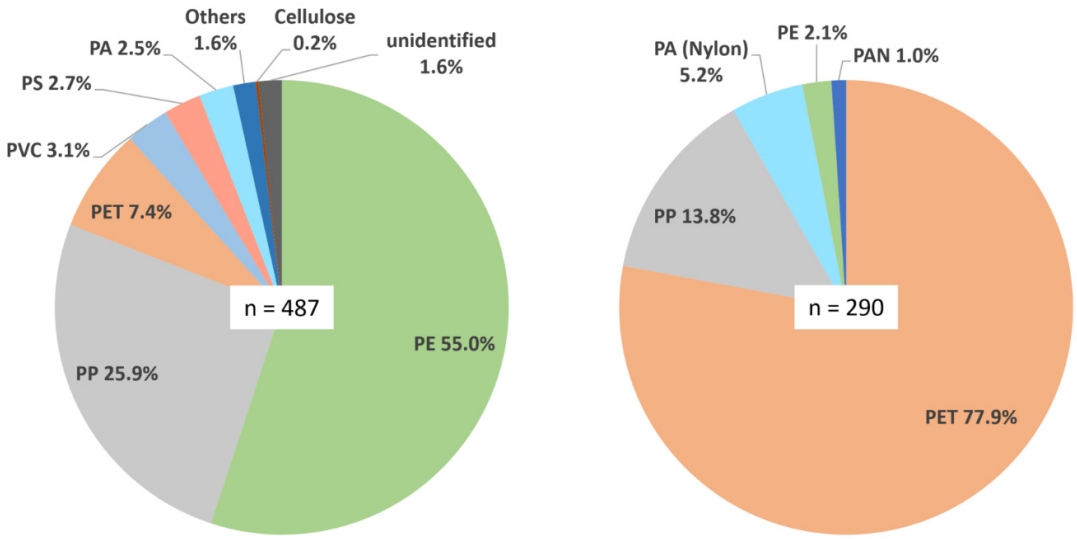

Fig. 3: Polymeric composition of particles from the Tollense catchment for irregular particles (left) and fibers (right).

In total, twelve different artificial polymers were identified across all samplings and their general shares are comparable to former studies in freshwaters (Koelmans et al. 2019, Jones et al. 2020). Polyolefins (PE and PP) dominated the composition of irregular particles beyond their market share (cumulative: 49.2%, PlasticsEurope 2020). Both polymers are widely used in various applications of which packaging or wrapping of consumer products is most prominent (e.g., food packaging or trays and plastic bags). In this regard, local human activities, such as recreation, sports, and tourism, are one likely source for microplastics in the Lake Tollense catchment. PE foils are additionally used in agriculture, which dominates land cover of the Tollense catchment and has thus to be considered as a further important source (see publication III, Jones et al. 2020). The remaining polymer types are underrepresented in water samples from the Lake Tollense catchment compared to their market share, which can be attributed to their non-buoyant characteristics, except for PA (Koelmans et al. 2019, Jones et al. 2020, PlasticsEurope 2020). PVC is commonly used in long-term applications, such as building and construction, which might lower the probability of PVC being discarded into the environment (cf. Jones et al. 2020). The use of chloroform as a solvent for Nile Red in this project (see original publications) might explain the low share of PS found, as chloroform can dissolve PS (cf. Tamminga et al. 2017).

The vast majority of microplastic fibers found were composed of PET, which is a common raw material in clothing production (Jones et al. 2020, PlasticsEurope 2020). Polyester clothing is omnipresent and

thereby a likely source for microplastic fibers within the study area. PP fibers can be related to shipping and (professional) fishing and both activities are carried on Lake Tollense (cf. Song et al. 2018). Nylon (PA) fibers were found less frequently in samples of the Tollense catchment. Nylon fibers are less sensitive to UV-induced fragmentation and thus might be less numerous in the environment (Sørensen et al. 2021).

6 Conclusion and Outlook

This dissertation provides one of the most comprehensive datasets on microplastic pollution within a freshwater catchment comprising biannual samples from three consecutive years and collected by three different sampling systems. A total of 21,825 microplastic particles were extracted from these samples and characterized concerning their shape and size, thus providing a broad and solid database with respect to the above formulated aims (A-1 to A-4). Specific conclusions with regard to this dissertation's aims are:

A-1 Development and evaluation of an *in situ* pump filtration system that is capable of filtrating 1,000 l of freshwater to collect microplastic particles smaller than common manta meshes.

The *in situ* pump filtration system applied in most samplings of this project enables the collection of particles down to 63 µm in size. For lake samples, at least 1,000 l of water could be filtered regardless of the sampling season and depth due to the implemented cascade of testing sieves. The system concentrates dozens to hundreds of particles in a few 100 ml of water, therefore, facilitating to record a strong environmental microplastic signal in each sample.

A-2 Evaluation of the representativeness of different sampling strategies for microplastics in Lake Tollense.

This dissertation emphasizes that representativeness is a complex network of factors in microplastic research, not only involving numerical environmental concentrations, but also relying on particle shape and size as well as further pollution characteristics. Therefore, none of the applied sampling systems is capable of providing representative information on the entire microplastic continuum present in the Lake Tollense catchment. Microplastic concentrations obtained by using these systems are partly convertible with respect to the targeted size range, but considerable variation remains. In fact, sampling concepts might induce the majority of observed variability of environmental microplastic concentrations amongst available studies, thus questioning concluded pollution patterns.

A-3 Investigation of microplastics in the water column of Lake Tollense.

Microplastic pollution in Lake Tollense showed distinct vertical patterns that are influenced by particle shape and the local wind field. This allows for a more robust estimation of the total amount of microplastics in the lake, which is necessary with respect

to the balancing approach of the MICROLIM project. In contrast, the simple extrapolation of surface concentrations towards the entire water column might lead to a severe overestimation of the total microplastic amount in the lake.

A-4 Assessment of microplastic concentrations and fluxes in the tributaries of the Tollense catchment.

Microplastic fluxes in the Tollense catchment were analyzed throughout the entire project duration and were related to the local hydrodynamics and land cover. Based on the sampled tributaries, it could not be verified that Lake Tollense serves as a (temporary) sink for microplastics, as output-fluxes generally exceeded inputs of microplastics into the lake. However, it was pointed out that the inclusion of additional environmental compartments, especially the atmosphere, might help to resolve this issue.

To finally understand the role that lakes play for microplastics along their journey from terrestrial sources towards the world's oceans, it is necessary to examine data of all relevant environmental spheres. In this concern, combining the data of all MICROLIM sub-projects is the next logical step. For the first time, it will then be possible to estimate a microplastic budget for an entire catchment based on solid empirical data and generated by harmonized methods.

This dissertation demonstrated that the validity of microplastic pollution characteristics depends on the applied methods, especially concerning sampling concepts. Though sampling is the starting point of all empirical evidence in microplastic research, sampling concepts have not received the attention necessary to allow for meaningful comparisons between studies. It is thus required to answer the call for harmonization to finally inform society if and to what extend microplastics pose a risk to the environment and to us humans.

Publications not associated with this dissertation

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Appendix: Original publications

Appendix: Publication I

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On the representativeness of pump water samples versus manta sampling in microplastic analysis☆

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ABSTRACT

To broaden the understanding of sources, pathways and sinks for microplastic pollution in the environment, the exact and representative determination of pollution levels is crucial. Still, sampling techniques differ greatly between studies and the influence of these differences is not fully understood. Thus, we evaluate the representativeness of manta trawling and pump sampling for microplastics in a freshwater lake. While large microplastics are not captured by most pump sampling approaches due to their low abundance, small and fibrous microplastics pass the relatively coarse nets of volume-reduced techniques. Testing different water volumes for pump samples, we show that sample volumes should be large enough to minimize overestimation induced by scaling up results. Moreover, we discuss the influence of sample numbers for microplastic analysis. Finally, we argue that manta trawling and pump sampling are complementary techniques, as they cover different parts of the overall microplastic pollution.

1. Introduction

The ubiquity of microplastics in a wide range of natural habitats has been proven in recent years (Graca et al., 2017; Zobkov and Esiukova, 2017; Bellas et al., 2016; Enders et al., 2016; Gusmão et al., 2016, Setälä et al., 2016). Microplastics have most frequently been defined as plastic particles <5 mm in their longitudinal extension (Arthur et al., 2009). Lately, the reliability and common understanding of this concept has been questioned and a framework of how to define microplastics on a more holistic basis has been suggested. This includes the chemical composition, solid state, solubility, size, shape and structure, color, and origin as criteria to define microplastics (Hartmann et al., 2019).

However, the description or measurement of size is not consistent across studies (Hartmann et al., 2019, Filella, 2015). Particle sizes are either reported as a result of actual measurements, e.g. via microscopes (Xiong et al., 2018; Enders et al., 2015; Isobe et al., 2014), or as fractions generated by sieves, nets or filters with a given mesh size (Li et al., 2018). The latter is often applied for practical sampling-related reasons but lacks the comparability of reported results as size fractions vary across studies (Koelmans et al., 2019; Eerkes-Medrano et al., 2015). Particles may coagulate with organic debris (Dris et al. 2018b; Heβ et al., 2018), and their shape is crucial in defining whether or not it is retained by a sieve/net (Dris et al. 2018a; Heβ et al., 2018; Setälä et al., 2016).

Common techniques applied for sampling microplastics in (fresh)water comprise the use of nets (e.g. manta or bongo trawls) towed behind a vessel (Baldwin et al., 2016; Fischer et al., 2016; Eriksen et al., 2013) or lowered from bridges as drift nets (Dris et al. 2018b; Heβ et al., 2018) and pump-based approaches using sieves or filters (Bordós et al., 2019; Lenz and Labrenz, 2018; Setälä et al., 2016; Enders et al., 2015; Desforges et al., 2014; Lusher et al., 2014). While the use of nets enables the filtration of large amounts of water, it mostly lacks the ability to cover plastic particles below 300 μm (Setälä et al., 2016), which is the typical mesh size of nets used in microplastic research (Mai et al., 2018). In contrast, most pump- or filtration-based approaches do not allow sampling large volumes of water (Dris et al. 2018b).

Thus, the question of how to sample microplastics representatively arises. Larger microplastics (e.g. > 300 μm) need to be concentrated to robustly assess their abundance in water, as their concentration is commonly below one particle per m^3. While several m^3 of sampling volume would be needed in this case, smaller microplastics (<300 μm) allow lower sampling volumes, since they present in higher concentrations (Lenz and Labrenz,

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2018). This calls for a minimum volume to be set to improve the representativeness and comparability of studies. Likewise, the influence of sample numbers should be assessed, especially when it comes to heterogeneous spatial distributions.

Koelmans et al. (2019) have assessed the quality of reported data across 50 studies on microplastics in drinking water and freshwater bodies recently, concluding that only few studies provide a satisfying data quality. Part of this analysis was the evaluation of the respective sampling method and sample size. Regarding surface freshwater, an absolute minimum volume of 500 l per sample is suggested based on reported concentrations of microplastics between $1*10^{-3}$ to 10 particles per l. Though, the authors state that for lower concentrations a larger volume might be needed.

We thus empirically assess the representativeness of manta trawling and a pump sampling system concerning the analysis of microplastics in a freshwater lake considering the sample volume and the sample number.

This study is associated to the DFG-funded project 'MICROLIM' (Matter budget of microplastics in limnic ecosystems: sources, flow paths and sinks of microplastic particles in the model catchment area of Lake Tollense, Mecklenburg Western-Pomerania).

2. Materials and methods

The general nomenclature for the analysis of microplastics has been adopted from Hartmann et al. (2019). In particular, this concerns the size range for the definition of microplastics, the definition of size as the largest dimension of a respective particle, and the categorization of shape into irregular particles (including often-used categories fragments and films) and fibers.

2.1. Study area

The Lake Tollense catchment is located in the northeastern part of the Mecklenburg Lake District in Mecklenburg-Western Pomerania, Germany, and comprises a total area of 374 km^2 (see S 1 in the supplementary information). The lake's longitudinal axis is oriented from south-west to north-east with a shoreline showing low levels of fragmentation. Most tributaries are located in the southern part of Lake Tollense, while the only discharge drains the lake at its northernmost point. The prevailing wind direction is west to southwest (DWD, 2018).

Land use within the catchment is dominated by agriculture and forestry (see S 2). Neubrandenburg, the only larger town (2017: 64,259 inhabitants, SAMV, 2018) partly situated within the catchment at the northern shore of Lake Tollense, is the main gateway for recreational activities on the lake. These include fishing, sailing, kayaking, and surfing, and are mostly carried out in the northern part of the lake. Regular ferry connections from Neubrandenburg towards the south and into the Lake Lieps are executed during summer.

2.2. Sampling strategy

Sampling was conducted on March 21st, 23rd, and 24th in 2018 on Lake Tollense. A total number of eight manta trawls were carried out transversely to the longitudinal axis of the lake and 46 pump samples were taken using a pump sampling system (Fig. 1). While manta samples (Chap. 2.2.1) served as a reference being a well-established sampling device for microplastics in water, pump sampling (Chap. 2.2.2) followed two basic strategies:

(1) To investigate the influence of sample volumes on the representativeness of pump samples, scale samples consisting of a sequence of four samples were collected at the midpoint of each manta transect (8). The first two samples comprised 500 l each, whereas the third and fourth sample comprised 1000 l respectively. This results in a total volume of 3000 l at each sampling station. Due to logistical reasons only a single 1000-l sample could be taken at manta transect 004 (VP 103) (n = 29).

(2) The relevance of sample numbers was assessed by carrying out a resampling experiment as described in Bancin et al. (2019; see also Walther, 1997). The sampling points were derived by a raster of 1 km grid size aligned to the southern and western borders of the lake. All vertices being completely within the lake surface area were defined as sampling locations (n = 17). The sampling volume was set to 1000 l for all raster samples.

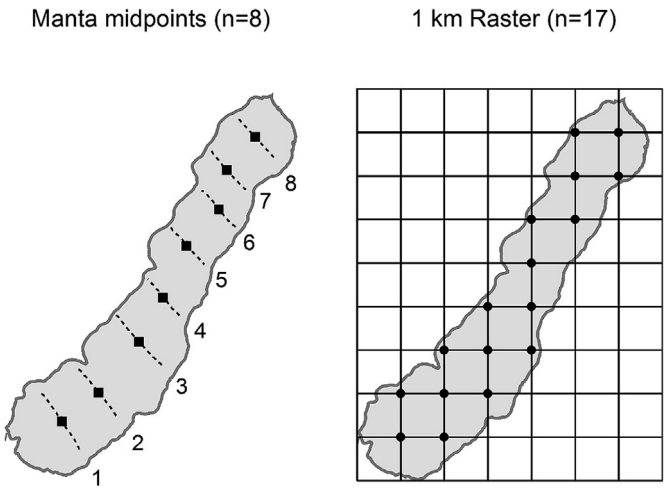

Fig. 1. Lake surface area (grey), manta transects (MT; dashed lines and numbers), scale sample stations (black squares) and sampling stations (black points) for resampling experiment (right) including the underlying raster grid.

To create resampling curves 1–17 (the complete data set) samples were randomly drawn with and without replacement using the sample function of the R base package (R Core Team, 2018). This was repeated 100 times so that the mean and 95% confidence interval of the mean microplastic concentration for the respective sample numbers could be calculated.

2.2.1. Manta sampling

All sampling-related data is given in S 3. Manta trawls were carried out from northwest to southeast transversely to the lake's longitudinal axis, starting with the southernmost transect. Trawling tracks were recorded via GPS (GARMIN GPSmap 62s) and comprised a mean (±SD) distance of 1334 ± 186 m depending on the width of the lake at the respective transect. The trawling speed was similar across all trawls with a mean value of 5.3 ± 0.3 kph. The trawled area and volume were calculated by multiplying the recorded distance with the width and height of the manta opening, respectively.

To avoid contamination and ensure undisturbed sampling conditions, the manta was kept outside the vessel's wake at all times during trawling. When a trawl was completed, the manta was recovered on deck, the cod end was detached and its content was thoroughly rinsed with Milli-Q water into a stainless steel bowl. Large debris (e.g. reed) was rinsed as well and discarded on site. The sampling material was then transferred into brown glass jars by rinsing with little Milli-Q water, treated with 10 ml of hydrochloric acid (HCl, 37%, Merck Emsure) for fixation and stored in a cooling

box until further processing in the laboratory.

2.2.2. Pump sampling system

As there was no established, commercially available pump system for sampling microplastics at the time of the investigation, it was designed setting the following requirements: The system had to be capable of sampling large amounts of water at a reasonable time (at least several cubic meters per hour), microplastics in the size range <300 μm needed to be included, the system should be at least largely plastic-free and be operable on small vessels or from bridges. A schematic depiction of the sampling device set up on this basis is given in S 4. The system consisted of a submersible pump (Ebara Idrogo M40/06, capacity max. 4.8 m^3/h) connected to a sieving cascade by a metal nozzle (VWR, test sieve, DIN ISO 3310-1) via a PVC hose. The PVC hose was considered an acceptable compromise between consistency and cost-effectiveness, since PVC is rare in surface water samples due to its density (Bond et al., 2018). The actual flow rate of the pump was determined on site by filling of a calibrated container (28 l) with water while measuring the time (mean of three replicates per location).

At each station, the pump was lowered into the water until the top row of inflow openings was submerged about 5 cm. When pumping was completed, the sieves were unmounted, all debris present was transferred into brown glass jars by rinsing with little Milli-Q water, treated with 1 ml of hydrochloric acid for fixation and the samples were stored in a cooling box until further processing.

2.3. Purification and contamination control

All samples were purified in the same manner following a two-step protocol using (1) 60 ml hydrogen peroxide (H_2O_2, 30%, Merck, 7 d at room temperature) followed by (2) 16.7 ml sodium hypochlorite solution (NaClO, 6–14% active chlorine, Merck Emplura, 24 h at room temperature) per 50 ml sample volume. This protocol has been applied successfully in different environmental matrices before (Hengstmann et al., 2018; Tamminga et al., 2018; Collard et al., 2015; Tagg et al., 2015; Nuelle et al., 2014). Finally, the samples were filtered (VWR, qualitative filter paper 413, 5–13 μm particle retention) using a stainless steel filter funnel (Sartorius Stedim) and left to dry for 48 h.

To prevent contamination, the samples were kept covered except during processing. Moreover, only cotton laboratory coats were worn, an air purifier (Philips, AC3256) was installed, and the laboratory was cleaned prior to any analysis step. Throughout all rinsing steps, Milli-Q water was used. To quantify the remaining contamination, procedural laboratory blanks (n = 3 for manta samples; n = 27 for pump samples) were run in parallel with the actual samples and treated equally, except for starting with 50 ml of Milli-Q water instead of sample material. The mean number of particles on blank filters was subtracted from plastic counts in each sample.

2.4. Nile Red tagging and spectroscopic validation

The dried filters were stained with 1 ml of Nile Red solution (1 mg/ml in chloroform) as described in Tamminga et al. (2018). After 24 h, the filters were examined via fluorescence microscopy (Zeiss, AxioLab A.1, 2.5x/006 A-Plan) with a TRITC HC filterset (AHF) by taking overlapping images (Canon EOS 80D, exposure time 1", ISO 500, resolution 1 × 1 μm) of the whole filter. The images were investigated visually for stained particles using Adobe Photoshop CS5. Prior to examination a set of stained reference plastic particles (LDPE, HDPE, PP, PET, PVC) purchased as virgin pellets (Goodfellow Inc.) and produced from post-consumer items (bottle caps, pipes, freezing bags, etc.) were investigated. Moreover, biogenic reference materials (algae, leaves, wood, and chitin) were stained and examined. As fluorescence was distinctly weaker for biogenic materials it was possible to distinguish between plastics and non-plastics. A summary of the reference library is provided in S 5. The shape (irregular particle (IP) or fiber) and size (precisely length and width) of each particle were recorded. The sum of both shapes is referred to as particles.

A subsample of 277 particles (~9% of all particles) was analyzed using μRaman spectroscopy to verify the artificial nature (Thermo Fisher Scientific, DXR2xi Raman Imaging Microscope). Of these, 273 particles could be confirmed as plastics and four could not be determined.

Nile Red-induced fluorescence, which was hindering the spectroscopic analysis, could be attenuated via previous photo-bleaching (Fig. 2). Therefore, particles were placed between microscope slides and the laser power was slowly raised to 10 mW for 2–10 min. In the presence of very dark surfaces, laser power was reduced to 5 mW with prolonged exposure time (up to 30 min, in rare cases up to 60 min). Spectra were recorded using a 365 nm laser at 5–8 mW and 100-10 Hz integrating 500 measurements. A signal-to-noise ratio (SNR) of at least 40 was aimed for and achieved in 80% of all cases (88% SNR > 30). The spectra were auto-compared to 16 libraries containing spectra of plastics, as well as natural organic and inorganic substances. A minimum library match of 70% was considered sufficient for a validation, though in the majority of cases (77% of confirmed particles) values above 80%

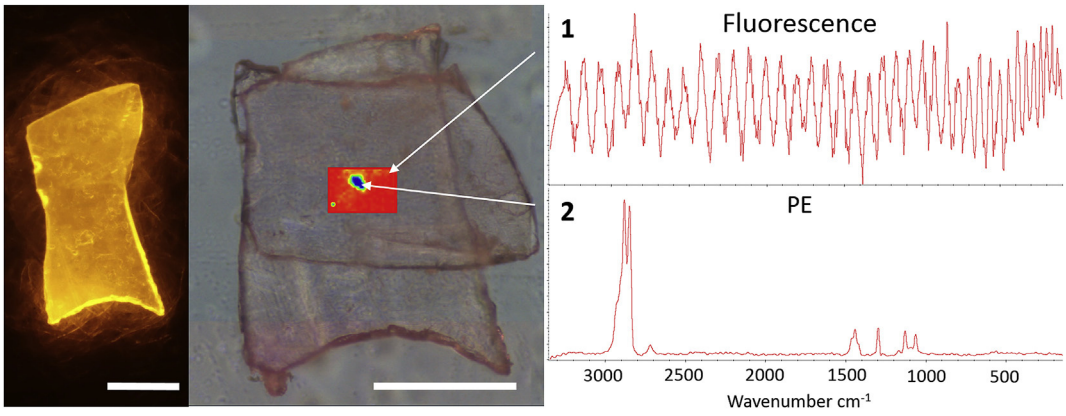

Fig. 2. Example of Nile-Red-tagged PE film (left) and spectroscopic validation after photo-bleaching (scale bar indicates 200 μm).

could be reached. Besides, library matching results were reviewed by the operator to secure valid assignments.

2.5. Statistical analysis and visualization

All data, split by shape (IPs, fibers), were tested for normality (Shapiro and Wilk, 1965) and homogeneity of variance. The Mann-Whitney *U* test was performed to test for differences between two groups (sampling device comparison), and the Kruskal-Wallis test was applied in case of more than two groups (e.g. different sample volumes).

Data were analyzed using the scripting language R (Version: 3.5.1; R Core Team, 2018) in an RStudio environment (Version: 1.1.423; RStudio Team, 2018). Geodata were processed and visualized with the software ArcGIS by ESRI (Version: 10.3).

3. Results and discussion

The background contamination during sample processing was deemed acceptable. The manta sample blanks showed an average of 0.7 IPs and 1.2 fibers per sample. Regarding the pump samples, a mean contamination load of 0.5 IPs and 0.5 fibers per sample was found. In both cases, IP contamination tended to rise with declining size of the particles, while fibers were sporadically present in all size fractions.

3.1. Inter-device comparison

A total number of 231 microplastic particles were found across all manta samples, of which 177 were classified as IPs and 54 as fibers. Particles being smaller than the manta mesh size ($n = 136 \triangleq 37\%$) were excluded. This equals to a median (IQR – interquartile range) abundance of 0.14 (0.15) particles m^{-3} comprising 0.12 (0.15) IPs m^{-3} and 0.02 (0.01) fibers m^{-3} (see S 6 for station values). The occurrence of fibers shows great variations and no clear spatial pattern. For IPs, the lowest concentration was recorded at the southernmost transect (MT 001) with 0.01 particles m^{-3}, while the maximum abundance was documented at the northern end of the lake (MT 008) with 0.36 particles m^{-3}. Moreover, the values for transects in between show a rather distinct gradient of rising microplastic concentrations towards the north of Lake Tollense (Fig. 3, left).

To enable the comparison of microplastic concentrations as derived by manta trawling and pump sampling, only particles >300 μm in length were included. Moreover, the 3000 l sample volumes were considered as we assumed that these results are more reliable compared to smaller sample volumes. Scale sample results are presented in more detail in Chapter 3.2.1.

Based on the criteria defined above, 284 particles including 85 IPs and 199 fibers were counted in all pump samples at the manta transect midpoints. As a result, a median concentration of 9.8 (2.9) particles m^{-3} constituted by 3.7 (1.3) IPs m^{-3} and 6.1 (2.6) fibers m^{-3} was calculated. Due to this obvious difference compared to trawling results, a direct pairwise comparison is not possible. On the one hand, the concentration of microplastics >300 μm, especially IPs, is most likely below the detection limit of the pump system, as the lowest possible concentrations except zero is 0.3 particles m^{-3} for a 3000 l sample ($\triangleq$1 particle). In freshwater, concentrations below this value are likely (Horton et al., 2017). On the other hand, physical properties of the respective particle play a key role. Particularly the shape of each respective particle determines whether it passes a sieve/net or is retained. While on average (±SD) 70 ± 8% of all microplastics found within the pump scale samples had a fibrous shape, their share in the manta samples was distinctly lower and more heterogeneous accounting for only 23 ± 21% (note that only particles >300 μm are considered; see also Fig. 4). Accordingly, Setälä et al. (2016) found that fibers are only poorly retained in coarse nets, as their small diameter enables them to pass through the mesh. Moreover, Dris et al. (2018a) stated that "using the 80 μm mesh size rather than the 330 μm increases the probability of sampling fibers by 250 times." The sieving cascade implied here considerably increases the probability to collect fibers by imposing higher retention capacities. This finding is supported by the size distribution of microplastics sampled by both the manta trawl and the pump system (Fig. 4). While fibers detected in the manta samples are unevenly spread across the whole size range, fibers found in the pump samples show a distinct positively skewed distribution peaking at >500–600 μm in length. Results concerning fiber widths are similar to values reported by Setälä et al. (2016) and approximately tend to normal distribution.

Despite the differences in the abundance of microplastics derived by both sampling approaches, comparable patterns are identifiable. A similar spatial gradient with rising microplastic concentrations to the north of Lake Tollense can be observed (see Fig. 3, right). The southernmost scale pump sample (VP 100) is an exception in this regard, as it contained the second-highest concentration of microplastics, whereas a minimum was assessed by manta trawling. We hypothesize that this is due to the specific position of VP 100 in line with the plume of the southern tributaries. Its punctiform character makes the pump sample more

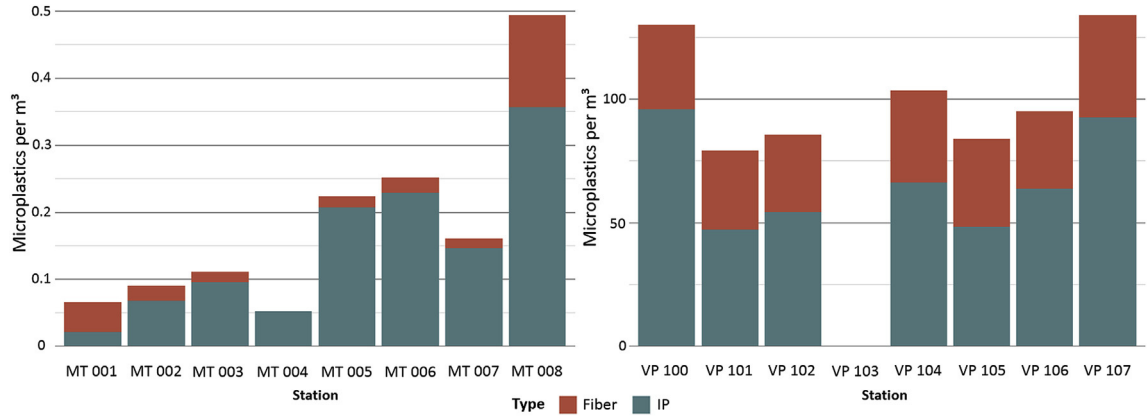

Fig. 3. Comparison of microplastic concentration by station from south (MT 001/VP 100) to north (MT 008/VP 107) for manta (left) and pump samples (VP 103 was excluded as only 3000 l results are shown).

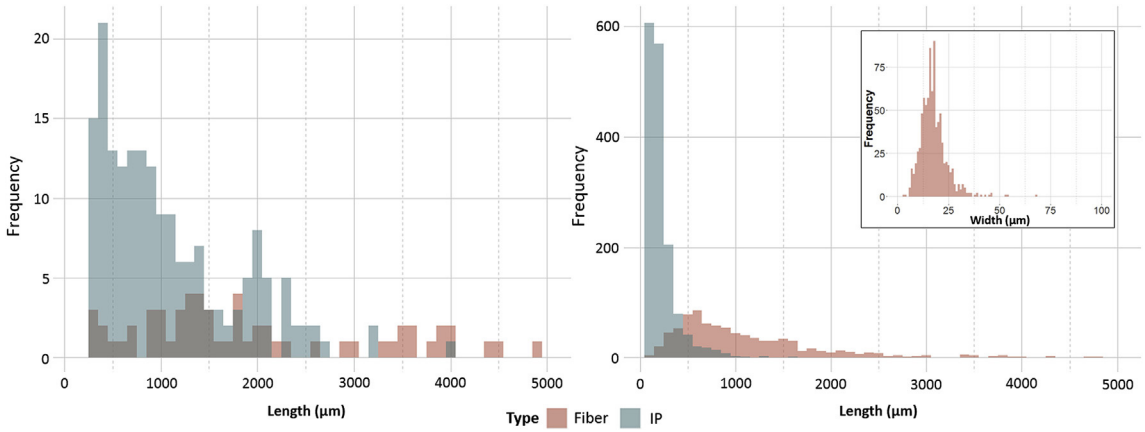

Fig. 4. Size (length) distribution of microplastics sampled by manta trawling (left) and the pump system by particle shape including the distribution for fiber widths (top right), note that the class width was set to 100 µm (1 µm for fiber width).

sensitive to extreme values compared to transect-based manta trawling.

The polymer composition (Fig. 5) for both sampling systems is noticeably influenced by the divergent abundance of fibers, of which about 80% were PET, followed by PP with a share of 12%. Across all analyzed particles, PE and PET dominate with a cumulative share of 57% and 23%, respectively.

3.2. Pump sampling representativeness

3.2.1. Scale samples

In all scale pump samples, 2439 microplastics consisting of 1578 IPs and 861 fibers were counted. This corresponds to an overall median of 94.9 (32.1) particles m^{-3} constituted by 63.7 (28.1) IPs m^{-3} and 34.2 (4.7) fibers m^{-3} (see S 7 for station values). The microplastic abundance in all samples strongly depends on size (here length), with smaller IPs being more frequent than larger ones (see Fig. 4, right). Though fibers do not show a similarly steady distribution, their abundance is characterized by distinct differences between size fractions. In order to show the influence of sample volumes on microplastic abundances according to particle size, Fig. 6 provides the results of scale pump samples by sample volume and size fraction.

Microplastic particle concentrations do not differ significantly ($\alpha = 0.05$) between sample volumes. This might be due to the relatively low sample number and probably the limited volume range investigated (500-3000 l). Volume-related patterns are still apparent. Concentrations across all size fractions and for both particle shapes tend to decrease with rising sample volume, indicating possible overestimations for low sample volumes. Moreover, the variance, which is used as an indicator for repeatability here (see also S 8), is distinctly reduced with increasing sample volumes. This is consistent across all size fractions and for both shape categories. The strongest decrease in microplastic concentration can be observed between 500 l and 1000 l. This could be an indication to avoid extrapolation at least when referring to concentrations per m^3 which has been suggested as a standard unit for microplastics in water (Ivleva et al., 2017) and has been a common reporting unit in the past (Li et al., 2018).

Lusher et al. (2014) carried out a similar experiment for microplastics in marine sub-surface water by filtering five different volumes between 100 l and 2000 l. In accordance with the results presented in this study, median concentrations were higher for smaller water volumes. Furthermore, the variance of microplastic abundance in low-volume samples was considerably higher than in large-volume samples.

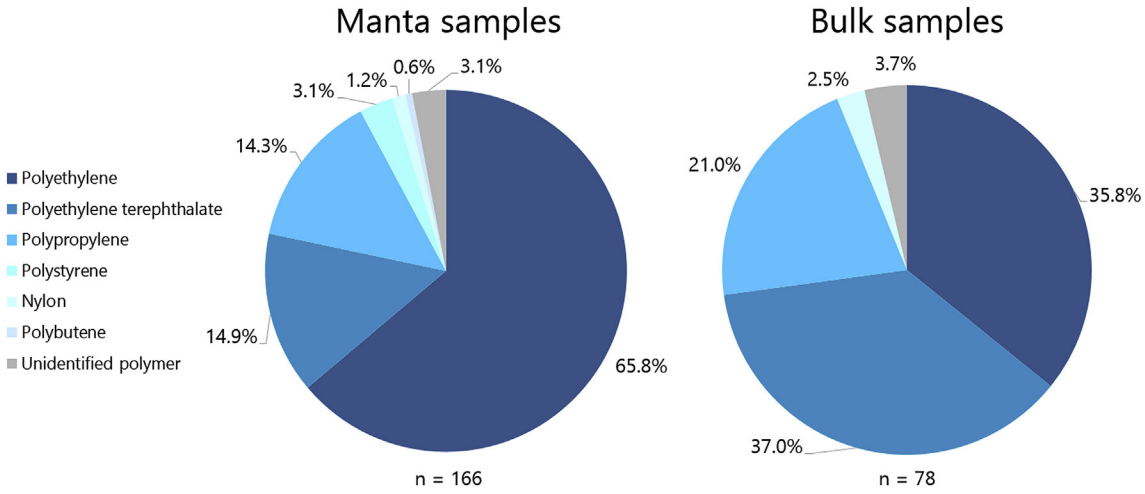

Fig. 5. Polymer composition of microplastic particles by sampling device according to µRaman-spectroscopy.

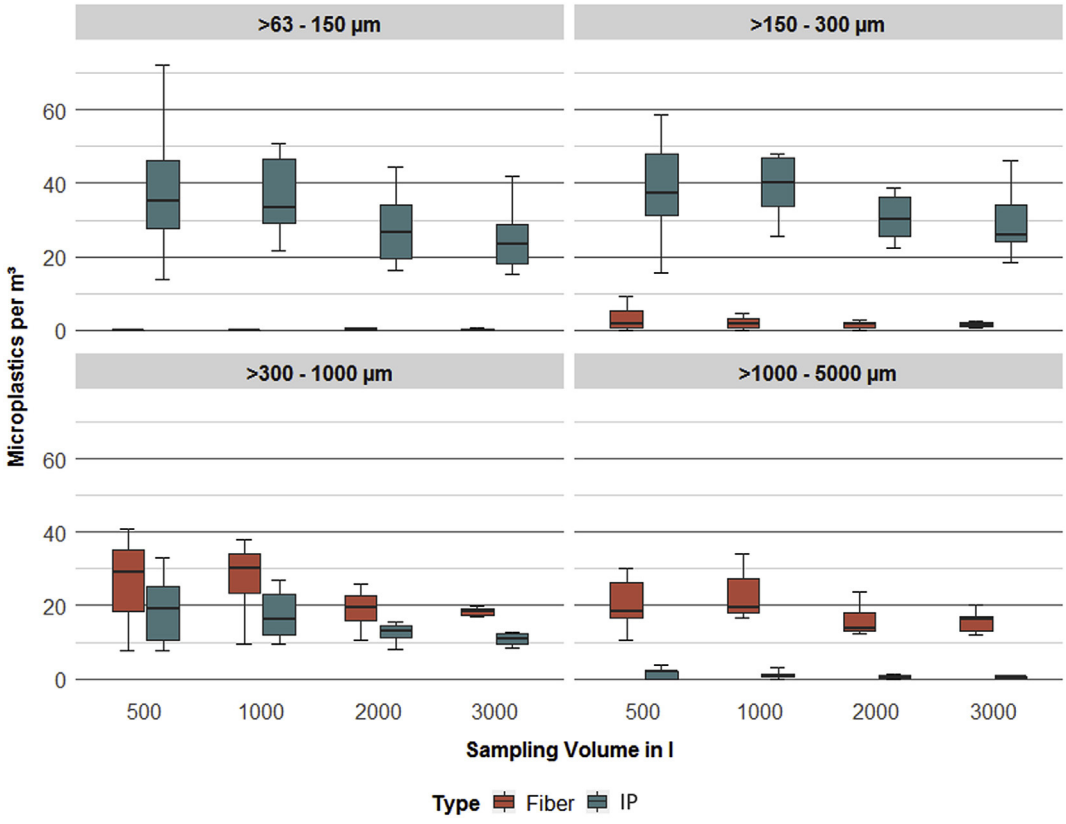

Fig. 6. Microplastic concentrations in pump samples (n = 7) by sample volume and size class; Boxes indicate the range of the 25–75% quantiles, whereas whisker give the smallest/highest observation at a maximum of 1.5*IQR (inter quartile range) from the box.

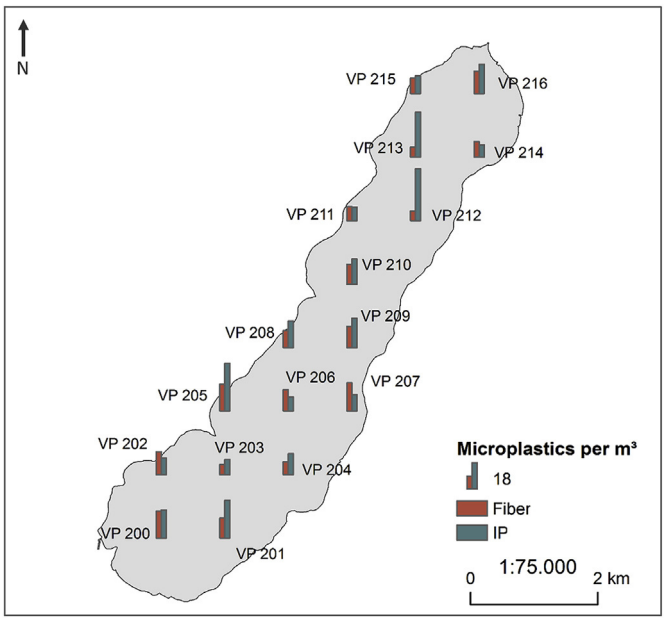

Fig. 7. Microplastic particle concentrations in raster samples by station and particle shape.

3.2.2. Sampling raster

An overview of the microplastic abundance at all stations being part of the raster experiment is given in Fig. 7. A total of 560 microplastics, comprising 338 IPs and 222 fibers, were counted. With a median of 30.8 (14.6) particles m^{-3} (17.2 (8.8) IPs m^{-3} and 13.6 (5.8) fibers m^{-3}), the samples collected on March 24th were considerably less polluted than samples of the previous days (see S 9 for by station values). The formation of ice layers throughout the nights before March 22nd and 23rd (first two sampling days), may have resulted in both, lower wind-induced vertical mixing and a possible accumulation within the ice layer, as well as a subsequent release from the ice, as has been stated before (Obbard et al., 2014).

The spatial distribution of microplastic particle concentrations is heterogeneous and no distinct pattern can be identified. This emphasizes the relevance of evaluating the influence of sample numbers on the representativeness of the overall sampling strategy. The maximum abundance was observed at station VP 205 (51.3 particles m^{-3}) which is situated more sheltered behind a small headland, whereas the lowest concentration was detected in proximity at VP 203 (17.2 particles m^{-3}) in the center of the southern lake part.

Fig. 8 displays the results of the resampling experiment. For resampling with replacement, a consolidation of the mean at $n \geq 9$ is visible, while the confidence interval steadily declines until reaching the mean of the complete dataset (30.8 particles m^{-3}). If resampling is carried out with replacement, the mean stabilizes at $n \geq 6$. Here, the confidence interval continues to narrow until $n \geq 13$, but changes are minor. At $n = 17$, a considerable variation remains (min: 25.8 particles m^{-3}; max: 36.2 particles m^{-3}). This is comparable to the findings of Bancin et al. (2019).

These results indicate that sample numbers of $n = 5$ or less are not sufficient to obtain sound results. At $n > 5$, the mean tends to remain relatively stable, but higher sample numbers will be needed for more robust estimations depending on the respective approach or the specific research interest. Moreover, the present experiment

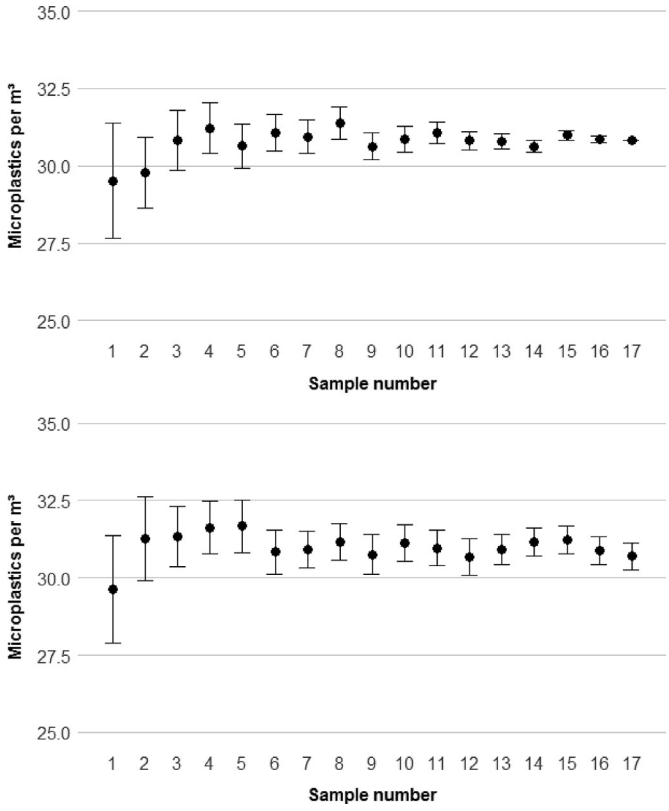

Fig. 8. Resampling results of 100 replicates without (top) and with replacement. Black dots indicate the mean number of microplastics, while whiskers correspond to the 95% confidence intervals of the means.

is limited to 17 samples with a volume of 1000 l each. It has to be assumed that greater changes could be observed for $n > 17$, and that the sample volume affects and influences the numbers of samples needed.

3.3. Limitations of the present approach

As for any experimental design, *a priori* assumptions and methodical limitations restrict the transferability of the present approach.

Generally, the concept is adapted to a specific lake and its catchment, which are characterized by specific conditions determining both the presence and distribution of microplastics at a specific time. Yet the approach provides useful information on how to solve the question of sample representativeness. The limited sample number of this investigation is rather induced by technical/logistical constraints than by conceptual considerations, and the results therefore need to be validated at different locations using approaches that enable larger sample numbers and volumes.

4. Conclusions

The representativeness of sampling strategies for microplastics in freshwater was assessed in this study. Although the manta trawl is a commonly used sampling device, it fails to deliver the overall pattern of microplastic pollution in an area, since it is not sufficiently retaining fibers and small microplastics. However, these constitute a great share of all particles. The pump sampling approach with the filtration of large water volumes ($\gg 3\ m^3$) is logistically challenging, but necessary to generate comparable numbers for manta and pump sampling. Consequently, each of the two approaches encompasses a subdivision of the overall population of microplastics. The pump sampling covers small microplastics, which are greater in number and the volume-reduced sampling covers large microplastics, being less abundant but still important when it comes to weight estimates. We therefore conclude that both techniques are rather complementary than substitutable.

Concerning pump samples, the filtrated water volume has a considerable influence on the obtained results. Sampling volumes should be adapted to the specific approach, particularly to the size range addressed and the expected concentrations of microplastics in the respective water body. However, volumes should be large enough to minimize overestimation induced by scaling up results (here to $1\ m^3$).

Sample numbers appear to have a less pronounced importance for achieving representative results, but the conducted resampling experiment suggests that more than five samples are needed to limit the variation range.

More research is needed to validate current findings and to broaden the understanding of representativeness concerning microplastics analysis, which is crucial to produce reliable data in the future.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Proofreading has been done by the agency 'topcorrect.com'.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.112970.

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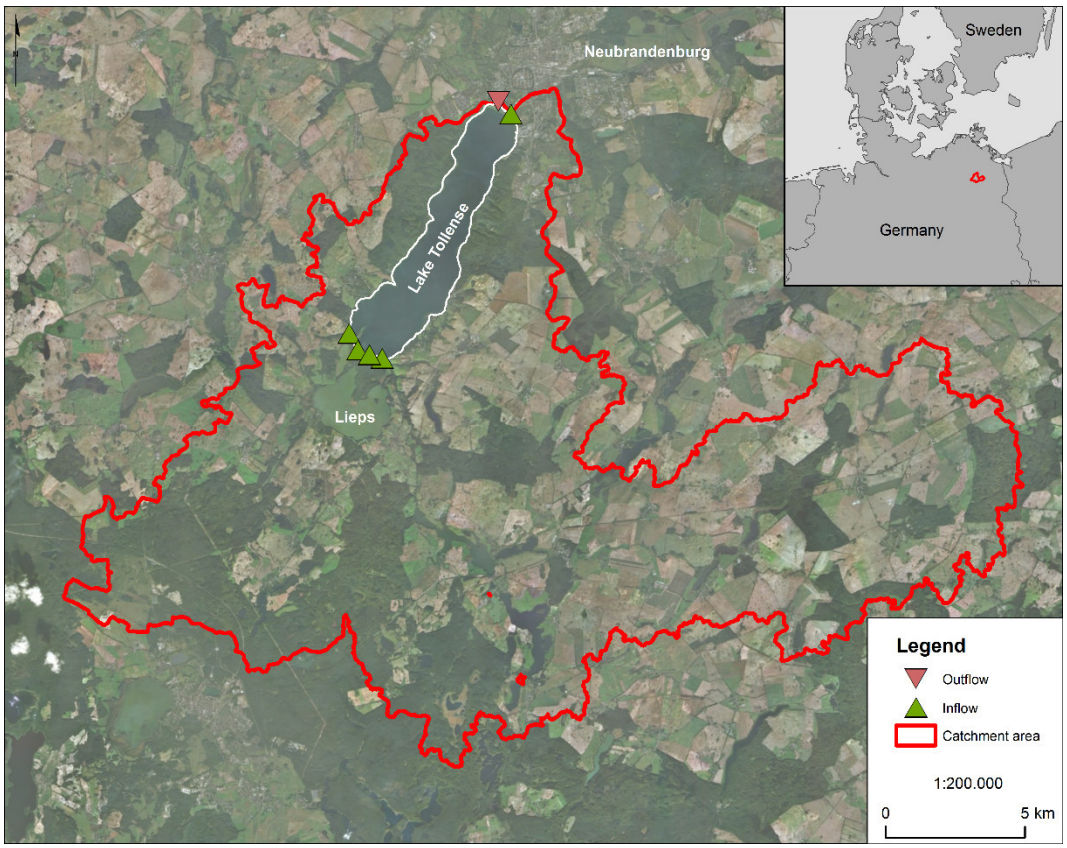
Supplementary to Tamminga et al. 2019: On the representativeness of bulk water samples versus manta sampling in microplastic analysis

S 1: Main characteristics of Lake Tollense and its catchment. (MLUMV 2017).

Lake surface area (km²)	17.8
Max. depth (m)	31.3
Mean depth (m)	17.8
Water volume (m³)	315,626,577
Shore Length (m)	26,755
Catchment area (km²)	373.8
Long-term discharge (m³/s)	2.4
Residence time (a)	4.1

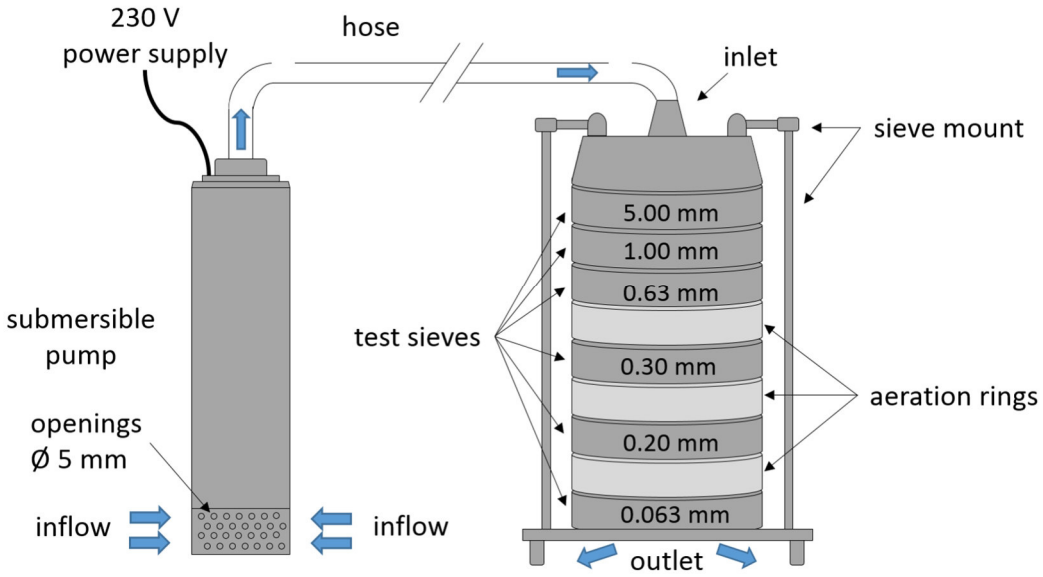
S 2: Lake Tollense, its tributaries, discharge and catchment. (Catchment data: MLUMV 2017, background: ESRI 2018)



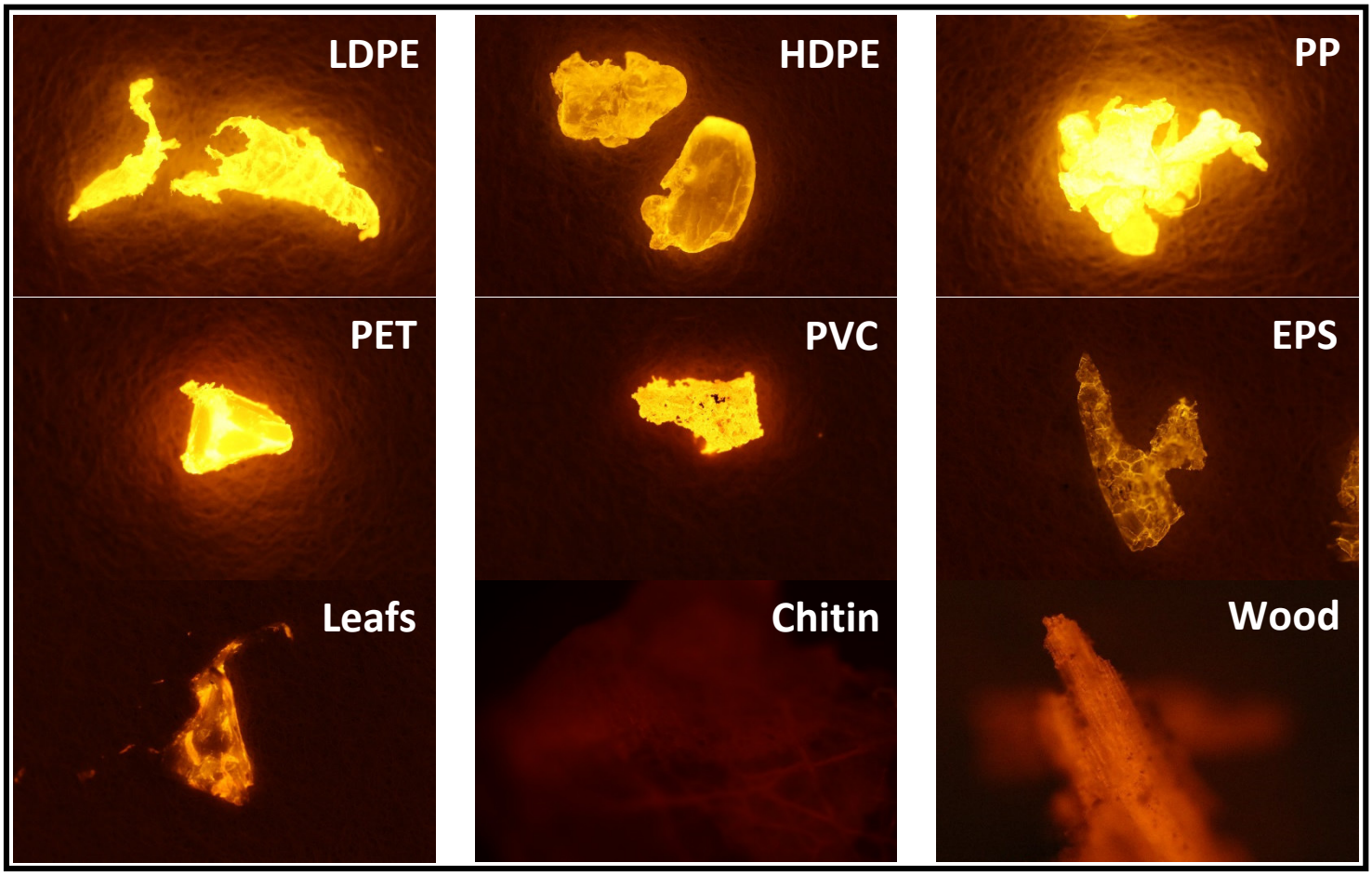
S3: Sampling data of all manta trawls.

Trawl no.	Date	Trawling information					Wind conditions	
		Duration (hh:min)	Distance (m)	Area (m^2)	Volume (m^3)	Mean speed (kph)	Beaufort no.	Direction (°)
1	2018-03-21	00:21	1,575	945.2	174.9	4.7	3	250
2	2018-03-21	00:23	1,439	863.6	159.8	5.0	4	260
3	2018-03-21	00:23	1,681	1008.4	186.6	5.2	4	260
4	2018-03-21	00:15	1,171	702.3	129.9	5.2	3	270
5	2018-03-23	00:15	1,159	695.6	128.7	5.5	2	70
6	2018-03-23	00:13	1,174	704.5	130.3	5.5	2	70
7	2018-03-23	00:15	1,272	763.4	141.2	5.4	2	110
8	2018-03-23	00:14	1,282	769.4	142.3	5.6	2	120

S 4: Schematic depiction of the used pump sampling system including the mesh widths of the test sieves.



S 5: Exemplification of Nile Red Staining Reference library. Note that the images were obtained with a Zeiss, AxioLab A.1, 2.5x/006 A-Plan microscope using an orange filter. Before applying Nile Red, samples were treated with H2O2 and NaClO as described in the main publication.



S 6: Results of Manta trawling by trawling, size fraction and shape.

	Fibers per m³		IPs per m³	
Trawl no.	>300-1000 µm	>1000-5000 µm	>300-1000 µm	>1000-5000 µm
MT_001	0.02	0.03	0.01	0.01
MT_002	0.01	0.01	0.05	0.02
MT_003	0.00	0.02	0.06	0.03
MT_004	0.00	0.00	0.02	0.03
MT_005	0.00	0.02	0.12	0.09
MT_006	0.00	0.02	0.13	0.10
MT_007	0.00	0.01	0.05	0.09
MT_008	0.00	0.13	0.18	0.18

S 7: Results of bulk scale samples by station, size fraction and shape (sample volume: 3 m³).

	IPs per m³				Fibers per m³			
Sample no.	**>63-150 µm**	**>150-300 µm**	**>300-1000 µm**	**>1000-5000 µm**	**>63-150 µm**	**>150-300 µm**	**>300-1000 µm**	**>1000-5000 µm**
VP 100	29.71	46.21	18.99	0.97	0.32	2.46	17.80	13.58
VP 101	17.37	18.29	10.88	0.65	0.00	0.58	19.10	12.28
VP 102	18.67	25.11	9.90	0.32	0.65	2.00	16.83	11.96
VP 104	27.76	25.76	12.18	0.32	0.32	1.42	19.10	16.50
VP 105	15.10	23.48	8.93	0.65	0.00	0.91	18.45	16.18
VP 106	23.54	31.28	8.60	0.32	0.00	1.10	12.93	17.15
VP 107	42.05	36.80	12.82	0.65	0.00	1.81	19.75	20.08

S 8: Variance of microplastic abundances by sample volume and size fraction.

Type	Size fraction (µm)	500 l	1,000 l	2,000 l	3,000 l
IP	>63-150	357.8	135.3	107.1	86.5
	>150-300	586.9	317.9	173.6	88.5
	>300-1000	95.8	49.2	20.0	12.6
	>1000-5000	2.2	1.1	0.3	0.1
Fiber	>63-150	2.2	0.6	0.1	0.1
	>150-300	13.5	3.1	0.9	0.4
	>300-1000	367.7	100.5	30.3	5.4
	>1000-5000	49.9	43.9	17.1	8.6

S 9: Results of bulk raster samples by station, size fraction and shape (sample volume: 1 m³)

	IPs per m³				Fibers per m³			
Sample no.	**>63-150 µm**	**>150-300 µm**	**>300-1000 µm**	**>1000-5000 µm**	**>63-150 µm**	**>150-300 µm**	**>300-1000 µm**	**>1000-5000 µm**
VP 200	4.87	10.71	3.57	0.00	0.00	0.97	13.64	3.90
VP 201	4.87	13.64	5.52	1.95	0.00	0.00	3.90	9.74
VP 202	4.87	2.92	3.57	0.00	0.00	0.00	7.79	7.79
VP 203	3.90	4.87	1.62	0.00	0.00	0.00	5.84	0.97
VP 204	7.79	4.87	0.65	0.97	0.00	0.97	5.84	1.95
VP 205	13.64	13.64	5.52	0.00	0.00	0.97	9.74	7.79
VP 206	2.92	6.82	0.00	0.00	0.00	0.00	5.84	8.77
VP 207	2.92	5.84	2.60	0.00	0.00	0.00	6.82	12.66
VP 208	3.90	10.71	2.60	0.97	0.00	0.00	3.90	7.79
VP 209	4.87	10.71	3.57	0.97	0.00	0.00	8.77	5.84
VP 210	7.79	5.84	3.57	0.00	0.00	0.97	2.92	9.74
VP 211	2.92	2.92	3.57	0.00	0.00	1.95	4.87	2.92
VP 212	12.66	15.58	7.47	0.00	0.00	0.00	1.95	4.87
VP 213	17.53	10.71	2.60	0.00	0.00	0.00	3.90	2.92
VP 214	0.97	6.82	0.65	0.00	0.00	0.00	4.87	5.84
VP 215	3.90	6.82	1.62	0.00	0.00	0.00	2.92	7.79
VP 216	4.87	8.77	6.49	0.00	0.00	0.00	5.84	9.74

Appendix: Publication II

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Microplastics in a deep, dimictic lake of the North German Plain with special regard to vertical distribution patterns☆

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ABSTRACT

The investigation of microplastics (MPs) in freshwater has received increased attention within the last decade. To date, sampling is mainly conducted at the surface of both rivers and lakes and only a few studies assessed the vertical distribution of MPs in the water column of freshwater bodies. To contribute to the understanding of MP pollution in the water column of freshwater lakes, this study evaluated the vertical profile of MPs in Lake Tollense considering particles between 63 and 5000 μm in size. Sampling was conducted on three occasions at three depths (surface, 7 m and 10 m) along a transect including eight sampling stations. The retrieved samples were digested with hydrogen peroxide and sodium hypochlorite and investigated via Nile Red staining and fluorescence microscopy. Subsequently, a subsample of stained particles was verified by μRaman-spectroscopy. The vertical distribution of MPs in Lake Tollense differed considerably between particle shapes (irregular particles (IPs) and fibers). Fibers did not show a noticeable pattern with depth and ranged between 22 fibers m^{-3} at 0 m to 19 fibers m^{-3} at 10 m. In contrast, IPs were distinctly less abundant in sub-surface samples with concentrations between 50 IPs m^{-3} at 0 m to 29 IPs m^{-3} at 10 m. Concerning IPs, buoyant polymers (mainly PE and PP) and concerning fibers PET and PP dominated the polymeric composition. Besides particle inherent properties, wind-induced mixing is likely affecting the intensity of vertical concentration gradients. This study highlights the need for depth-integrated sampling approaches in order to achieve representative data without over- or underestimating the overall abundances.

1. Introduction

Microplastics (MPs) are ubiquitous pollutants in natural environments. So far, MPs were documented in fresh- and seawater (Bordós et al., 2019; Enders et al., 2015; Faure et al., 2015; Kanhai et al., 2018; Setälä et al., 2016), beach and bed sediments (Hengstmann et al., 2018; Vaughan et al., 2017; Woodall et al., 2014), organisms (Bessa et al., 2018; Leslie et al., 2017; Li et al., 2018), soils (Corradini et al., 2019) and atmospheric deposition (Zhang et al., 2020; Allen et al., 2019; Cai et al., 2017; Klein and Fischer, 2019).

Commonly, MPs are defined as artificial polymer particles <5 or <1 mm in their longitudinal direction (Arthur et al., 2009; Hartmann et al., 2019). Hartmann et al. (2019) extended this definition by taking further criteria into account, namely the chemical composition, solid state, solubility, size, shape and structure, color, and origin of the investigated particles.

The abundance of MPs in global freshwater bodies has been increasingly demonstrated in recent years (Eerkes-Medrano and Thompson, 2018). The ubiquity and persistence of MPs in freshwater have raised concerns about potential threats to the environment as well as to humans. These concerns include the potentially harmful effects of ingested/inhaled MPs and related sub-pollutants or microorganisms on the receiving organism (Diepens and Koelmans, 2018; Murphy et al., 2020; Gasperi et al., 2018; Wright and Kelly, 2017). Many of these effects are likely dose-dependent and an accurate assessment of exposure levels or MP concentrations in freshwater is therefore crucial (Koelmans et al., 2019).

The vast majority of studies assessing MP pollution in freshwater are focusing on surface sampling only (Lenaker et al., 2019; LfU, 2019; Uurasjärvi et al., 2020). Surface sampling mostly was conducted using manta trawls or similar neuston nets. These nets often have a rather coarse mesh-width (≈300 μm) adapted from

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marine surface trawling and are thus neglecting a large number of MPs with smaller sizes (Horton et al., 2017; Ivleva et al., 2017; Li et al., 2018). The water column of freshwaters was sampled only in few cases (Dris et al., 2018; Eo et al., 2019; LfU, 2019; Lenaker et al., 2019; Liedermann et al., 2018). In most of these studies, the vertical distribution of MPs in rivers was assessed, while the water column of freshwater lakes only was investigated twice (Lenaker et al., 2019; LfU, 2019). In the marine environment several studies investigated MPs in the water column and identified wind (and wave) driven mixing as an important driver for the vertical distribution of (mostly buoyant) MPs (Egger et al., 2020; Kooi et al., 2016; Kukulka et al., 2012; Lattin et al., 2004; Song et al., 2018). In addition, particle properties (mainly shape, size and composition) and biofouling were reported to affect the MPs buoyancy and thus on the vertical distribution of MPs in the water column (Kooi et al., 2016; Song et al., 2018). Whether these findings can be transferred to limnic environments of utterly different scales is uncertain so far and has to be investigated in the future.

To enhance the understanding of the vertical distribution of MPs in freshwater lakes, this study evaluates the vertical concentration profiles of MPs ($\geq$63–5000 μm) in a deep dimictic lake of the North German Plain considering both spatial and temporal variations.

2. Material and methods

2.1. Study area

Lake Tollense is located in northeastern Germany approximately 100 km north of Berlin. The lake covers an area of 17.8 km^2 and extends ca. 10 km from southwest to northeast while maintaining a width of 1.3–2.2 km (MLUMV, 2017). The depth profile of Lake Tollense essentially follows its outer shape due to the lake's formation as a tunnel valley (U-shape) in the last glaciation (StALU MS, 2017) (Fig. 1). In general, the lake's depth (mean 17.8 m) increases rapidly from its shorelines to a maximum of 31.3 m and values $\geq$ 20 m in large parts of Lake Tollense. Shallower parts are mainly limited to the northeastern and southwestern ends of the lake, where its main tributaries (discharge – Nonnenbach (0.57 m^3/s), Gaetenbach (0.55 m^3/s), Lieps Channel (0.49 m^3/s) and Wustrower Bach (0.10 m^3/s) – cause sediment accumulation (Nixdorf et al., 2004). The depth of Lake Tollense enables a thermal stratification that forms after the spring overturn (March/April) and stagnates until the end of October when declining water temperatures lead to a second mixing (IGB, 2018; see supplementary material (SM) 1 for climatic information).

Land-use within the catchment of Lake Tollense is characterized by agriculture and forestry and comprises only minor settlements, except for the city of Neubrandenburg (see Tamminga et al., 2019 for a more detailed description of the catchment). Neubrandenburg (population: 64,086; StatA MV, 2019) is partly located within the lake's catchment at its northern shore and is the main access for recreational activities on the lake, which include fishing, sailing and kayaking.

2.2. Sampling

Sampling was conducted on September 10th and 11th 2018 (stratified phase), March 22nd and 23rd 2019 and March 17th and 18th 2020 (stage of overturn) at Lake Tollense, Germany. At each two-day sampling campaign, a set of eight vertical profiles was sampled that comprised three individual samples at 0 m, 7 m and 10 m below lake surface (n = 72). Sampling stations were evenly distributed on a SW to NE transect spanning across almost the entire extension of Lake Tollense (Fig. 1).

To enable sampling in well-differentiated depths, an *in situ* pump filtration system was used, which is described in detail in Tamminga et al. (2019). In brief, a submersible pump (Ebara Idrogo M40/06, capacity max. 4.8 m^3/h) was connected to a cascade of testing sieves (mesh widths: 1.0, 0.63, 0.3, 0.2, and 0.063 mm, VWR, test sieve, DIN ISO 3310–1) via a PVC hose. A total volume of 1000 l of lake water was filtered at each position. The time required to filter this volume was determined with regard to the pump's flow rate (cf. Tamminga et al., 2019 for more information).

All material retained within the sieves was pooled and transferred into 500 ml brown glass jars by rinsing with little MilliQ water (filtered <0.2 μm). For preservation 1 ml of hydrochloric acid (HCl, 37%, VWR) was added to each sample, before storing them at dark and cool place until further processing.

Water temperatures were recorded (WTW GmbH, Multiline P4, universal meter) using additional small aliquots of water from the respective sampling depth filled into a glass beaker (after 1 min of pumping) and immediately measuring the temperature.

2.3. Sample purification and contamination control

Reducing the biogenic content of a sample that could interfere in the identification of artificial polymers is an essential step in MP analysis. Here, an established protocol was used comprising a two-step treatment with hydrogen peroxide (H_2O_2) and sodium hypochlorite (NaClO; Hengstmann et al., 2018; Tamminga et al., 2018, 2019).

First, samples were volume-reduced (63 μm sieve) and transferred into glass beakers by rinsing with little MilliQ water. Afterwards, 60 ml of H_2O_2 (30%, Merck) per 50 ml sample volume were added and samples were left to react for 7 days. Subsequently, samples were volume-reduced again, were treated with 16.7 ml of NaClO (6–14% active chlorine, Merck Emplura) per 50 ml sample volume, and left to react for 24 h. Finally, samples were filtered (VWR, qualitative filter paper 413, 5–13 μm particle retention) using a stainless steel filtration device and filters were placed into glass petri dishes. The glass petri dishes were closed and left to dry for at least 24 h. All steps were conducted at room temperature to prevent the degradation of artificial polymers (Munno et al., 2018; Pfeiffer and Fischer, submitted).

Anti-contamination measures were taken to prevent the unintended introduction of MPs from the laboratory environment. In brief, all material in contact with the samples was made of glass or metal, samples were covered with a watch glass except when handled, laboratory rooms were cleaned before analysis, an air purifier (Philips, AC3256) was installed, and cotton laboratory coats were worn at any time. Procedural laboratory blanks were carried out in parallel with the actual samples, to account for remaining contamination potential. Blank samples (n = 26) were treated in the same manner as field samples except starting with 50 ml of MilliQ water. Mean blank contamination was subtracted from counts of field samples by particle shape and size fraction.

2.4. Nile Red staining and μRaman-spectroscopy

The filters were treated with 1 ml of Nile Red solution after they had completely dried (1 mg/ml in chloroform; see Tamminga et al., 2018). Subsequently, all filters were photographed under a fluorescence microscope (Zeiss, AxioLab A.1, 2.5×/006 A-Plan) connected to a digital camera (Canon EOS 80D, exposure time 1", ISO 500, 6000 x 4000 μm) and using a TRITC HC filter set (AHF, ex.: 532–554 nm, em.: 573–613) and a broadband white light source

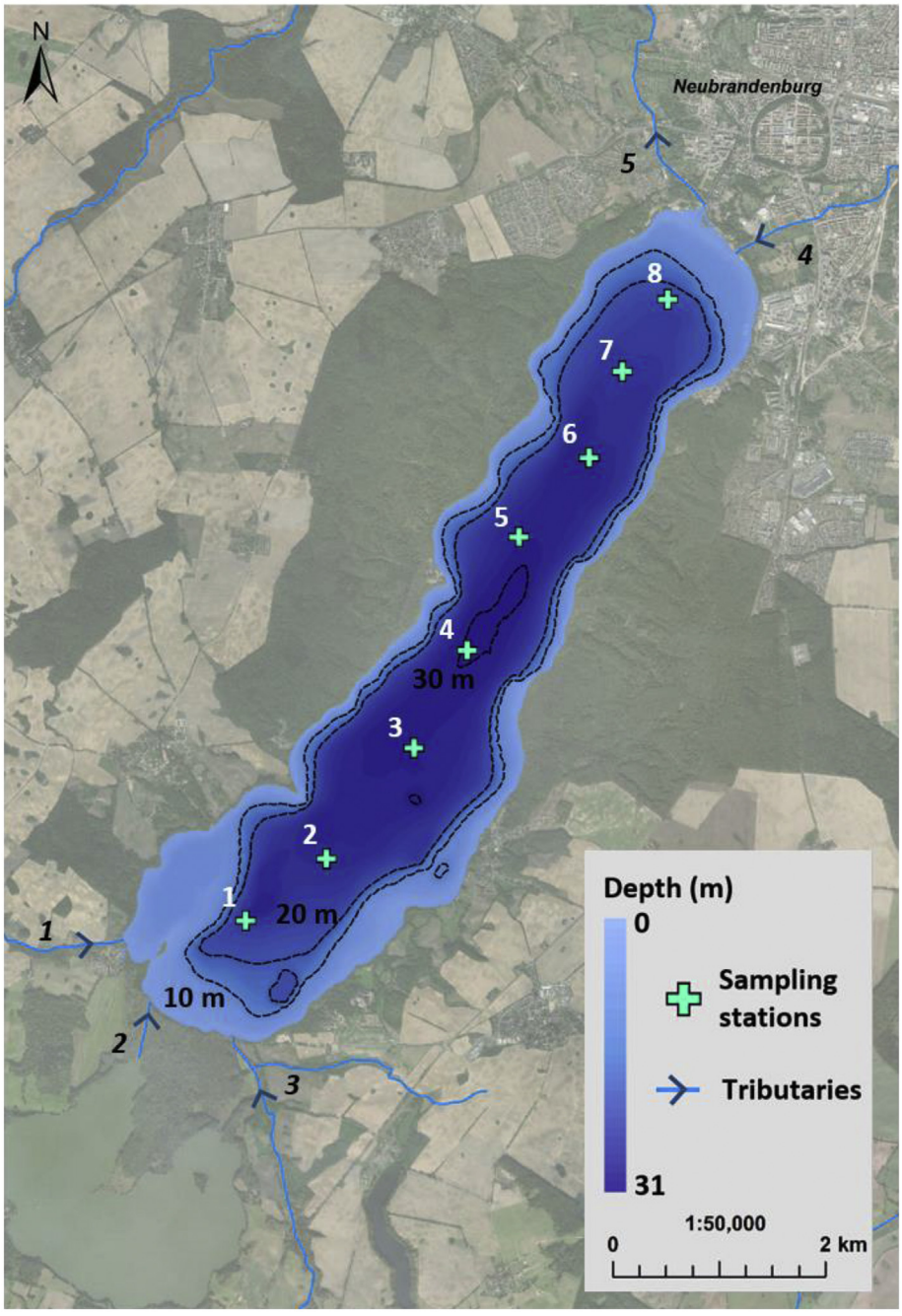

Fig. 1. Bathymetry of Lake Tollense including sampling stations (white numbers). Dotted lines indicate points of equal depth; Italic numbers mark tributaries: 1 = Wustrower Bach, 2 = Lieps Channel, 3 = Nonnenbach, 4 = Gaetenbach, and 5 = Tollense. Coordinate System: ETRS 1989 UTM Zone 33N; Bathymetry: MLUMV, 2017, background: ESRI, 2020).

(Photonic, F5100Endo). For each sample, the set of photos was examined for MPs in Photoshop (Adobe, Version CS5). MPs appear in shades of yellow under the described conditions, while natural debris shows only a weak signal in orange to dark red. The appearance of all particles was compared to a set of stained reference material as described in Tamminga et al. (2019). During counting, particles were classified as irregular particles (IPs) or fibers following Hartman et al., (2019) and their size was measured (length and width). Subsequently, counts per filter were grouped into three size fractions, namely >63–300 μm, >300–1000 μm, and >1000–5000 μm.

A subset of 179 stained particles including 90 IPs and 89 fibers was removed from the filters using tweezers and placed onto microscope slides for verification by μRaman-spectroscopy (Thermo Fisher Scientific, DXR2xi Raman Imaging Microscope). Spectra were recorded using a 532 nm laser at 5–10 mW (25 μm confocal pinhole) and 100-10 Hz integrating 1000 measurements. Nile Red induced fluorescence could be erased by photo bleaching (a few seconds to several minutes). Afterwards, the spectra were auto-compared to 16 reference libraries comprising spectra of artificial polymers and natural organic and inorganic materials. The threshold for accepting the match was set to 70%, but all matches were verified by the operator as well.

Within the analyzed subset, 178 particles were verified as

artificial polymers, while one particle did not give a distinct signal and could therefore not be assigned.

2.5. Statistics and visualization

Statistical analysis and data visualization were carried out using R (version 3.5.2, R Core Team, 2018) in an RStudio environment (version 1.1.463, RStudio Team, 2018). Plots were created using the 'ggplot2' package (Wickham, 2016). Geodata were processed in ArcMap (version 10.5.1, ESRI, 2017).

All data were tested for normality (Shapiro et al., 1965) and homogeneity of variance (Bartlett's test). As either one or both of the criteria were not satisfied, the Kruskal-Wallis-test was applied to test for differences between groups (e.g. depths). In case of significant differences ($\alpha = 0.05$), a Dunn-post-hoc-test with a Bonferroni correction was carried out. Spearman's rho was used to investigate correlations.

3. Results

3.1. Blank samples

Despite strict measures, a certain degree of contamination was detectable in procedural laboratory blanks (Table 1). Counts for blanks samples were 10–20 times lower than counts for actual field samples and depended on both particle shape and size fraction. In general, fibers were less abundant than IPs. Less than one fiber was present in blank samples on average. The highest abundance of fibers was detected in the size fraction >300–1000 μm. For IPs, contamination tended to rise with declining particle size. For the smallest size fraction (>63–300 μm) a mean abundance of 2.3 IPs per sample was detected, whereas the larger size fractions showed little to no particle counts.

3.2. MP concentrations in Lake Tollense and their seasonal variation

Microplastics were ubiquitous in the water column of Lake Tollense. A total of 4582 MPs were found across all samples comprising 1612 fibers and 2970 IPs. IPs were the dominant particle shape at all sampling campaigns.

Considerable differences were identified between individual sampling campaigns (Fig. 2). Median (mean) concentrations varied between 12 (14) fibers m^{-3} to 25 (24) fibers m^{-3} and 22 (27) IPs m^{-3} to 42 (47) IPs m^{-3}. The concentration of fibers in September 2018 differed significantly from that in the March sampling campaigns. Regarding IPs, concentrations in March 2019 and 2020 differed significantly from each other, but no differences were present compared to September 2018. Thereby, no clear seasonal pattern (Sep.: stratified vs. Mar.: overturn phase) could be observed.

Particle size distributions differed distinctly according to particle shape (Fig. 3). Across all sampling campaigns, fibers showed similar median (mean) lengths, precisely 924 (1304) μm in September 2018, 986 (1217) μm in March 2019, and 979 (1462) μm in March 2020. The variability of fiber lengths was equally high at all sampling campaigns (IQR: Sep. 2018: 989, Mar. 2019: 942, Mar. 2020: 960). The abundance of IPs rose with declining particle length showing median (mean) lengths of 172 (206) μm in September 2018, 170 (210) μm in March 2019, and 176 (223) μm in March 2020. The variability of IP lengths was less pronounced compared to fibers (IQR: Sep. 2018: 102, Mar. 2019: 122, Mar. 2020: 114).

Fiber diameters showed similar distributions (median, mean) for September 2018 (17, 17) and March 2020 (16, 18), but differed in March 2019. At this time, the median (mean) diameter was considerably lower with 13 (15) μm. This can be mainly attributed to the fact that a large number of fibers with diameters between 5 and 8 μm were present in the samples of March 2019. In fact, these fibers constituted the most abundant group concerning fiber diameters. The lengths of these fibers did not vary substantially from that of fibers found in samples of September 2018 or March 2020. Moreover, the fibers with diameters between 5 and 8 μm were not equally distributed across samples in March 2019 but were most abundant in the central part of Lake Tollense (Fig. 4, Stations 4 and 5). Towards the southern (Station 1) and northern (Station 8) ends of the lake their abundance decreased steadily. A total number of 20 thin fibers were analyzed for their polymeric composition. All of them were made of PET.

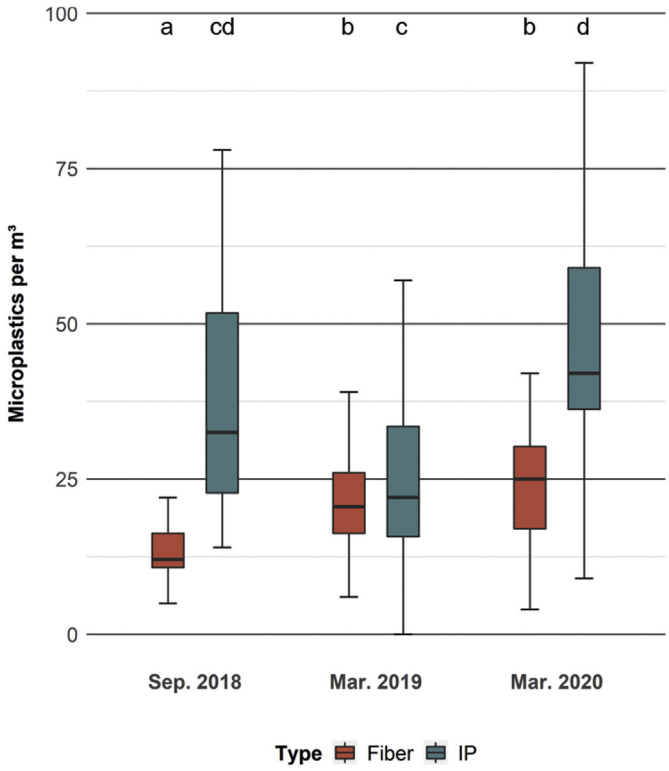

Fig. 2. Microplastic concentrations by particle shape and sampling campaign. Boxes indicate the range of the 25–75% quantiles, whereas whisker give the smallest/highest observation at a maximum of 1.5*IQR (inter quartile range) from the box. Letters at the top mark significant differences between groups.

Table 1
Mean and median blank sample contamination by particle shape and size fraction.

		Size fraction (μm)		
		>63-300	>300-1000	>1000-5000
IP	Mean (SD)	2.3 (2.7)	0.2 (0.4)	0 (0)
	Median	1.0	0	0
Fiber	Mean (SD)	0.1 (0.4)	0.5 (0.9)	0.3 (0.5)
	Median	0	0	0

3.3. Vertical profiles of MPs in Lake Tollense

The vertical distribution of MP in Lake Tollense revealed noticeable differences between depths that substantially depended on the particle shape (Fig. 5).

Fibers did not show a clear trend by depth but were almost

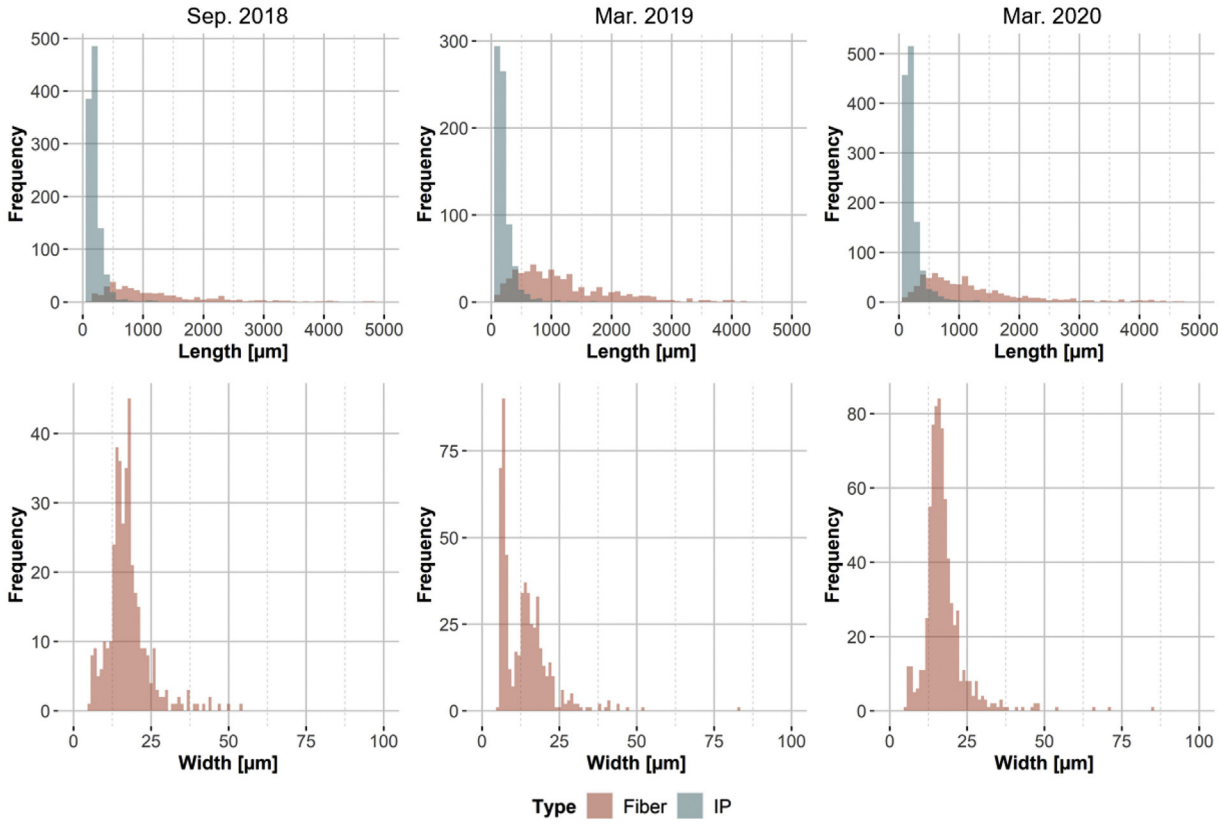

Fig. 3. Histograms of particle lengths by shape and sampling campaign (top) and fiber diameters by sampling campaign.

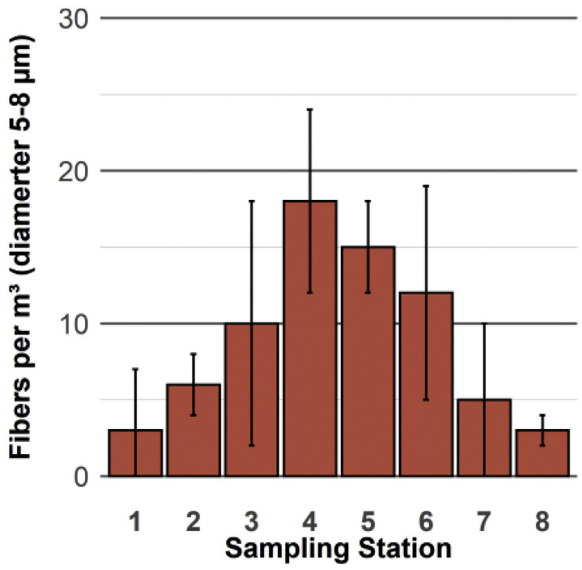

Fig. 4. Median concentrations of fibers with diameters between 5 and 8 µm by sampling station in March 2019 (depths integrated). Error bars represent the IQR.

equally distributed across the water column at all sampling campaigns. Considering all sampling campaigns their median (mean) abundance ranged from 16 (16) to 25 (24) fibers m^{-3} at 0 m, from 13 (12) to 26 (25) fibers m^{-3} at 7 m, and from 11 (13) to 22 (24) fibers m^{-3} at 10 m. For September 2018 and March 2019, the variability (IQR) of fiber concentrations was lower at 10 m samples (3 and 3) compared to 7 m (10 and 14) and 0 m samples (7 and 18).

IPs were more abundant in surface samples than in sub-surface samples. While these differences were significant for September 2018 when comparing 0 m and 10 m samples, they are less apparent in March 2019 and 2020. This could point to a seasonal component concerning the vertical distribution of IPs in Lake Tollense. The median (mean) abundance of IPs ranged from 43 (40) to 54 (51) IPs m^{-3} at 0 m, from 20 (19) to 46 (47) IPs m^{-3} at 7 m, and from 20 (21) to 35 (36) IPs m^{-3} at 10 m. The variability of IP concentration distinctly declined towards deeper water at all sampling campaigns, especially in the 10 m depth samples.

The particle sizes did not differ significantly by depth (see SM 2). IPs tended to be slightly larger (median length ca. 10% higher) in 7 and 10 m depth than at the water surface. For fibers, no such pattern was apparent.

As IP concentrations varied across both sampling campaigns and depths, 7 and 10 m samples were normed by surface concentrations at each respective sampling station to evaluate vertical concentration gradients (Fig. 6). Relative abundances >1 indicate that sub-surface samples contained higher amounts of IPs than surface samples, while values < 1 indicate a decline of IPs concentrations with depth. In some cases, sub-surface IP concentrations exceeded that of surfaces samples. This applies to three 7 m samples and one 10 m sample in September 2018, two 7 m samples in March 2019 and three 7 m samples and one 10 m sample in March 2020. In September 2018 and March 2019 relative abundances tended to rise from SW (station 1) to NE (station 8), while in March 2020 no such tendency was apparent. Local wind patterns seemed to have considerable influence on the intensity of the vertical gradients. In March 2020, e.g., strong SW winds coincided with higher relative IP abundances in sub-surface samples in the southern part of Lake Tollense. Likewise, relative IP abundances and wind speeds were higher towards the NE end of Lake Tollense in March 2019. In March

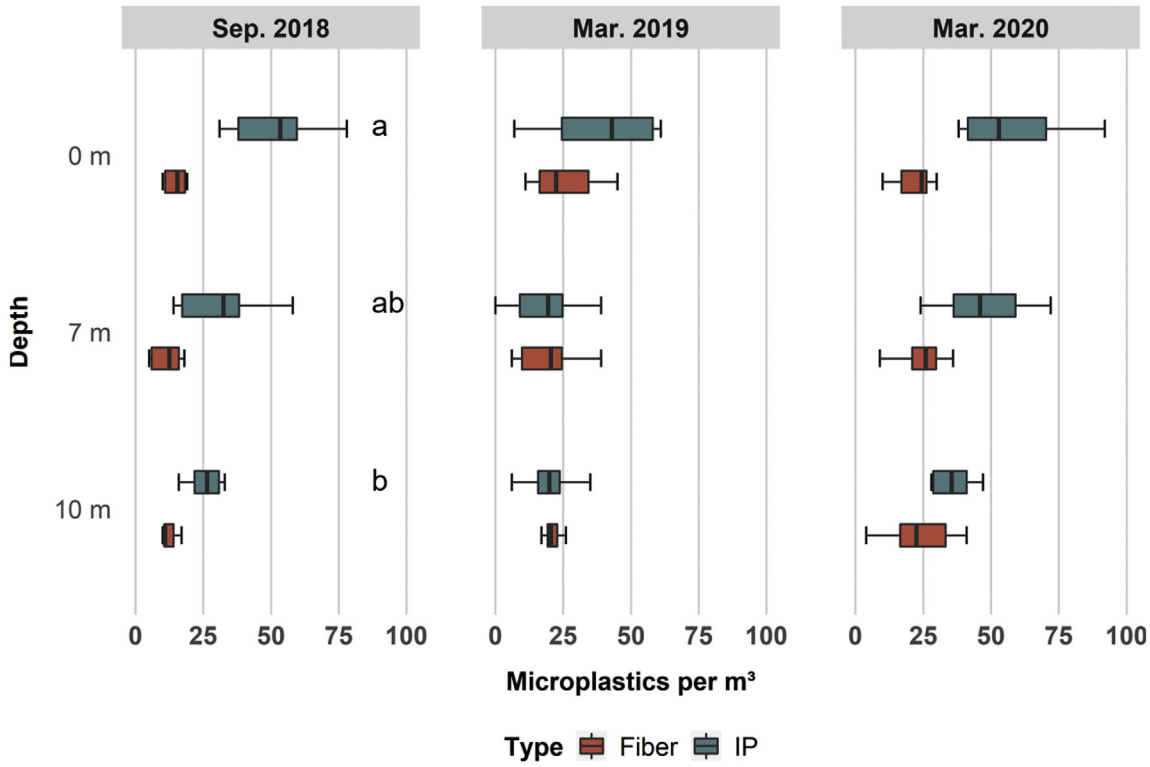

Fig. 5. Microplastic concentrations by depth, particle shape, and sampling season. Boxes indicate the range of the 25–75% quantiles, whereas whisker give the smallest/highest observation at a maximum of 1.5*IQR from the box. Letters mark significant differences between groups.

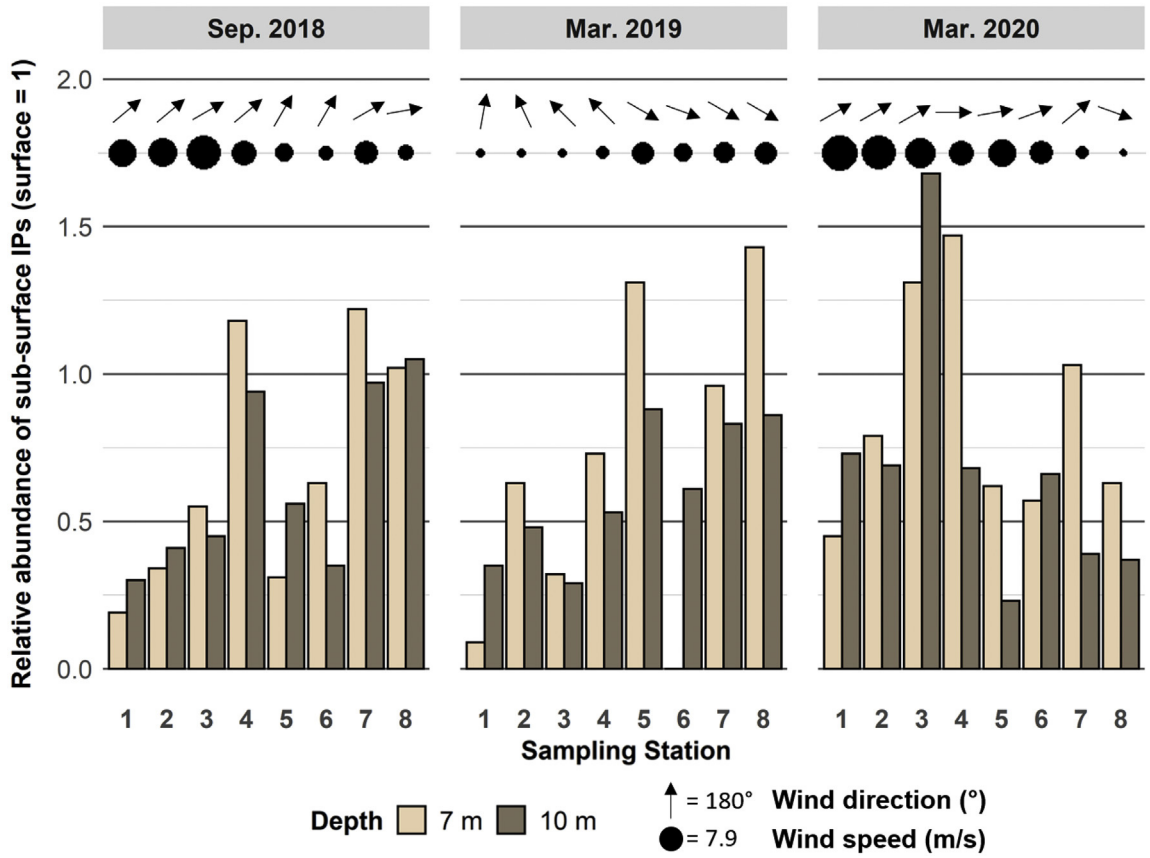

Fig. 6. Abundance of IPs in sub-surface samples normed by the IP concentration at surface and displayed by sampling season and station. (Note that Station 1 is the southernmost sampling stationd, while station 8 is the northernmost point along the transect). Wind speed and direction at Trollenhagen airport (7 km north of Lake Tollense) (DWD, 2020).

2019, a significant correlation (rho = 0.76) between wind speed and relative IP abundance in sub-surface samples was found.

3.4. Polymer composition and water temperatures

The results of the spectroscopic analysis were summarized for all sampling campaigns to enhance the sample size (see SM 3). Eight different polymer types were found across all samples. Concerning IPs, relative abundances were 59% PE, 20% PP, 12% PET, 4% PA, 1% PS, 1% epoxy resin and 1% POM. Fibers were dominated by PET (71%), followed by 18% PP, 7% PA (Nylon), 3% PAN, and 1% PE. No depth-related pattern was visible.

Water temperatures did neither reveal a relation to MP concentration nor relative abundances. In March 2019 and 2020 the water temperature was nearly constant across the sampled depths (Min.-Max; 5.2–5.6 °C in Mar. 2019 and 5.0–6.4 °C in Mar. 2020). In September 2018, the water temperature decreased with depth (14.4–20.2 °C).

3.5. Horizontal profiles of MP

SM 4 provides an overview of MP concentration by sampling stations. Fibers did not show a consistent horizontal concentration pattern across sampling stations (except thin fibers in March 2019). In September 2018 and March 2020, IP concentrations steadily increased from SW (station 1) to NE (station 8) with few exceptions. In contrast, no obvious pattern was detectable concerning IPs in March 2019.

4. Discussion

4.1. Spatio-temporal variation of MPs in Lake Tollense

MPs were found in all samples from Lake Tollense. In general, MP concentrations were in the range of previous reports from freshwaters, but methodical differences hamper a more insightful comparison of actual concentrations between studies. Most studies conducted so far, relied on net-based approaches with relatively coarse nets (Li et al., 2018). For example, Baldwin et al. (2016) found an average of 4.2 MP m^{-3} in 29 tributaries of the Great Lakes, which is about one order of magnitude less than in this study. However, they used a neuston net with mesh-size of 333 μm and it has been shown that sampling with finer nets/sieves may significantly increase the amount of captured particles (Dris et al., 2018). Bordós et al. (2019) used a comparable sampling system (lower detection limit 100 μm) as this study for investigating natural freshwaters as well as fishponds in the Carpathian basin and found MP concentrations of up to 32 MP m^{-3}. This is within the range of our results, especially when considering that 63 μm was the lower detection limit here.

Within Lake Tollense's water column distinct vertical concentration profiles were observed that seem to depend to a large degree on the particle shape. Whereas fibers were distributed relatively evenly across the water column, IP concentrations decreased with depth. Although some studies have investigated the vertical distribution of MP in the water column of the world's oceans, there is little information concerning the water column of freshwater lakes (Kooi et al., 2016; Kukulka et al., 2012; Song et al., 2018). A single study investigated the vertical distribution of MPs in Lake Michigan and the Milwaukee River and did not find significant differences between sampled depths (up to 13.7 m depth; Lenaker et al., 2019). Nevertheless, fibers constituted the vast majority of MPs in Lake Michigan, so that the absence of significant differences is in accordance with our results. It has been shown before that the shape of MP particles is a relevant factor concerning their terminal rising or settling velocity. Waldschläger and Schüttrumpf (2019) found that the terminal settling is distinctly lower for fibers when compared to fragments, cylinders or spheres in freshwater (see also Khatmullina and Isachenko, 2017). While buoyant IPs may rise to the surface, when no vertical mixing is induced (e.g. by wind), fibers may not rise with sufficient speed to establish noticeable vertical concentration gradients. Although the terminal rise velocity depends on the particle size as well, the differences in size found in this study were too small for a meaningful comparison.

Vertical differences in IP concentrations were only significant in September 2018. This might point to a certain seasonal component determining the intensity of the vertical concentration gradients, but more data is needed to assure this result. Several studies emphasized the importance of wind mixing concerning the vertical distribution of buoyant plastics in the oceans (Egger et al., 2020; Kooi et al., 2016; Kukulka et al., 2012; Lattin et al., 2004; Song et al., 2018). In general, MP concentration decreased exponentially with depth and Kooi et al. (2016) reported that the steepness of the decrease was related to particle properties and sea state. A general exponential decrease could not be observed in this study. The influence of wind-induced mixing is still apparent, but local wind conditions (e.g. due to land surface characteristics) seem to have influence as well. Taking the dominant wind direction, southwest, into account, the south of Lake Tollense is more sheltered by surrounding vegetation (see e.g. stations 1 and 2 in March 2020) and therefore vertical gradients are more pronounced. Moreover, relative IP abundances (see Fig. 6) in sub-surface samples followed a diurnal course in September 2018 (sampling on two consecutive days with station 1 and 5 sampled in the morning and stations 4 and 8 sampled in the afternoon). This is likely to be related to wind-induced mixing as wind speeds followed a similar diurnal pattern (see SM 5) while wind direction stayed rather constant.

Nevertheless, the described relationship of wind speed and relative MP abundance is not constant. The confluence of Lake Tollense's tributaries (three in the south and one in the north) might potentially cause turbulence that alters the vertical distribution of MPs in the water column (Lenaker et al., 2019). Given the rather small dimensions and discharge of these tributaries, it is unlikely that this effect plays a key role in Lake Tollense. Vertical gradients were only pronounced at the southern and northern end of the lake, when coinciding with higher wind speeds. This contradicts a strong influence of inflowing waters, as well.

Moreover, biofouling was reported to likely alter the MP vertical distribution. Song et al. (2018) stated that a solely wind-driven model underestimated the amount of low-density MPs present in the water column of six bays and two near-shore waters around South Korea and that biological interactions might explain this effect. Similarly, low-density polymers dominated the polymeric composition of particles throughout the water column in Lake Tollense. Lagarde et al. (2016) demonstrated that MP particles might be colonized by the freshwater microalgae *Chlamydomonas reinhardtii* within a few weeks under laboratory conditions and that PP particles formed hetero-aggregates (microalgae, microplastics and exopolysaccharides) with densities greater than freshwater. Moreover, the high surface to volume ratio of small MP particles might enhance the probability and the speed with which the buoyancy of these particles is affected by biofouling (Egger et al., 2020; Fazey and Ryan, 2016). Lake Tollense can be classified as meso-to eutrophic. Chlorophyll-a concentrations rise rapidly in spring (March/April) reaching values of up to 16 μg per L (IGB, 2018). This degree of biological productivity makes biofouling in

Lake Tollense plausible.

Fibers in the water column of Lake Tollense were predominantly composed of PET and to a lesser degree of PP, PA (Nylon), and PAN, which is in accordance with results from Lake Michigan and the Milwaukee River (Lenaker et al., 2019). Similar polymer types were found for fibers in coastal waters around South Korea, but PP was more abundant there than in this study (Song et al., 2018). The high prevalence of PP was attributed to oceanic activities such as professional fishing and shipping, which have minor relevance at Lake Tollense.

IPs in the water column of Lake Tollense consisted mainly of buoyant polymer types, namely PE and PP. Both polymers are widely found in freshwater and the oceans (Bordós et al., 2019; Di and Wang, 2018; Egger et al., 2020; Mason et al., 2016; Song et al., 2018). PE and PP are among the polymers with the highest demand in Europe and are used for a wide range of applications such as packaging, building construction and agricultural applications (Plastics Europe, 2019). All these applications must be considered as potential sources for MPs found in Lake Tollense.

Various studies have emphasized the fact that relying on surface sampling only can lead to severe overestimations of the number of MPs present in the water column, when surface concentrations are extrapolated (Lenaker et al., 2019). In contrast, the abundance of buoyant MPs can be underestimated, when assuming that all of them afloat at the water surface (Egger et al., 2020; Kooi et al., 2016; Kukulka et al., 2012). Lenaker et al. (2019) calculated relative differences between the water surface and depth-weighted concentrations and found overestimations of 22–162% for estuarine and lake locations. The same relative values for Lake Tollense result in an overestimation of 2–61% for fibers and 7–108% for IPS when neglecting surface concentrations that are lower than depth-weighted concentrations. Both, the results from Lake Michigan and Lake Tollense emphasize the need for depth-integrated sampling to ensure a more reliable quantification of MPs in the water column.

The horizontal distribution of MPs across the sampling stations can partly be attributed to prevailing winds and, with rising concentrations towards Neubrandenburg, the proximity to the city and related anthropogenic activities (at least in Sep. 2018 and Mar. 2020). The importance of human activities concerning the abundance and composition of MPs in freshwaters has been demonstrated before (Eerkes-Medrano et al., 2015). Moreover, the only outlet of Lake Tollense that drains the lake at its northern end might represent a bottle-neck for the discharge of MPs from the lake.

Agricultural areas and forests mainly surround Lake Tollense. Several agricultural applications include the use of artificial polymers such as plastic foils for silage bales. Silage bales e.g. are present on fields around the lake and fragments thereof might enter the water column via the tributaries and surface run-off, especially when considering intense precipitation events. Hitchcock (2020) emphasized the importance of such stormwaters as contributors for MPs in aquatic environments. At the time of sampling (and one week prior), no heavy rainfall occurred (see SM 6). Therefore, assessing the importance of MP inputs to Lake Tollense by stormwaters is not feasible, although a certain influence is plausible.

Atmospheric deposition (both dry and wet) may as well add to MP pollution in Lake Tollense. Brahney et al. (2020) found mean deposition rates of 132 particles per day and m^2 for (remote) protected areas of the western United States. Likewise, Allen et al., (2019) suggested that MPs might reach remote areas via atmospheric transport by analyzing MP deposition in a mountain catchment of the French Pyrenees. Due to these reasons, it is very likely that parts of the MPs present in Lake Tollense were deposited from the atmosphere.

4.2. Prevalence of thin fibers in March 2019

In March 2019, a considerable number of thin fibers (5–8 μm) were collected during sampling. Fibers of the same diameter did not occur in similar concentrations in September 2018 and March 2020. The same operators wearing the same clothes and using the same equipment on the same vessel were performing sampling at all three campaigns. Moreover, fibers with the given diameter were not present in blank samples and we are thus confident that these fibers represent an actual MP pollution within Lake Tollense.

The spatial distribution of the fibers that peaks in the center of the Lake could point to a potential proximal source, though, the actual source can hardly be identified. The sampling campaigns in March were conducted before the beginning of the tourist season. In this time of the year, a large campground, including several fishing huts, on the central-western shore of Lake Tollense (between stations 4 and 5) is the main pathway for activities on the lake (mainly fishing). The observed presence of thin fibers may be related to recreational activities at this campground. This might be supported by the fact that all fibers in the given diameter-range were composed of PET.

5. Methodical considerations and limitations

In this study, a total number of 72 samples were taken in three sampling campaigns at three depths across eight sampling stations. Thereby, we rely on one of the most comprehensive data sets concerning MPs in the water column of freshwater lakes. Nevertheless, more data would be needed to address e.g. the question of seasonality driven vertical MP concentration gradients caused by thermal stratification within the water column. This refers to both, a repeated sampling in the stratified season as well as sampling of the deeper water column including the hypolimnion. Moreover, a larger number of depth levels might draw a more detailed picture of MP vertical variation in lakes.

The contamination as measured by procedural laboratory blank samples was rather low and seemed therefore acceptable within the study's framework. Still, for future investigations more effort concerning anti-contamination measures might be needed, especially when MPs <63 μm get into focus.

6. Conclusions

This study investigated MP pollution within the water column of a deep dimictic lake and found distinct differences between sampled depths. Thereby, the importance of depth-integrated sampling approaches is highlighted to gain a more complete picture of MPs present in the water column of lakes and freshwaters in general. Moreover, severe over- and underestimations may bias the extrapolation of results from surface samples towards the water column.

The particles shape and wind patterns are likely influencing the vertical distribution of MPs in Lake Tollense. Moreover, variations of this distribution can occur on rather small spatio-temporal scales. To resolve this variation more accurate sampling should be conducted in future investigations with higher vertical resolution (sampled depths) and, if applicable, shorter time intervals between samples.

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Author statement

Matthias Tamminga: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization Elke Fischer: Conceptualization, Resources, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.115507.

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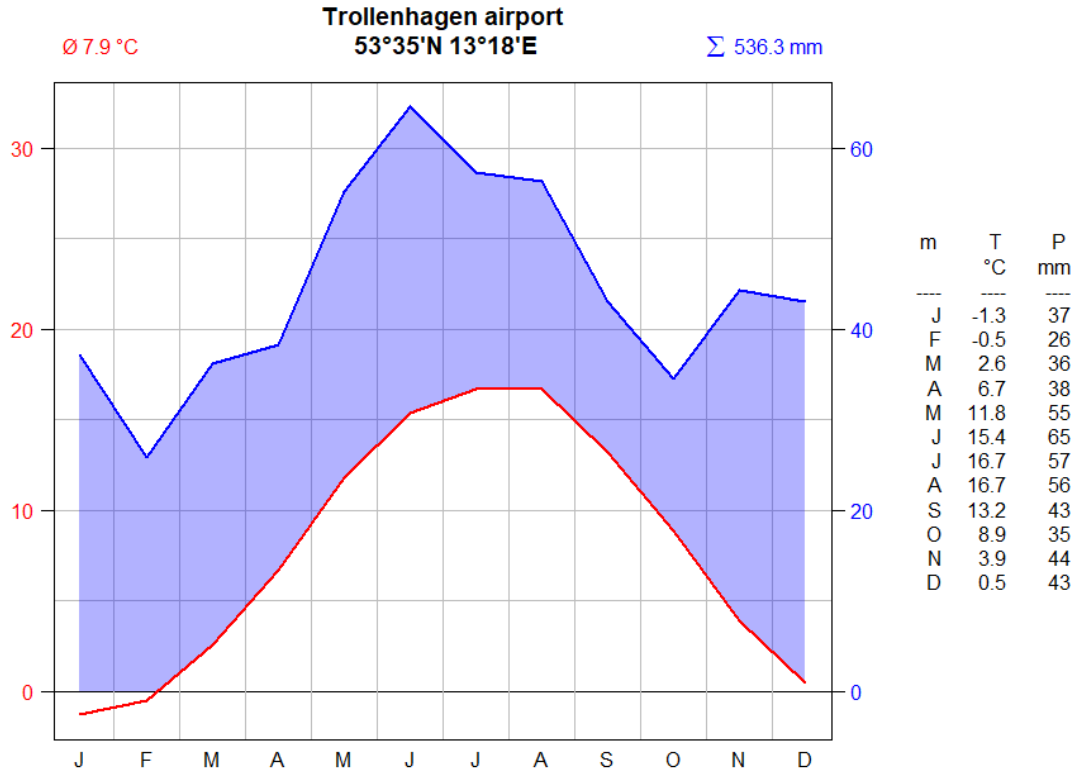
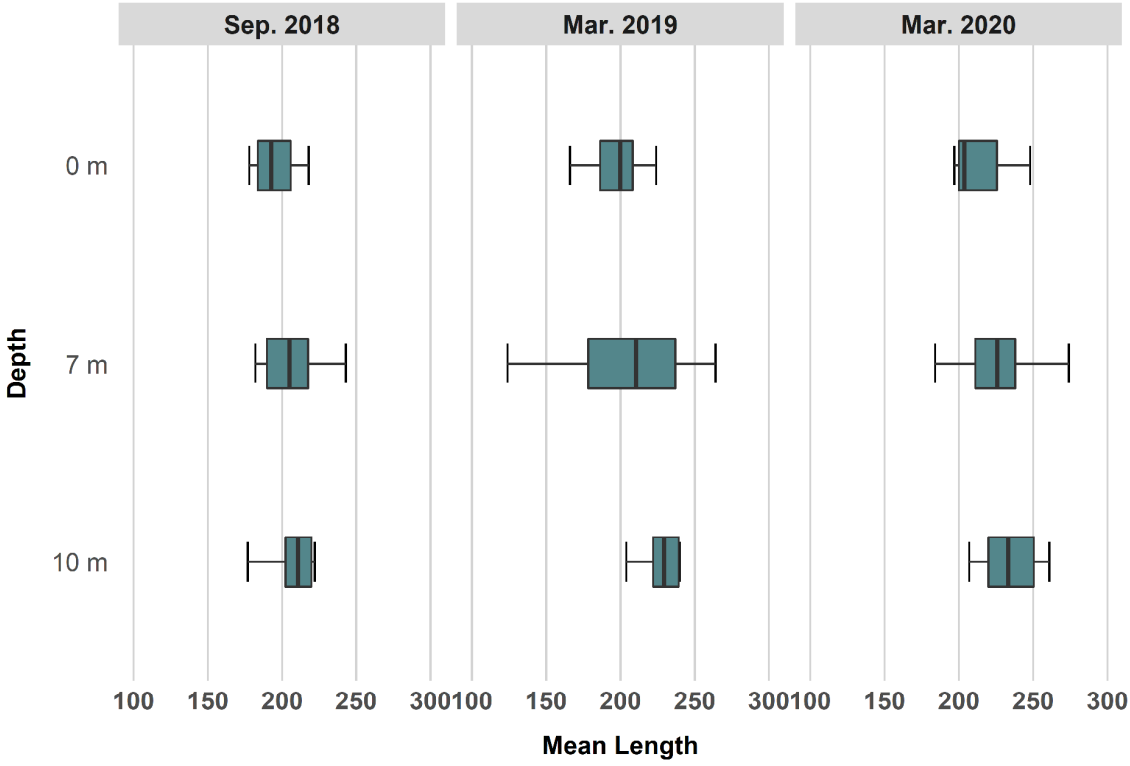
Supplementary to Tamminga and Fischer 2020: Microplastics in a deep, dimictic lake of the North German Plain with special regard to vertical distribution patterns

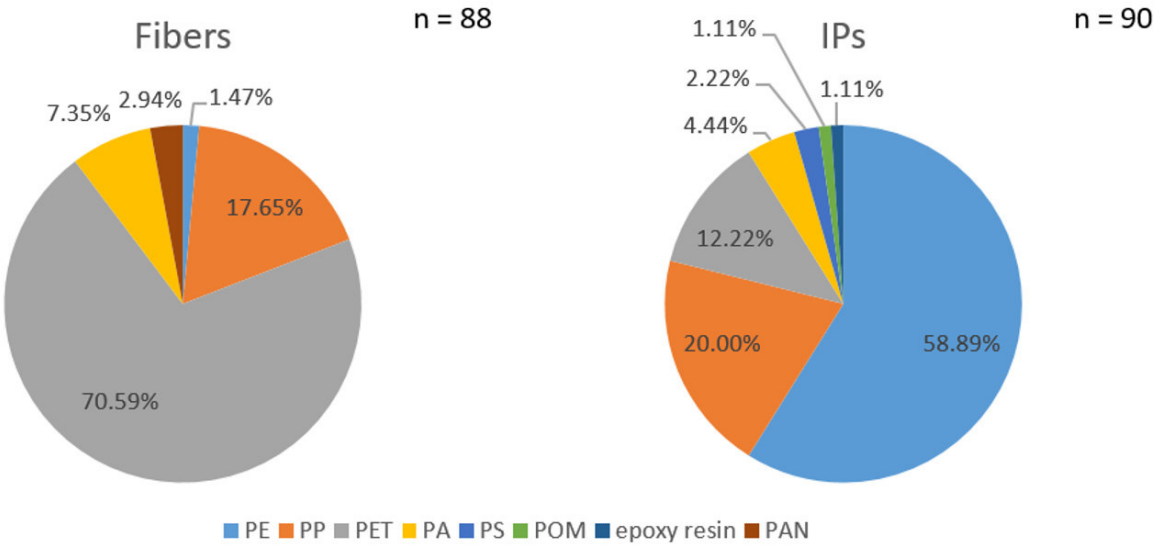


m	T °C	P mm
J	-1.3	37
F	-0.5	26
M	2.6	36
A	6.7	38
M	11.8	55
J	15.4	65
J	16.7	57
A	16.7	56
S	13.2	43
O	8.9	35
N	3.9	44
D	0.5	43

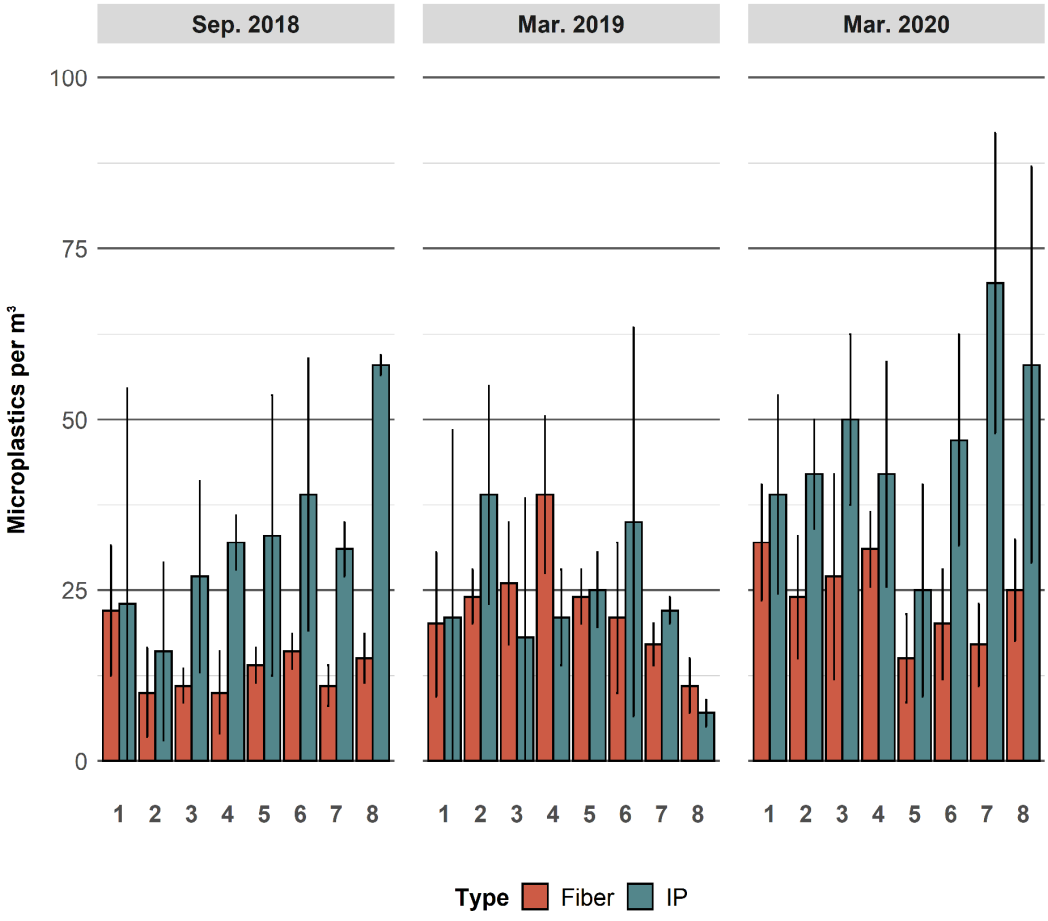
SM 1: Thirty-year (1976-2005) monthly mean of air temperature and precipitation sum at Trollenhagen airport (DWD 2020a). Plot created using the 'climateGraph' function as part of the 'berryFunctions' package in R.



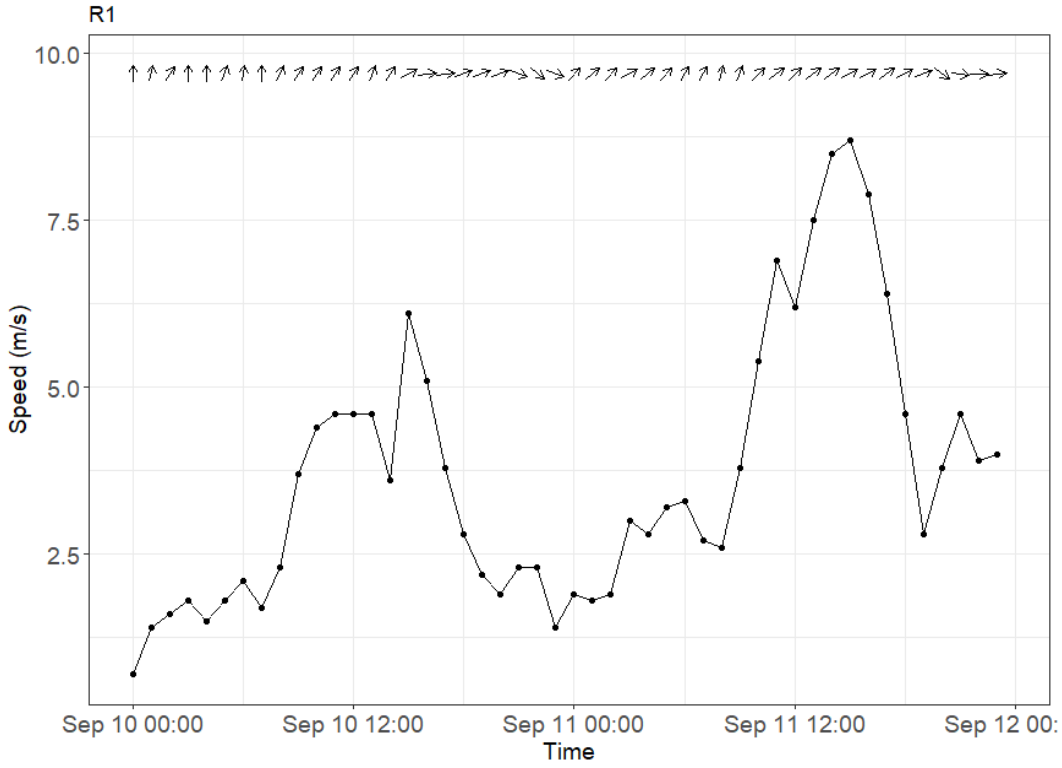
SM 2: Mean length of irregular particles found in water column samples of Lake Tollense by depth and sampling campaign.



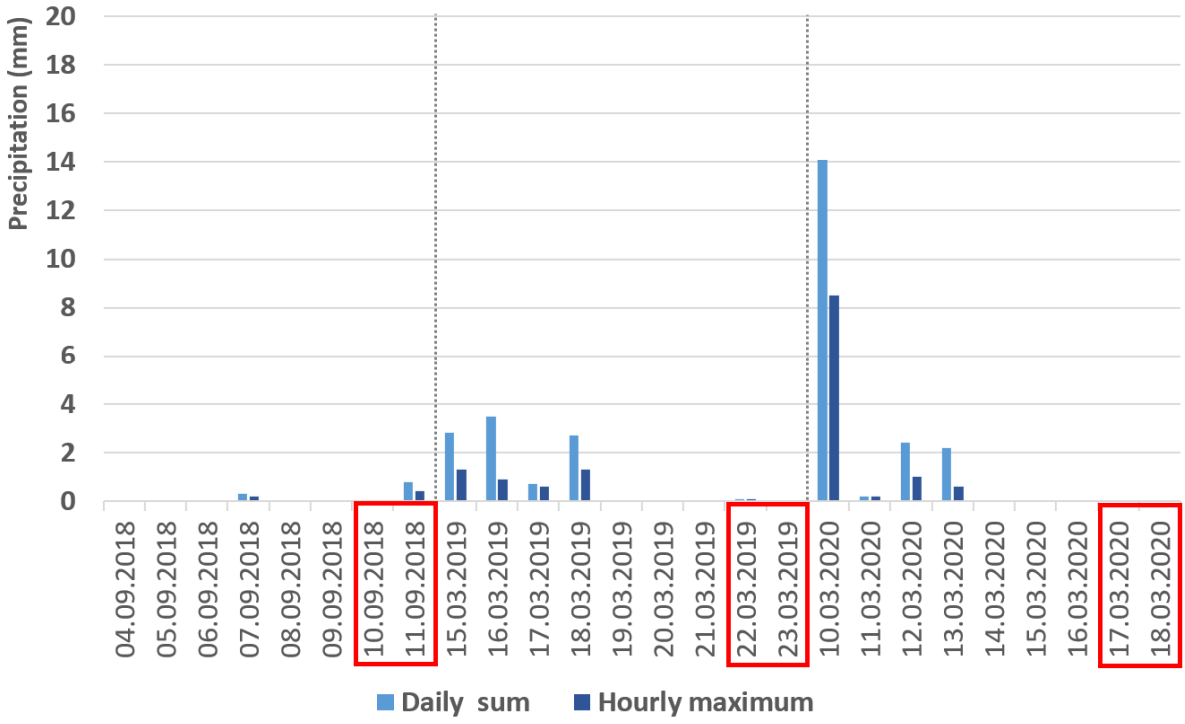
SM 3: Polymeric composition of microplastics in the water column of Lake Tollense by shape category.



SM 4: Mean microplastic concentration by sampling station, campaign, and shape category.



SM 5: Wind speed and wind direction at Trollenhagen airport during the sampling campaign in Semptember 2018 (DWD 2020b).



SM 6: Rainfall at Trollenhagen airport one week prior to sampling and during sampling expressed as daily sums and hourly maximums (DWD 2020c).

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Deutscher Wetterdienst (DWD) 2020b. Wind speed and wind direction for climate station 5109, Trollenhagen.

Deutscher Wetterdienst (DWD) 2020b. Precipitation as daily sums and hourly maximums for climate station 5109, Trollenhagen.

Appendix: Publication III

Environmental Science and Pollution Research
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RESEARCH ARTICLE

Microplastic concentrations, characteristics, and fluxes in water bodies of the Tollense catchment, Germany, with regard to different sampling systems

Matthias Tamminga[1] · Elena Hengstmann[1] · Ann-Kristin Deuke[1] · Elke Kerstin Fischer[1]

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Abstract

The widespread presence of microplastics in multiple environmental compartments has largely been demonstrated. Assessing the ecological risk that microplastics pose is, at the present stage, hindered due to methodical differences. Moreover, different methods hamper meaningful comparisons between studies and data on microplastics <300 μm is scarce. Therefore, we focused on microplastics >20 μm in freshwater and sampling-related aspects in this concern. Sampling was conducted between 2018 and 2020 in the Tollense catchment in northeastern Germany and was carried out by in situ pump filtration. Two different sampling systems (cutoff sizes 20 μm and 63 μm) were applied to filter water volumes of 0.075–1.836 m^3. Retained particles were analyzed by a combination of Nile red staining and micro-Raman spectroscopy. Thereby, we found microplastic concentrations between 123 and 1728 particles m^{-3} using the 63-μm cut-off size and between 1357 and 2146 particles m^{-3} using the 20-μm cut-off size. Local hydrodynamics (discharge and flow velocity) and land cover are likely influencing the observed microplastic concentrations and fluxes. The variability between both sampling systems cannot fully be explained by the different mesh sizes used. We argue that differentiation between a theoretical cut-off size (finest mesh) and a factual cut-off size (reliable quantification) can help to understand sampling related differences between studies.

Keywords Freshwater · Lake · Nile red · Data reliability · Pump filtration · micro-Raman-spectroscopy · Mesh size · Cut-off size

Introduction

The widespread presence of microplastics, plastic particles <5 or 1 mm (Arthur et al. 2009; Hartmann et al. 2019), has been documented in various environmental compartments. The affected environmental compartments comprise sediments and soils (Woodall et al. 2014; Vaughan et al. 2017; Corradini et al. 2019), fresh-and seawater (Enders et al. 2015; Faure et al. 2015; Setälä et al. 2016; Kanhai et al. 2018; Bordós et al. 2019; Park et al. 2020), ice (Kanhai et al. 2020), organisms (Leslie et al. 2017; Bessa et al. 2018; Li et al. 2018), and the atmosphere (Cai et al. 2017; Allen et al. 2019; Klein and Fischer 2019).

As the presence of microplastics in the environment is evident, the assessment of associated risks got into focus, recently (GESAMP 2019; de Ruijter et al. 2020; Koelmans et al. 2020). Assessing the risk of a contaminant needs information on its (1) toxicity and (2) environmental abundance (de Ruijter et al. 2020). Moreover, both aspects need to be relatable to one another, for example, by effect thresholds that are linked to environmental concentrations (Koelmans et al. 2020). (1) In terms of toxicity, laboratory-based studies have been carried out for various freshwaters species in the past, but particle sizes, shapes, and polymer types are often simplified and therefore not fully congruent with environmental microplastics (Murphy and Quinn 2018; Redondo-Hasselerharm et al. 2018; Mateos-Cárdenas et al. 2019). To solve this concern, Koelmans et al. (2020) proposed a method for transferring laboratory effect thresholds into environmentally realistic thresholds (in terms of sizes, shapes, and polymer types). (2) Concerning the environmental abundance of microplastics in freshwater, a growing amount of data became available by an increasing number of studies (Eerkes-Medrano and Thompson 2018). However, the majority of these studies relies either on sampling with

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relatively coarse nets (>300 μm) or, when targeting particles <300 μm, on small sample volumes (Li et al. 2018; Stock et al. 2019; Boyle and Örmeci 2020).

Various studies have shown that microplastic concentrations in water depend on the respective sampling method in general and on the applied mesh size specifically (Vermaire et al. 2017; Cai et al. 2018; Green et al. 2018; Covernton et al. 2019; Tamminga et al. 2019; Lindeque et al. 2020; Prata et al. 2020, 2021a). In this context, Green et al. (2018) compared 1 l grab samples (filter pore size 0.45 μm) to common net sampling techniques (mesh size 200–500 μm), with microplastic concentrations in grab samples being ca. 3 orders of magnitude higher than in net samples. Similarly, Lindeque et al. (2020) found that microplastic concentrations in seawater increased up to 10-fold when comparing nets with mesh sizes of 500 μm and 100 μm for surface trawling. Within these studies, reducing the mesh size was often accompanied by drastically reducing the sample volume. Covernton et al. (2019) identified a decreasing microplastic concentration with increasing sample volume and mesh size by reviewing the available literature. Thereby, both, sampling volume and the applied mesh size, influence reported microplastic concentrations. Moreover, data on microplastics <300 μm based on larger sampling volumes (>10 l) is scarce and methodical differences further limit the comparability of data.

The variability and reliability of reported environmental microplastic concentrations need better comprehension to identify potential risks associated with microplastics. Thus, this study focuses on microplastics smaller than common manta mesh sizes and sampling-related aspects in this concern. We evaluated microplastic concentrations in tributaries and a lake down to a particle size of 20 μm while maintaining a reasonably high sampling volume. Moreover, comparing two sampling systems with different cut-off sizes, we discuss the implications connected to using different mesh sizes on resulting microplastic concentrations and fluxes.

Material and methods

Study area and sampling locations

The present study was conducted within the catchment of the river Tollense in northeastern Germany. The Tollense drains a catchment of 1829 km^2 and is a tributary of the river Peene, which flows into the Baltic Sea (LUNG 2004). The Tollense's upper catchment is characterized by Lake Tollense that covers 17.9 km^2 and drains an area of 525 km^2. Lake Tollense is fed by several small tributaries (mean discharge) of which the Gaetenbach (0.55 m^3/s), Nonnenbach (0.57 m^3/s), Liepskanal (0.49 m^3/s) and Wustrower Bach (0.10 m^3/s) contribute the largest share of surface inflows (Nixdorf et al. 2004). The only outlet of Lake Tollense is the river Tollense draining the lake at its northern end.

A total of five locations were sampled along the course of the Tollense and its tributaries (Fig. 1). Three of these sampling locations are tributaries of Lake Tollense (Gaetenbach, GB; Nonnenbach, NB; and Wustrower Bach, WB), while two sampling locations are situated downstream of the lake (Tollense at Neubrandenburg, TN and Tollense at Woggersin, TW). Additionally, eight locations within Lake Tollense were sampled.

For each sampling location (exclusive lake samples), individual sub-catchments draining toward the respective point were calculated (Fig. 1). Land cover within these sub-catchments was derived from the CORINE land cover data set (EEA 2021; LUNG 2021; see supporting information (SI) 1 for catchment area sizes and SI 2 for land cover distribution within the study area). Agricultural areas and forests dominate land cover within the study area (Table 1). The sub-catchments GB, TN, and TW show higher shares of urban and industrial areas, which can be attributed to the fact that GB, TN, and TW comprise parts of the city of Neubrandenburg. Neubrandenburg has a population of 64,086 and is the economic and touristic center of the area (StatA MV 2019).

Sampling

Two different pump filtration systems were used for sampling in this study. The first system (hereinafter 63-μm system) has been used for sampling microplastics in Lake Tollense before and is described in detail in Tamminga et al. (2019). The 63-μm system consists of a submersible pump connected to a cascade of analytical testing sieves (mesh widths: 1.0, 0.63, 0.3, 0.2, and 0.063 mm) via a PVC hose. The pump's flow rate (verified in the field, cf. Tamminga et al. 2019) was used to quantify the volume of filtered water. The second system (hereinafter 20-μm system) is a commercially available in situ filtration system for sampling microplastics in water (Microplastic particle pump, KC Denmark). The 20-μm system is a modified version of the sampling device described in Karlsson et al. (2020) and Schönlau et al. (2020). It consists of a stainless steel pump placed above a cascade of up to four stainless steel sieves (mesh widths: 0.3, 0.1, 0.05, and 0.02 mm) followed by an inductive flow meter. The 20-μm system is connected to a computer terminal where precise programming of the desired sample volume can be done (see SI 3 for a depiction of the in-field setup).

Sampling has been conducted in March 2018, 2019, and 2020 using the 63-μm system at the Tollense and its tributaries. The sampling station WB could only be sampled in March 2018 due to low water levels in the subsequent years. Moreover, TW was only sampled in March 2019 and 2020. The 20-μm system was used to sample the same stations

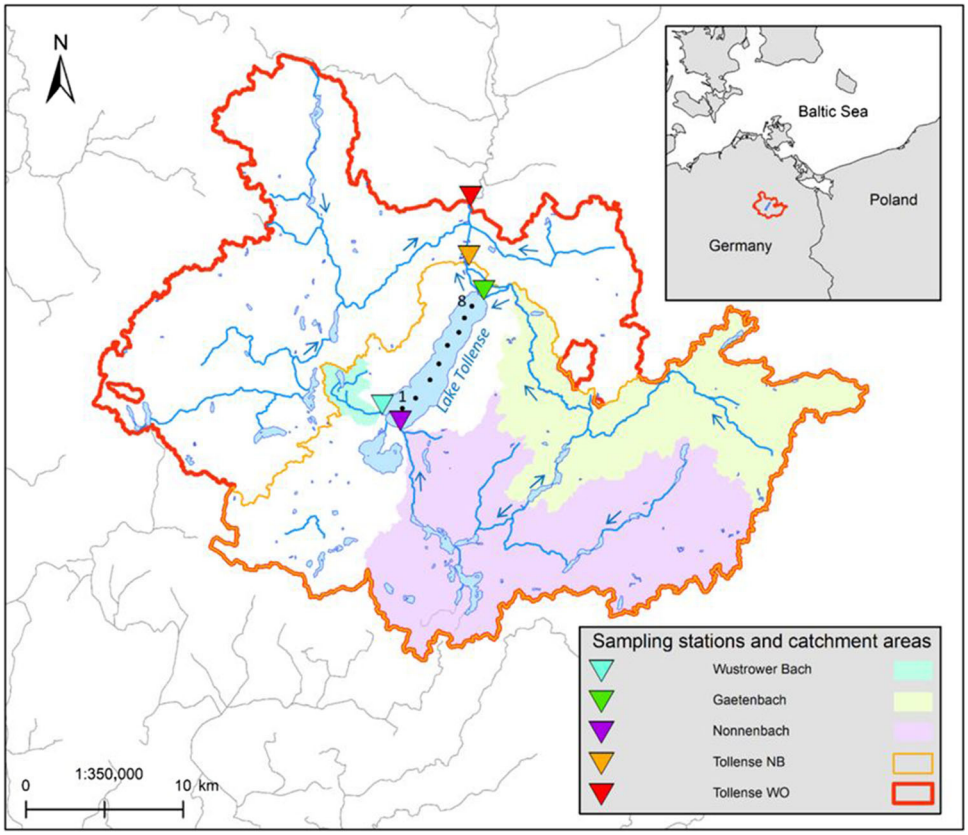

Fig. 1 Sub-catchments of the Tollense and its tributaries with respect to sampling locations (triangles). Sampling locations at Lake Tollense are represented by black dots and numbers indicate relative sampling positions from 1 = south to 8 = north. Flow directions are shown as blue arrows. Coordinate system: ETRS 1989 UTM Zone 33N; Water bodies and catchments: LUNG 2021)

(except WB) as well as Lake Tollense in September 2019. At Lake Tollense, eight stations distributed across the entire lake (Fig. 1) were sampled (results for the same stations are available from Tamminga et al. 2019 and Tamminga and Fischer 2020).

The infield procedures for both sampling systems were similar. Sampling was carried out at bridges in the case of tributaries and from a small vessel in the case of Lake Tollense. The pumps were lowered toward the water surface using a stainless steel winch (including a stainless steel wire) until the water inlets were fully submerged (0–15 cm below the surface). Applying the 20-µm system, pumping stopped automatically after filtrating 1 m^3 of water. Concerning the 63-µm system, pumping was stopped manually after eleven minutes (aiming for at least 1 m^3). At the tributary locations, small particles were sometimes clogging the finest sieves (see supplementary information 4 for sample volumes). If clogging was observed (decrease of the flow rate), pumping was interrupted and sieves were replaced by clean ones (max. twice). If sieves tended to clog again quickly, sampling was continued without the finest sieve. Thereby, using the 63-µm system, between 0.075 and 1.836 m^3 of water were filtered per station for the smallest size fraction >0.063–0.2 mm. For particles >0.2 mm, between 0.990 and 1.836 m^3 were filtered with the exception of WB. At WB, the total sample volume did not exceed 0.417 m^3. The 20-µm system was capable of filtering 1 m^3 at every station, except NB, where 0.7 m^3 were filtered for the smallest size fraction >0.02–0.05 mm.

Table 1 Land cover (%) within the catchment of the Tollense based on individual sampling locations and CORINE land cover data (EEA 2021)

Land cover in %	GB	NB	WB	TN	TW
Agriculture	78.9	72.2	89.7	63.7	69.5
Forest	14.6	22.6	6.6	24.9	20.0
Water	1.1	3.2	3.7	6.0	3.9
Parks and leisure facilities	1.0	0.2	0.0	0.8	0.9
Natural vegetation	0.2	0.6	0.0	1.1	1.4
Industrial or commercial	0.8	0.0	0.0	0.8	1.3
Urban	3.3	1.2	0.0	2.7	3.0

The content of sieves was rinsed into brown glass jars (500 ml) using ultrapure water (finest filter: 0.2 µm). One milliliter of hydrochloric acid (HCl, 37%, VWR) was added to each glass for preservation, and glasses were stored at 4°C until further processing in the laboratory.

At each sampling location, flow velocity and discharge were recorded by an ADC (Acoustic Digital Current Meter, OTT HydroMet GmbH) immediately after the microplastic sample was retrieved.

Sample purification and QA/QC measures

For digesting biogenic organic matter, the sample material was recovered from brown glass jars and transferred into glass beakers via rinsing with little ultrapure water. Here, we followed a digestion protocol that has been successfully applied in former studies and is described therein in detail (Hengstmann et al. 2018; Tamminga et al. 2018, 2019; see as well SI 5). In brief, the protocol comprises two digestion steps using oxidizing agents at room temperature. First, 60 ml hydrogen peroxide (H_2O_2, 30%, Merck) per 50 ml sample volume was added to the beakers. After an exposure time of 7 days, samples were poured through a sieve (mesh width according to the respective sampling system's finest mesh) to eliminate the remaining H_2O_2 and were rinsed into the beakers again. Then, 16.7 ml of sodium hypochlorite (NaClO, 6–14% active chlorine, Merck Emplura) per 50 ml sample volume were added and the reaction was allowed to proceed for 24 h. Finally, samples were filtered onto qualitative filter papers (VWR, qualitative filter paper 413, 5–13 μm particle retention) using a stainless steel filtration funnel. The filters were transferred into glass Petri dishes, covered with a watch glass, and left to dry at room temperature.

Assuring and controlling the quality of analyses is of particular importance in microplastic research (Koelmans et al. 2019). Prata et al. (2021b) analyzed 50 recent publications dealing with microplastics in various environmental compartments regarding ten contamination control parameters. In conclusion, they formulated seven essential aspects of contamination control that were also used as a guideline in this study. Cotton laboratory coats were worn by all personnel; samples were processed in a room with limited access and equipped with an air purifier (Philips, AC3256). Moreover, used materials were made of glass and metal whenever applicable and sieves were washed before as well as in between sample processing, and samples were covered with watch glasses at any time except when directly handled. To account for remaining contamination, procedural laboratory blanks were processed alongside field samples. Blanks underwent the same steps as actual samples, except for starting with 50 ml of ultrapure water. The mean number of microplastics present on blank filters was subtracted from field sample counts.

Quantification and qualification of microplastics

Microplastics on filters were quantified by Nile red staining. Nile red (Nile red, extra pure, Carl Roth) was solved in chloroform ($CHCl_3$, AnalaR NORMAPUR, VWR) as described in Tamminga et al. (2018, 2019). One milliliter of Nile red solution (1 mg/ml) was applied on each filter; filters were covered with a watch glass and dried for at least 24 h at room temperature. Afterwards, filters were photographed under a fluorescence microscope (Zeiss, AxioLab A1, 2.5x/006 A-Plan) connected to a digital camera (Canon EOS 80D, exposure time 1′, ISO 500, 6000 × 4000 μm) and equipped with an external light source (Photonic, F5100Endo, broadband) and a TRITC HC Filter Set (AHF, ex.: 532–554 nm, em.: 573–613). For particle counting, photos were examined in Adobe Photoshop (version CS5) and stained particles were compared to a set of artificial and natural reference materials (see supporting material of Tamminga et al. 2019). Plastics appear in shades of yellow, while natural debris if stained at all, appears in orange to dark red (see Supplementary Information 6 for illustration of the differentiation between microplastics and natural particles as well as assigned Raman spectra). Microplastics were classified as irregular particles (IPs) or fibers according to Hartmann et al. (2019) and their length and width were recorded. Within this study, the sum of both shape categories is referred to using the term particles.

A subset of 336 stained particles (2.24% of all particles) comprising 188 IPs and 148 fibers were subsequently analyzed by μRaman spectroscopy (Thermo Fisher Scientific, DXR2xi Raman Imaging Microscope) to verify their artificial origin and to gain information on their polymeric composition. A 532 nm laser was applied at 5–10 mW using a 25-μm confocal pinhole. Either ×10 or ×50 magnification was used depending on the respective particle size. Spectra were obtained at 10–100 Hz integrating up to 1000 scans. The results were compared to multiple spectral libraries, including artificial and biogenic polymer spectra as well as mineral signals. The best match was chosen for assigning a polymer type to each particle. In general, a match of 70% was perceived as sufficient to accept an assignment, but a trained operator verified the auto-assignments as well.

Data analysis and processing

All data were analyzed in R (R Core Team 2018) in an RStudio environment (RStudio Team 2018). Plots were generated using the ggplot2 package (Wickham 2016). Geodata were processed in ArcMap (Esri, Version 10.5.1).

Numeric concentrations reported for microplastics in freshwaters often span several orders of magnitude. Besides variability related to actual differences of environmental conditions, methodical discrepancies hamper comparisons between studies. Thus, Koelmans et al. (2020) proposed an alignment method based on the power law character of microplastic size distributions to account for some of the variability between studies targeting different size ranges. Following their proposal, we fitted a linear model to log-log plot of relative microplastic abundances by size classes as described in Kooi and Koelmans (2019). Thereby, we calculated α-values of 2.0 ($R^2 = 0.914$) for the 63-μm system and 2.4 ($R^2 = 0.975$) for the 20-μm system that were used to determine correction factors in the following.

Results

Results are described separately for both sampling systems, as distinct differences in measured microplastic concentrations were visible. These differences are discussed in the following.

Blank contamination was deemed acceptable compared to the number of microplastics that were found on filters of field samples. Concerning the 63-µm system, 1.6 ± 2.7 IPs and 1.2 ± 1.5 fibers were detected in blanks on average (±SD, see SI 7 for detailed blank results). For IPs, contamination tended to rise with decreasing size, but for fibers no such trend was visible. Blank samples of the 20-µm system showed a similar size-related distribution. Mean contamination consisted of 10.6 ± 10.0 IPs and 2.1 ± 3.0 fibers. Contamination was higher for the 20-µm system, which is in general expectable due to the increased number of smaller particles (cf. Prata et al. 2021b). Such small particles are also more likely to be transported through the air, thus inducing a higher risk for airborne contamination (e.g., Klein and Fischer 2019). Still, the number of microplastics on filters from field samples exceeded the number of microplastics on blank filters by 8–20 times.

Results applying the 63-µm system

Microplastic concentrations were in a similar range when comparing the three sampling campaigns in which the 63-µm system was used (Table 2). Microplastic concentration was highest in March 2019, followed by March 2020 and March 2018. Microplastics were dominantly composed of IPs (mean ± SD share: 80 ± 11% IPs and 20 ± 11% fibers). Moreover, microplastic concentrations were more variable in March 2019 when compared to March 2020 and March 2018. This was mainly due to the concentration measured at GB in March 2019, which was considerably higher compared to the other sampling points.

Figure 2 displays the spatial distribution of microplastics retrieved with the 63-µm system. Microplastic concentrations at GB were by far above those at other sampling locations. Moreover, a slight increase of microplastic concentrations toward downstream locations within the catchment is visible. Mean (median where applicable) concentrations were 1110 (1059) particles m^{-3} at GB, 320 particles m^{-3} at TW, 247 particles m^{-3} at WB (single measurement), 225 (200) particles m^{-3} at TN, and 157 (154) particles m^{-3} at NB. GB showed the highest microplastic concentrations across all sampling campaigns, while NB showed the lowest concentrations in March 2019 and 2020. In March 2019, the lowest concentration was measured at TN (123 particles m^{-3}), but the concentration at NB was only slightly higher (124 particles m^{-3}).

Based on the discharge measured in the field (available in SI 8), we calculated microplastic fluxes by multiplying concentrations (particles m^{-3}) with discharge values (m^3/s). Subsequently, results were referred to a flow of particles per day. The derived fluxes differed considerably across sampling locations and tended to increase toward downstream locations within the catchment. Mean (median where applicable) fluxes were 129.9 million particles per day at TW, 58.4 (58.5) million particles per day at TN, 47.4 (42.6) million particles per day at GB, 5.0 (5.3) million particles per day at NB, and 1.5 million particles per day at WB. TW showed the highest microplastic fluxes in all sampling campaigns (not sampled in March 2018), while the lowest fluxes were calculated for NB (excluding WB).

Calculating absolute flux values enables generating a simple budget for in- and outputs of microplastics at Lake Tollense. TN is located close to the only outlet of Lake Tollense and can therefore be used to approximate outputs of microplastics from the lake. All major tributaries of Lake Tollense were sampled as well (GB, NB, and WB). The balance was then calculated by subtracting the output fluxes from the sum of the input fluxes. In March 2018 (Δinputs-output: −6.0 million particles per day) and 2020 (−35.5 million particles per day), microplastic fluxes out of Lake Tollense exceeded inputs. In contrast, input fluxes were higher than the output flux in March 2019 (24.8 million particles per day).

Flux values were further normed to the catchment size (hectare) to allow for evaluating the pollution intensity with respect to land cover patterns. The ranking of the sampling locations changes when considering particle fluxes per day

Table 2 Microplastic concentrations by sampling device, sampling campaign, and particle shape (*Fib*. fibers; *Par*. particles) expressed in particles per m^3

	63-µm system						20-µm system					
	March 2018			March 2019			March 2020			September 2019		
	IPs	Fib.	Par.	IPs	Fib.	Par.	IPs	Fib.	Par.	IPs	Fib.	Par.
Mean	247	39	**286**	512	56	**567**	403	85	**488**	1612	200	**1812**
Median	183	37	**224**	160	49	**209**	228	85	**349**	1620	195	**1872**
SD	175	18	**176**	752	29	**778**	398	45	**388**	333	60	**339**

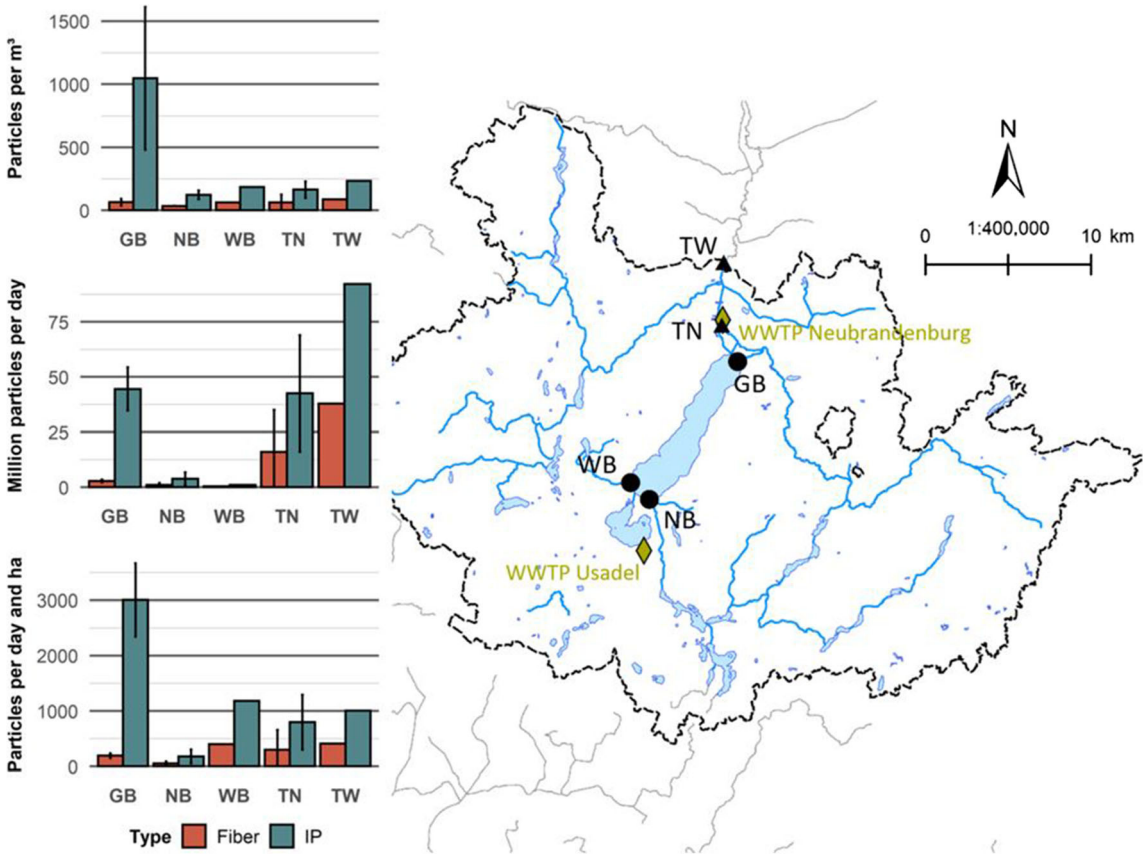

Fig. 2 Spatial distribution of microplastics expressed as particle concentrations and fluxes by shape categories and sampling location. Error bars indicate the standard deviation. Sampling locations are displayed as upstream (circles) or downstream (triangles) of Lake Tollense. Yellow diamonds represent wastewater treatment plants (WWTP). Note that the WWTP in Neubrandenburg is the only larger WWTP in the area with a population equivalent (p.e.) of 100,000 inhabitants (WWTP Usadel p.e.: 1400 inhabitants). Coordinate System: ETRS 1989 UTM Zone 33N; water bodies and catchments: LUNG 2021)

and hectare. In this manner, GB is characterized by the highest flux rates, followed by WB, TW, TN, and NB. With a mean of 227 particles per day and hectare, NB is well below fluxes at the other locations (>1000 particles per day and hectare).

Microplastic concentrations showed a significant ($p < 0.05$) positive correlation ($r = 0.69$) with discharge data (Fig. 3, left). This correlation was valid at all sampling locations except for GB. Here, microplastic concentrations tended to decline with rising discharge. This tendency was more pronounced when

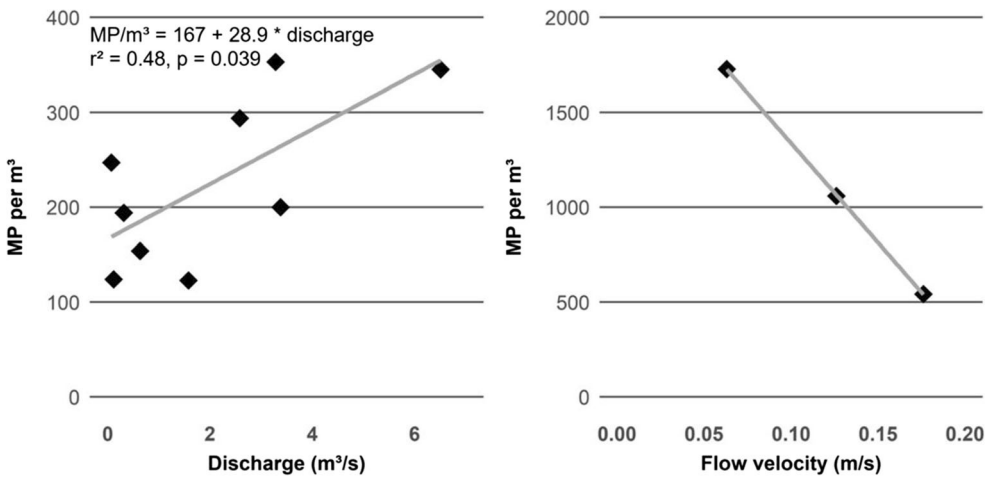

Fig. 3 Microplastic concentrations in relation to the current discharge at sampling locations for all locations except GB (left) and microplastic concentrations in relation to the mean flow velocity at GB (right)

choosing the mean flow velocity instead of discharge (Fig. 3, right).

Results applying the 20-μm system

Microplastic concentrations retrieved with the 20-μm system were considerably above values of the 63-μm system (Tab. 2). Again, microplastics were dominantly composed of IPs (mean ± SD share: 89 ± 3% IPs and 13 ± 4% fibers), but the share of fibers was even lower than using the 63-μm system. The spatial distribution was similar to the March sampling campaigns when the 63-μm system was applied. The highest concentration was measured at GB (2146 particles m^{-3}) and the lowest concentration was measured at TW (1357 particles m^{-3}).

In principle, the spatial distribution of microplastic fluxes retrieved with the 20-μm system was similar to patterns observed with the 63-μm system. The highest fluxes were observed at the downstream locations TN (120.1 million particles per day) and TW (109.7 million particles per day). Concerning the tributaries of Lake Tollense, fluxes at GB (15.6 million particles per day) were above those at NB (2.0 million particles per day). With −103.2 million particles per day, the deficit of the input-output balance is more pronounced compared to values gained with the 63-μm system. Fluxes normed to catchments were higher at the downstream locations TN (2267 particles per day and hectare) and TW (1195 particles per day and hectare) when compared to GB (1050 particles per day and hectare) and NB (91 particles per day and hectare).

Within Lake Tollense, microplastic concentrations were three- to fourfold lower than in its tributaries and the Tollense. The mean (median) concentration was 489 (496) particles m^{-3}. No distinct spatial pattern of microplastic concentrations in the lake was observed (see supplementary information 9). The highest concentration was measured at Station L7 (625 particles m^{-3}) in the north of the lake, while station L4 in the center of Lake Tollense showed the lowest microplastic abundance (234 particles m^{-3}).

Polymeric composition and particle size distribution

The results of the spectroscopic analysis were summarized for both sampling systems to enlarge the sample size (Fig. 4). Of the analyzed particles, 332 were verified as artificial polymers, three did not show a conclusive signal and one particle was composed of cellulose. Mean (SD) match quality was 84 ± 12% and the mean signal-to-noise ratio was 69 ± 44.

Particle shape did significantly influence the material composition. IPs were dominantly composed of polyolefins (78%) and to a lesser degree of PET (polyethylene terephthalate) and PVC (polyvinyl chloride). PS (polystyrene) was only observed in samples of TW in the form of spherical particles. The chemical composition of fibers was less variable with the majority of fibers being made of polyester. PP (polypropylene) fibers had considerably larger diameters (>40 μm) compared to PET fibers (mostly below 30-μm in diameter).

Particle size distributions by sampling campaign and particle shape are displayed in the supporting information (SI 10). In general, size distributions differed according to the particle shape. IP abundance was exponentially increasing with decreasing particle size, except for particles whose size was close to the mesh size of the respective sampling system. In contrast, fibers were widespread across the size range and showed the highest abundance between 300 and 500 μm.

Particle size distributions were similar for both sampling systems concerning their principal structure. As expected, particles were larger in samples of the 63-μm system. Mean ± SD fiber length was 1007 ± 700 μm for the 63-μm system and 727 ± 673 μm for the 20-μm system (fiber diameters: 19 ± 11 μm and 17 ± 9 μm). Likewise, the average IP was 229 ± 217 μm long and 106 ± 77 wide within samples of the 63-μm system, compared to 104 ± 101 μm and 47 ± 35 μm for the 20-μm system.

Discussion

Comparison with other studies

Numerical concentrations for microplastics in freshwaters differ vastly among studies, spanning several orders of magnitude on a global scale (Cera et al. 2020; Scherer et al. 2020). To compare data of this study with previous findings, a selection of studies was chosen based on either methodical and/or regional comparability (Table 3). Moreover, a correction following Koelmans et al. (2020) was applied to compensate for a share of the variability induced by different size ranges targeted. Applying this correction, microplastic concentrations measured by both the 63-μm system and the 20-μm system are in a comparable range. Due to this conformity, the general comparison to previous studies is carried out jointly for the two sampling systems. The comparison is further conducted with respect to the water body type, which means that microplastic concentrations of Lake Tollense are compared to studies carried out in lakes, only. Results from tributaries of the Tollense catchment were analogously compared to studies carried out in rivers.

Microplastic concentrations in the Tollense and its tributaries are in an equal range as in earlier studies. Most of the studies reporting similar results used a similar methodical setup concerning the cut-off size of the sampling device (Rodrigues et al. 2018; Eo et al. 2019; Mintenig et al. 2020; Park et al. 2020; Stanton et al. 2020). Mintenig et al. (2020) found concentrations between 67 and 11,532 particles m^{-3} investigating microplastics in the Dommel and Meuse (the

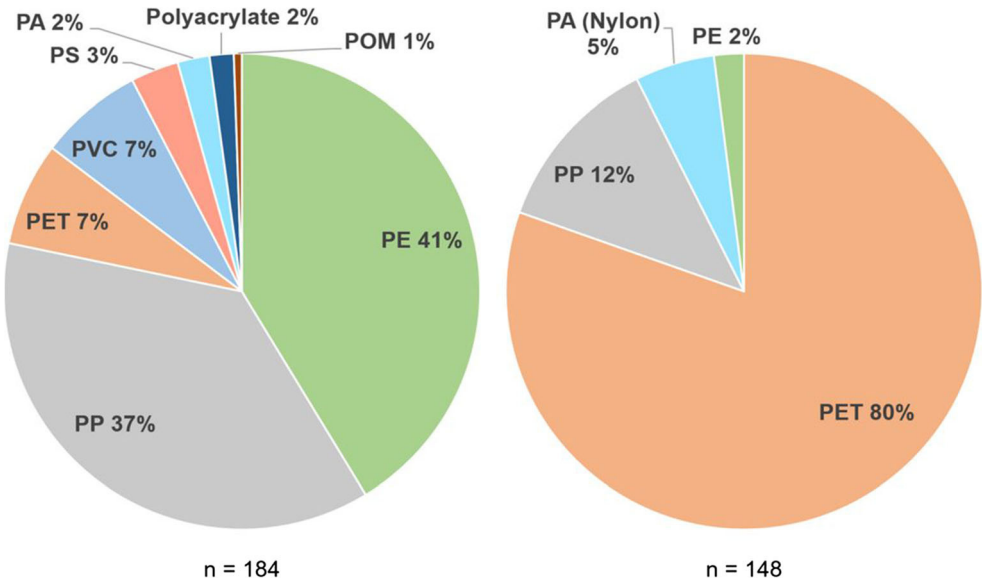

Fig. 4 Polymeric composition of particles from the Tollense catchment for IPs (left) and fibers (right)

Netherlands). These comparably high numbers are similar to our results. This can be explained by the fact that both studies used lower size limits down to 20-µm while maintaining a reasonably high sample volume. Likewise, Rodrigues et al. (2018) found microplastic concentrations of up to 1265 particles m^{-3} in the Portuguese Antuã River using a 55-µm mesh and sampling more than 1 m^3 of water. Finally, Eo et al. (2019) reported microplastic concentrations between 293 and 4760 particles m^{-3} in the Korean Nakdong River, filtering 100 l of river water through a 20-µm mesh.

Methodological differences seem to account for an important part of the variability between studies, as differences mainly occur when applied methods differ. Among those studies reporting lower concentrations than this study, some applied a visual preselection before particles were spectroscopically qualified (Mani et al. 2015; Mao et al. 2020; Scherer et al. 2020). This may lead to underestimating particles that are transparent, colored similarly to organic debris, or bleached due to aging by UV irradiation. Moreover, in some cases, comparably intensive digestion methods (in terms of applied chemicals or temperatures) were implemented (Baldwin et al. 2016; Scherer et al. 2020). The degradation of plastics in this process may cause an underestimation of the actual microplastics abundance (cf. Munno et al. 2018; Pfeiffer and Fischer 2020). Mao et al. (2020), for example, sampled various stations along the Yulin River, a tributary of the Yangtze River in China, applying similar methods as this study in terms of sampling (pump filtration), quantification (Nile red), and qualification (µRaman). Considering that the authors state to expect high abundances of microplastics due to the insufficient waste management in their study area, it is rather surprising that the corrected values of this study are in a similar range. Still, high temperatures (100 °C) were applied for digesting organic matter in the samples from the Yulin River so that a degradation of polymers is plausible. This is strengthened by the fact that only a few polymer types (PE, PP, and PS) were positively identified. Studies characterized by comparably low sample volumes report higher microplastic concentrations than this study (Pan et al. 2020; Sekudewicz et al. 2021). Extrapolating results based on few liters to one cubic meter may lead to overestimating the microplastic abundance due to a non-representative sample volume.

Microplastic concentrations in Lake Tollense are in a comparable range as previous reports for Bavarian Lakes (LfU 2019). However, reported concentrations for the Bavarian lakes included microplastics below the sampling-related cut-off size (300-µm) and may thus be not fully comparable to values from Lake Tollense. Uurasjärvi et al. (2019) sampled microplastics in Lake Kallavesi, Finland, using a similar in situ pump filtration approach (cut-off size 20-µm) as this study. Microplastic concentrations in Lake Kallavesi are below those for Lake Tollense. Still, the authors state that their actual cut-off size might be higher than 20-µm due to the applied optical microscopy and the manual selection of particles. Thereby, the actual microplastic concentrations in Lake Kallavesi might be higher than reported.

Numerical fluxes of microplastics in freshwater are rarely stated. Among the considered studies, only Stanton et al. (2020) and Eo et al. (2019) gave information on particle flows. Stanton et al. (2020) reported fluxes between 0 and 463 million particles per day for the rivers Leen, Soar, and Trent (UK). The overall range is thereby comparable to fluxes for the Tollense and its tributaries. While zero fluxes might be due to the low sample volume (30 l) enhancing the probability of not collecting any microplastics, maximum fluxes are roughly 3–4 times higher than in the Tollense (at TW). This coincides with the difference in the discharge between TW (mean: 4.5

Table 3 Comparison of microplastic abundance in freshwaters. Temperature (temp.) refers to the highest temperature applied. For comparison with other studies values at GB were excluded (see below). *Mean of sampled volumes, **original values were reported in MP/L, ***following Koelmans et al. 2020

Reference	Country	Type	Sampling			Sample processing		Conc. (MP/m^3)	This study (corrected)***	
			Sampling method	Sample volume (l)	Cut-off (μm)	Digestion/temp.	Identification	Mean/median min–max	63 μm-system (min–max)	20 μm-system (min–max)
Mao et al. 2020	CHN	River	Pump, filtration	50	64	H_2O_2 (30%)/100 °C	Nile red, Raman	n.r. 7–700**	121–347	272–430
Pan et al. 2020	CHN	River	Metal bucket, filtration	20	330	H_2O_2 (30%) with 0.05 M $FeSO_4$	Visual, Raman	246/n.r. 50–275	22–64	28–44
Stanton et al. 2020	GBR	River	Metal bucket, filtration	30	63	H_2O_2 (30%)	Visual, FTIR	n.r. 0–400**	-	278–440
Scherer et al. 2020	GER	River	Plankton net	3200–32,700	150	KOH (10 M) and H_2O_2 (30%)	>500 μm: visual, FTIR; >150 μm: visual, FTIR	5.57/5.11 0.9–13.24	51–146	83–132
Heß et al. 2018	GER	River	Manta-Trawl	n.r.	300	<500 μm: enzymes	>500 μm: visual, FTIR <500 μm: FTIR	37.8/19.2 2.9–214.2	25–71	32–50
Eo et al. 2019	KOR	River	Beaker, Pump	100	20	H_2O_2 (35%) with 0.05 M Fe(II)/~75 °C	Visual, FTIR	n.r. 293–4760	391–1121	1357–2146
Park et al. 2020	KOR	River	Pump, filtration	100	100	H_2O_2 (30%)/62.5 °C	FTIR	n.r. 0–234.5	77–221	147–232
Mintenig et al. 2020	NLD	River	Pump, filtration	>100 μm: 1300–8000; >20 μm: 30–2250	20	SDS (5%), KOH (10%), H_2O_2 (32%)/35 °C	>300 μm: visual, FTIR; <300 μm: FTIR	n.r./862 67–11,532	391–1121	1357–2146
Sekudewicz et al. 2021	POL	River	Metal bucket, filtration	20	55	H_2O_2 (30%)	Visual, Raman/FTIR	n.r. 1600–2550**	141–405	336–532
Rodrigues et al. 2018	PRT	River	Pump, filtration	~1200	55	H_2O_2 (30%) with 0.05 M Fe(II)/~75 °C	Visual, FTIR	n.r. 58–1265	141–405	336–532
This study	GER	River	Pump, filtration	>200 μm: 990–1836; >63 μm: 75–1836	63	$H2O_2$ (30%) and NaClO (6–14%)	Nile red, Raman	226/200 123–353	-	278–440
This study	GER	River	Pump, filtration	>50 μm: 1000; >20 μm: 700–1000	20	H_2O_2 (30%) and NaClO (6–14%)	Nile red, Raman	1812/1872 1357–2146	391–1121	-
Uurasjärvi et al. 2019	FIN	Lake	Pump, filtration	6–468*	20	n.a.	Visual, FTIR	168.8/n.r. n.r.	-	-
LfU 2019	GER	Lake	Manta trawl	n.r.	300	<500/1000 μm: enzymes	>500/1,000 μm: visual, FTIR <500/1000 μm: SWIR, FTIR	n.r./4 <1–42	-	5–15
Bordos et al. 2018	HUN	Lake River	Pump, filtration	1012–1990	100	H_2O_2 (30%)/80 °C	Visual, FTIR	13.8/n.r. 3.5–32.1	77-221	Lake: 25–68 river: 147–232
This study	GER	Lake	Pump, filtration	1000	20	H_2O_2 (30%) and NaClO (6–14%)	Nile red, Raman	489/496 234–625	-	-

m^3/s) and the river Soar (11.7 m^3/s), where the maximum fluxes were measured. Eo et al. (2019) reported annual fluxes of 1.7×10^{13} particles for the Nakdong River in South Korea based on surface sampling and covering four different seasons. This would roughly correspond to 47 billion particles per day, assuming equal fluxes throughout the year. As Eo et al. (2019) found a considerable seasonal variability, this comparison must be interpreted with caution. Still, fluxes within the Nakdong River are at least two orders of magnitude higher than the maximum fluxes observed within the Tollense catchment. This difference coincides with the differences in catchment size (Nakdong River: 21.588 km^2, Tollense at TW: 918 km^2). Additionally, the Nakdong River catchment is densely populated containing major cities like Daegu and Busan (Eo et al. 2019).

Comparison of tributaries and potential sources

Concentrations and fluxes within the Tollense catchment are spatially variable. The sampling station GB showed exceptionally high pollution levels, which cannot be explained by the sole size of its catchment area. These high pollution levels are plausible due to different reasons: (1) the highest share of urban area among all investigated catchments (see Table 1) characterizes the catchment of the GB. Moreover, the last stretch of the GB is located in Neubrandenburg, the economic and touristic center of the region. Several studies have emphasized a relation of urbanization toward pollution levels (Mani et al. 2015; Baldwin et al. 2016; Kataoka et al. 2019). (2) The construction of a road bridge crossing the GB started in early 2018, approximately 2 km upstream of the sampling location. This construction site may have acted as a continuous point source for microplastics in the GB by ongoing activity on-site until the end of 2019. Construction sites are considered an important source for microplastics due to the processing of plastic materials (e.g., insulation and pipes) or the abrasion of plastics from machinery and tools (Bertling et al. 2018). (3) The hydrodynamic conditions in the lower part of the GB may promote the accumulation of microplastics. Measured discharges and especially the mean flow velocities (0.06–0.18 m/s) were comparably low at GB (see SI 8). In contrast to all other sampling locations, lower flow velocities coincide with higher microplastic concentrations at GB (see Fig. 3). Winds are typically blowing from southwesterly directions in the study area, which was, in fact, the case at days before sampling (see SI 11). The last trench of the GB faces southwest so that water from Lake Tollense can be pushed into the GB. Hence, microplastics might be enriched here. This is strengthened by the fact that occasionally surficial upstream flows in opposite directions were observed in the field at GB.

At all sampling locations except GB, a positive correlation of microplastic concentrations with the current discharge was observed, which is plausible since dynamic hydrological conditions lead to the increased mobilization of microplastics within the riparian zone of receiving waters. Discharge within the Tollense catchment is generally controlled by precipitation. It was demonstrated before that rainfall can lead to increased microplastic concentrations within freshwaters (Hitchcock 2020; Xia et al. 2020).

The relation of microplastic concentrations to land cover patterns within the sub-catchments is difficult, while fluxes show a better concordance with land cover. Urban areas, as centers for human activities, concentrate sources for microplastic emissions (Mani et al. 2015; Baldwin et al. 2016; Kataoka et al. 2019). Accordingly, the area (in hectares) covered by the urban land cover class coincides with the mean absolute fluxes (million particles per day) within the sampled catchments. Moreover, mean fluxes normed to the catchment's size coincide with the share of urban land cover within the respective catchment (with the exception of WB).

Among the polymers found in samples of the Tollense catchment PE and PP dominate beyond their market demand concerning IPs (cf. PlasticsEurope 2020). Both polymers are widely used in various applications but are also strongly connected to consumer-related applications such as food wrapping/packaging and single-use items (Jones et al. 2020; PlasticsEurope 2020). These consumer-related applications are plausible sources of microplastics within the Tollense catchment due to the absence of large-scale industry. Moreover, land cover within the study area is dominated by agriculture, and PE and PP are widely used in related applications (e.g., plastic foils for silage bales). Among all samples, 13 particles were identified as PVC. Two of those particles originated from samples of the 63-μm system. Consequently, it cannot be ruled out that these particles originate from the used PVC-hose. The majority of analyzed fibers were composed of polyester, which is largely applied for clothing. PP fibers, having the second-highest share, were related to shipping and fishing before (Song et al. 2018). Both activities are carried out within the Tollense catchment (especially on Lake Tollense) but at low intensities.

Comparison of sampling devices and methodical considerations

Microplastic concentrations were severely different when comparing the two sampling systems with respect to the lower cut-off size. It has been emphasized before that lowering the mesh size of a sampling system may lead to significantly higher microplastic abundances (e.g., Dris et al. 2018; Green et al. 2018; Covernton et al. 2019; Prata et al. 2020, 2021a). However, we were able to achieve comparably high sampling volumes for both systems, despite lowering the mesh size in this study. Comparing the mean concentrations of microplastics derived by the 63-μm system to concentrations of the 20-μm system, values are 1.9–11.3 (mean 6.5) times

higher in samples of the 20-μm system. Using the alignment method suggested by Koelmans et al. (2020) with an α of 2.4 concentrations 5 times higher would be expected using a 20-μm instead of 63-μm mesh. An important share of the discrepancy is therefore related to the targeted size range. Still, taking into consideration that the correction factor would be smaller using the α-value of the 63-μm system (2.0), less difference would be explained. Thus, extrapolating microplastic abundance toward smaller size fractions may underestimate the actual abundance.

When comparing both systems, a noticeable difference became apparent concerning the input-output balance of Lake Tollense, which was considerably higher applying the 20-μm system. Seasonal patterns may influence such results, as for the comparison of the sampling systems in general, but based on present data, seasonal differences cannot be assessed. We rather hypothesize that the larger input-output difference is related to atmospheric inputs on Lake Tollense and those parts of its catchment that were not included by sampling its tributaries. Klein and Fischer (2019) showed that some hundreds of particles per m^2 and day might be deposited through the atmosphere (both dry and wet deposition) and that the majority of these particles were <63 μm in size. The general dimension of these deposition rates is comparable to other studies (Cai et al. 2017; Allen et al. 2019). Thereby, billions rather than millions of particles may be deposited considering the surface of Lake Tollense alone (17.9 km^2). While the exact numbers are somewhat speculative, this points to the fact that the importance of input pathways may considerably shift when very small particles are targeted.

For both systems, we observed that particles with a size (length) close to the mesh width of the finest sieve were less effectively collected compared to larger particles, which is in line with previous studies (Heß et al. 2018; Stanton et al. 2020). Particles close to the cut-off size of a system cannot be reliably quantified and thus, a difference between a theoretical and a factual cut-off size exists. While the first refers to the minimum mesh opening of a system, the latter is the size limit at which particles larger than this limit can be reliably quantified. The factual cut-off size can be approximated using the width of a particle, as it will better explain whether a particle may pass a sieve compared to using length (for particles may align in flow direction). In this study, we calculated a mean width-to-length ratio of 0.46 for the 63-μm system and 0.45 for the 20-μm system. These values are comparable to previous studies (0.67 in Simon et al. 2018 and 0.56 in Mintenig et al. 2020). Conversely, this implies a factual cut-off size that may be up to twice as large as the mesh width of the finest size (the theoretical cut-off size). This assumption may not be applicable to spherical particles, as these have higher width-to-length ratios. Moreover, especially long fibers can be collected using mesh widths far above their diameter and it is rather their length (often several 100-μm) that increases the probability that they are retained.

Conclusions

This study analyzed the abundance, characteristics, and fluxes of microplastics within the Tollense catchment using two different sampling systems. Microplastic concentrations ranged between 123 and 1728 particles m^{-3} using the 63-μm system and between 1357 and 2146 particles m^{-3} using the 20-μm system. Microplastic abundance significantly increased with rising discharge in the tributaries of the catchment. Moreover, higher microplastic concentrations were found in those tributaries that had a higher proportion of urban land cover.

Microplastic concentrations showed a large variability when comparing the two sampling systems used in this study. Sampling with the 20-μm system yielded in up to 10 times higher microplastic concentrations than sampling with 63-μm system. This variability can mostly be explained by the different cut-off sizes being used. Still, the applied correction, precisely its α-value, is influenced by the respective mesh size (higher α-value for smaller mesh size). Therefore, extrapolating the microplastic abundance toward smaller size fractions may underestimate the actual abundance. Considering the particle size distributions of both sampling systems, we suggest differentiating between a theoretical cut-off size (finest mesh applied) and a factual cut-off size (size limit for reliable quantification) in future studies. More research is needed to verify if the observed difference is site-specific or can be generalized. Consequently, whether environmental microplastic concentrations exceed effect thresholds or not can be related to sampling methods and the assessment of ecological risks may change when sampling is adapted to smaller microplastics.

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Declarations

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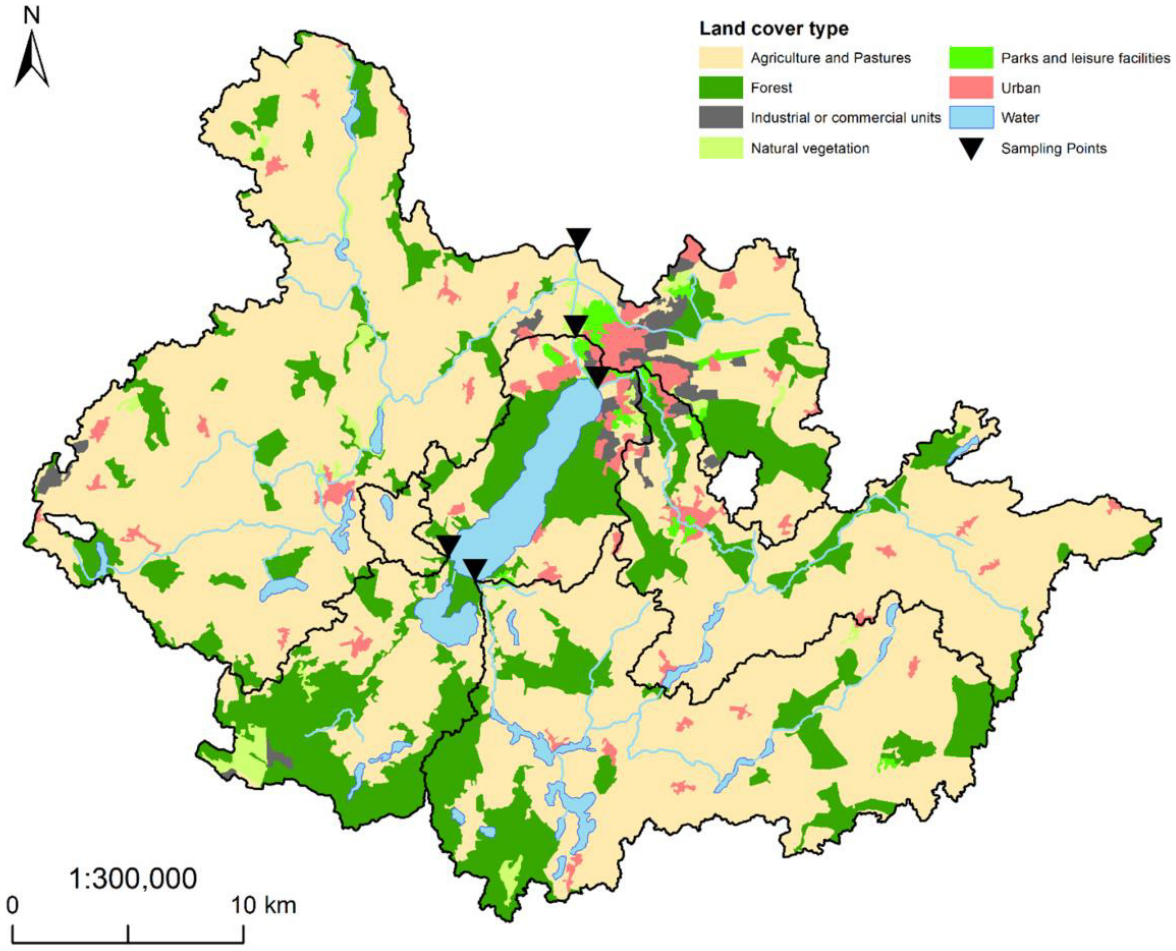
Supplementary to Tamminga et al. 2021: Microplastic concentrations, characteristics, and fluxes in water bodies of the Tollense catchment, Germany, with regard to different sampling systems

Catchment	Area (km²)	Area (ha)
Wustrower Bach	9.33	933
Nonnenbach	218.97	21897
Gaetenbach	148.28	14828
Tollense at Neubrandenburg	532.7	53270
Tollense at Woggersin	917.7	91770

SI 1 Size of investigated catchments



SI 2 Landcover within the study area based on the CORINE land cover data set (EEA 2021, LUNG 2021)

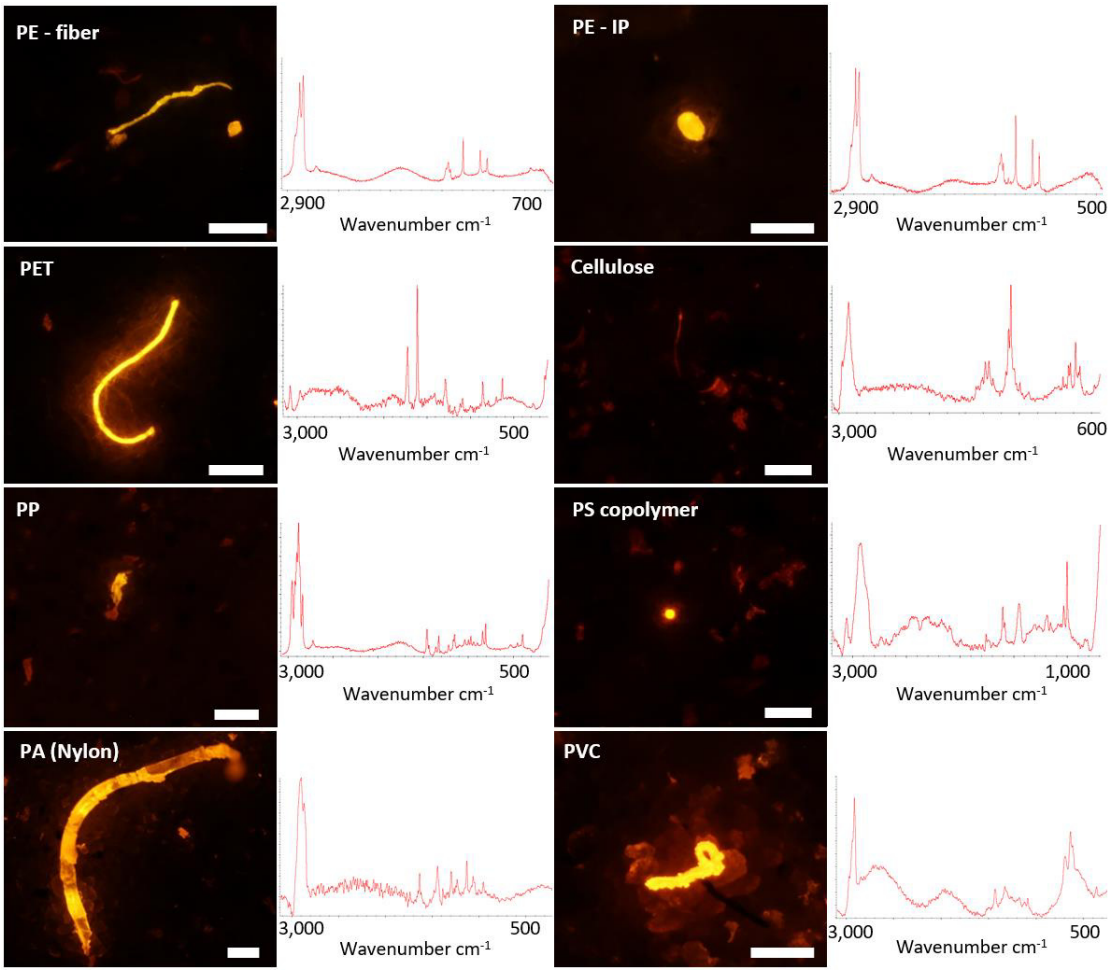
SI 3 Set-up of the 20 µm-system in the field

Sampling station	Mar. 2018	Mar. 2019	Sep. 2019*	Mar. 2020
Gaetenbach	990 (97)	1069 (75)	1000	1163 (183)
Nonnenbach	1066 (321)	1043	1000 (700)	1152
Wustrower Bach	417	-	-	-
Tollense NB	1027 (786)	1836	1000	1228
Tollense WO	-	1305	1000	1260
Lake Tollense	-	-	1000	-

SI 4 Sample volumes (in liter) by sampling stations and sample time, numbers in brackets give sample volumes for the finest sieves, if clogging hindered the filtrations of 1,000 l, *20 µm-system

SI 5 Digestion protocol applied in this study according to Hengstmann et al. 2018 and Tamminga et al. 2018, 2019

For digesting biogenic organic matter, the sample material was recovered from brown glass jars and transferred into glass beakers via rinsing with little ultrapure water. At first, hydrogen peroxide (H_2O_2, 30%, Merck, 60 ml per 50 ml sample volume) was added to the sample. The beaker was covered with a watch glass and incubated for seven days at room temperature. Subsequently, the hydrogen peroxide was removed by pouring the beaker content through an analytical sieve (63 µm or 20 µm) and rinsing with little ultrapure water. After transferring the sample into the beaker again by rinsing with little ultrapure water, the sample was treated with 16.7 ml sodium hypochlorite solution per 50 ml sample volume (NaClO, 6–14% active chlorine, Merck Emplura). The samples were then incubated for 24 h at room temperature to remove the residual organic matter that was not digested by hydrogen peroxide. In the following, the sample was relocated to a stainless-steel vacuum filtration system via rinsing with little ultrapure water (Satorius Stedim, 500 ml funnel capacity). After filtration, filters were placed into glass petri dishes and covered with a watch glass until further analysis.



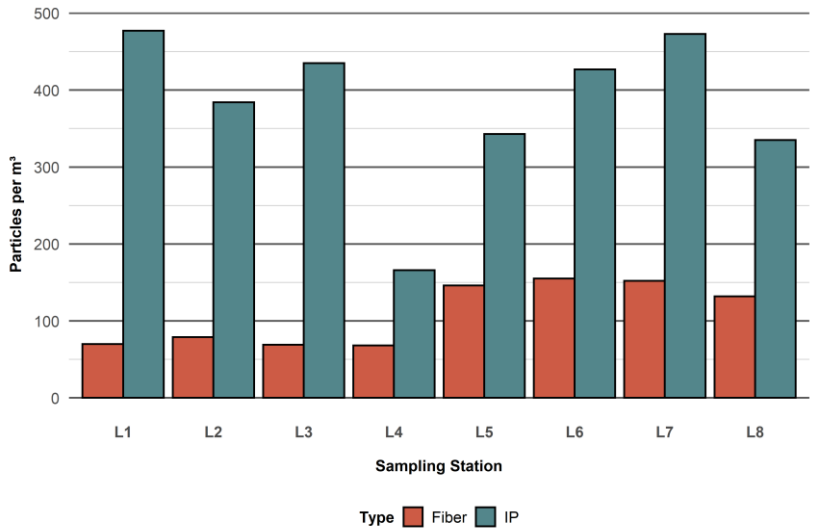
SI 6 Stained microplastic particles, a cellulose fiber, and associated Raman spectra, white scale bars indicate 200 µm

			Abundance (number) per size class (µm)			
Campaign	**Shape**	**Value**	**>63-200**	**>200-300**	**>300-1000**	**>1000-5000**
Mar. 2018 (n=3)	IP	Mean	0.0	0.0	0.0	0.0
		SD	0.0	0.0	0.0	0.0
	Fiber	Mean	0.7	0.3	0.3	0.0
		SD	0.6	0.6	0.6	0.0
Mar. 2019 (n=10)	IP	Mean	1.5	0.5	0.1	0.0
		SD	2.6	0.5	0.3	0.0
	Fiber	Mean	0.0	0.1	0.4	0.1
		SD	0.0	0.3	0.5	0.3
Mar. 2020 (n=5)	IP	Mean	1.2	0.4	0.0	0.0
		SD	2.2	0.5	0.0	0.0
	Fiber	Mean	0.0	0.0	1.6	0.8
		SD	0.0	0.0	1.5	0.4
			>20-50	**>50-300**	**>300-1000**	**>1000-5000**
Sep. 2019* (n=20)	IP	Mean	2.0	8.3	0.3	0.0
		SD	2.8	6.7	0.6	0.0
	Fiber	Mean	0.0	1.0	0.6	0.5
		SD	0.0	1.2	1.0	0.8

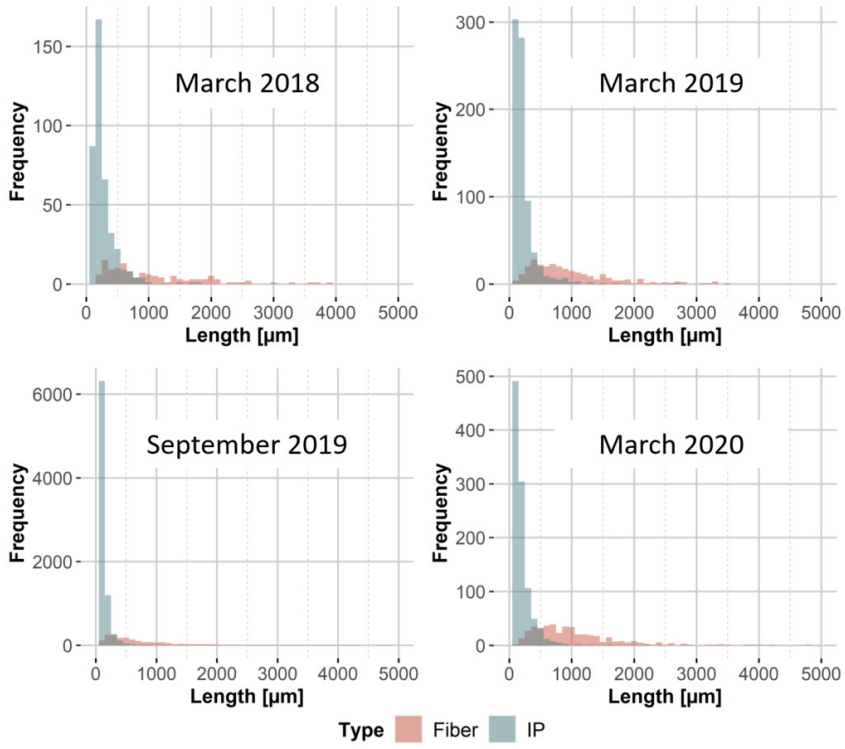
SI 7 Number of microplastics detected in procedural laboratory blanks by shape, size class and sampling campaign, *20 µm-system

	Discharge (m³/s)			
Sampling station	**Mar. 2018**	**Mar. 2019**	**Sep. 2019**	**Mar. 2020**
Gaetenbach	0.907	0.27	0.084	0.647
Nonnenbach	0.633	0.116	0.013	0.313
Wustrower Bach	0.069	-	-	-
Tollense NB	3.38	1.58	0.711	3.282
Tollense WO	-	2.583	0.936	6.509
Mean	**1.25**	**1.14**	**0.44**	**2.69**
SD	**1.46**	**1.17**	**0.46**	**2.87**

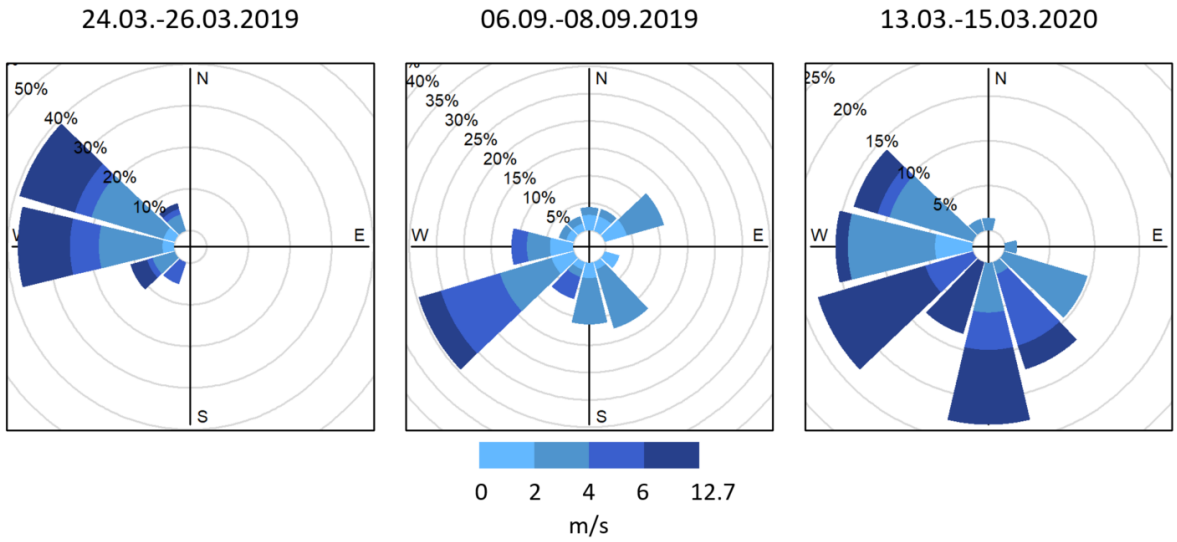
SI 8 Discharge measured in the field using the ADC device by sampling station and sampling campaign



SI 9 Microplastic concentrations within surface water of Lake Tollense in Sep. 2019 by sampling station and particle shape



SI 10 Particle size distributions by sampling campaign and particle shape



SI 5 Wind speed and direction at Trollenhagen airport averaged over sampling (at Gaetenbach) days and the two days before (DWD 2021)

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Versicherung an Eidesstatt (Declaration on oath)

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

I hereby declare upon oath that I have written the present dissertation independently and have not used further resources and aids than those stated.

Hamburg, den 02.11.2021