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Final report

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Microplastics as vector for microbial assemblages in Baltic Sea ecosystems (MikrOMIK)

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Executive summary

For about 10 years, the importance of microplastics (MP), plastic particles with a size of <5mm, has been studied for the marine environment more in detail, but the data situation is still dissatisfying. However, a hazard potential for marine ecosystems is not only based on MP, but also on the potential for the development of pathogenic biofilms on MP surfaces. The aim of MikrOMIK was to be able to assess the health risks for Baltic Sea countries due to MP as a vector of pathogenic microorganisms. MikrOMIK conducted an initial analysis of the distribution, as well as potential sources and sinks of MP in the Warnow estuary and determined the role of microplastics as carriers of specific microbial populations and their functions. Since the research experience in the field of MP was still quite young, it was hardly possible to refer to literature or experience in the international scientific community at the beginning of the project, e.g., how MP can be most effectively extracted, processed or identified from environmental samples. Therefore, the basis of MikrOMIK was a fundamental optimization of the processing of environmental samples, the spectroscopic identification, as well as the validation of MP distribution models by determining certain MP characteristics. For example, a new Si filter was developed that is IR-transparent in the entire mid-infrared range which enables clear differentiation of different polymers via FTIR and Raman microscopy. The comparability of FTIR and Raman microscopy for MP identification was determined. For the first time, validated data on sinking rates of polymer particles of different density, size and shape, including biofouling, at different salinities were determined for modelling. The study of MP transport processes in sediment (bioturbation) showed that they are similar to sediment particles and that the potential of marine invertebrates to bury MP can be extrapolated from already existing bioturbation performance data. Based on the validations for estuarine sediments, the Warnow MP ≥ 500 µm was spectroscopically quantified and identified and the MP distribution was related to hydrodynamic parameters and the effect of local sources. Significant correlations between the occurrence of specific sediment grain size classes and MP fractions were found, with the result that MP distribution patterns can largely be determined from simple sedimentological data. Conclusion is that the investigated sediments of the Warnow and the central Baltic Sea can be considered as a potentially permanent geographical sink for MP. It should be emphasized that a large proportion of the identified MP in the vicinity of ports or marinas were paint particles, which were also found in areas of dense shipping traffic in the more central Baltic Sea. The Matrix Scoring method also proved that tourism and recreation seekers are mainly responsible for the pollution of the German Baltic Sea beaches.

In the course of the project, an *in situ* experiment was conducted to investigate the microbial communities and their activities on MP along an environmental gradient cultivation dependent and independent. In addition, the influence of higher organisms on MP biofilms was determined by laboratory experiments. Compared to natural particles, it can be summarized that for the MP used by MikrOMIK no higher potential as a vector for pathogenic microorganisms could be derived. Consequently, the health risks for Baltic Sea countries due to MP as a vector of pathogenic microorganisms can be classified as being low. However, there are taxa that specifically colonize plastics and gene transfer, or genes that are typical for anthropogenic environmental influences, can also be significantly increased on MP. Moreover, MP has been found in high concentrations in some of the ecosystems studied by MikrOMIK. The potential consequences for aquatic ecosystems should be investigated in future studies.

Initial questions and objectives of the project

The enrichment of MP is proven for coastal (Dubaish & Liebezeit, 2013) and offshore marine systems (Lavender Law et al., 2010). However, sinks and dispersal pathways of these particles are still largely unknown, which is partly due to the difficulty of detection, as well as unclear sedimentation mechanisms and dispersal pathways in the food web. Although reliable assessments of the importance of MP for the marine food web are still lacking, its uptake by marine organisms is documented and ranges from filtering copepods (Pfaffenhöfer & Van Sant, 1985) to fish (Boerger et al., 2010; Lusher et al., 2012; Rummel et al., 2016). While some marine organisms excrete MP without visible damage (Andrady, 2011) in other organisms it can be absorbed by the body and trigger a violent immune response (Köhler, 2010).

Biodegradation of MP, especially via microbial decomposition, if occurring at all, is an extremely long process (Andrady, 2011). However, the interactions between marine microorganisms and plastic particles are not limited to their potential degradation. Compared to the pelagic, microorganisms can also accumulate selectively on plastic surfaces. Potential human pathogens such as *Vibrio* spp., which accounted for up to 25% of all Sargasso Sea bacteria associated with a plastic surface (Zettler, 2013), may affect the extent to which a critical infectious dose of pathogenic bacteria is reached in water, and thus have a direct social relevance. The reasons for selective growth are largely unknown. One hypothesis is that MP functions as a substrate analogue and is enriched by faecal contact with pathogenic bacteria. The anthropogenic influence takes place predominantly indirectly, but massively, via the joint discharge of faeces and MP into sewage systems before MP reaches the environment. MP in waters can directly pass through the digestive tract of filtering organisms such as zooplankton, worms or mussels where it can be inoculated selectively. It is known that copepods or mussels can, in comparison to the surrounding water, strongly accumulate e.g. *Vibrio* species (Sochard et al., 1979; Turner et al., 2009; Pruzzo et al., 2005). Thus, higher organisms could play an outstanding role as a potential link between MP and *Vibrio*. This can be particularly important for the southern Baltic Sea with relatively high *V. vulnificus* and *V. cholerae* human infections with partially fatal outcomes ((Brennholt et al., 2010). Besides vibrio's, this may also apply to other pathogenic microorganisms, including eukaryotic fungi, on MP.

Depending on its own density and the ambient density, MP occurs in the entire water column and is particularly enriched at the atmosphere/water or sediment/water interfaces. Microbial colonization in the form of biofilms strongly influences the buoyancy and sedimentation behaviour (Cole et al., 2011) and at the same time microbial populations on MP are spread differently in the marine environment than it would be the case under natural conditions. It would be possible that potential activity patterns and infection risks for pathogenic microorganisms may shift in this way. With regard to the Baltic Sea, there is currently little viable data on the distribution, abundance, type or colonisation of MPs. However, since around 85 million people live in the catchment area of the Baltic Sea, of which almost all of them discharge the waste water into the Baltic Sea, it seems obvious that MP also reaches the Baltic Sea in higher concentrations. I could accumulate there and, as carriers of certain microorganisms or even pathogenic germs, can greatly increase their spread and thus the risk of infection. It was the aim of the MikrOMIK project to be able to evaluate this on a solid basis. Milestones to be reached were planned as follows:

(I) Initial analysis of the distribution and potential sources and sinks of microplastics in the Baltic Sea.

(II) Determination of the role of microplastics as a carrier of specific microbial populations and their functions.

(III) The assessment of health risks for Baltic Sea countries by microplastics as a vector of pathogenic microorganisms.

These milestones were to be achieved by combining laboratory/field experiments and analyses of environmental samples. MikrOMIK's program consisted of five work packages (WP):

WP1. Basic investigation of the composition of MP in the Baltic Sea. In addition to sediment and water samples, sediment trap samples from the Gotland Deep, that have been stored in the IOW for a 15-year period, should reveal the dynamics of MP sedimentation. In order to establish work processes and analysis in WP1 as quickly as possible, the aim was to integrate the expertise already available at the Universities of Bayreuth and Oldenburg (ICBM) and the Alfred Wegener Institute (AWI) in the field of sampling of MP, extraction and purification in the laboratory and subsequent spectroscopic analysis and to adapt it to new ecosystems.

WP2. Method optimizations and properties of MP. For a validated qualitative and quantitative assessment of MP in marine environmental samples, established analytical methods (e.g. based on Fourier Transform Infrared Spectroscopy (FTIR)) should be optimized at the same time as WP1. New methods/techniques (e.g. based on Raman microscopy) should be developed, established and compared with each other. In parallel, laboratory experiments were to be conducted to determine the behaviour of MP in different water bodies in the Baltic Sea. In a first step it was planned to investigate the sinking behaviour of different polymers in water with different salinity and to clarify whether this behaviour can be predicted by theoretical calculations. In subsequent steps, the influence of individual factors that fundamentally influence the sinking behaviour of microplastics should be evaluated.

WP3. Biofilms on MP. The aim was to discover biofilm formation/properties on MP in combined field and laboratory experiments, as this determines the vector properties of MP. The plan was to experimentally investigate the anthropogenic influence on the microbial colonisation and activity of MP via a coastal Baltic Sea transect, representing different anthropogenic influences. To illustrate the *in situ* biofilm formation at the different sites, defined MP in size, density and composition should be exposed outdoors and the microbial structure and functions of the biofilms formed should be analysed at regular time intervals using 16S/18S rRNA and metagenomics. In particular, differences in microbial composition (Pro- and Eukaryotes) between different substrate types at different sites should be addressed. It was necessary to test whether there are MP specific indicator organisms, but also whether biodiversity differs between the substrate types and sites and what interaction possibilities exist for prokaryotes and eukaryotes on MP. Microbial activities should be shown by metaproteomic analysis of MP associated microbial communities. In particular, it should be investigated which metabolic processes play prominent roles and whether proteins of potentially pathogenic microorganisms or virulence associated proteins are detectable. This was coupled with the isolation of bacteria in order to investigate activity patterns of bacterial communities depending on the colonized substrate, as well as the succession of bacterial communities on plastic. The aim was to determine what demands towards the environment biofilm forming bacteria on plastics would have. These experiments should provide a better understanding of the composition and function of bacterial communities on MP in order to better assess the risks associated with MP.

It was completely unclear whether higher organisms have an influence on biofilm formation on MP. Therefore, the impact of key marine organisms of the Pelagic (copepods), the sediment surface (*Mytilus*) and the sediment (*Arenicola*) on the microbial colonization and activity of MP should be determined by laboratory experiments. These investigations were linked to the investigation of the bioturbation of MP in sediments and the resuspension behaviour. In particular, the potential of marine invertebrates for burying MP was in the foreground. The aim was to observe and systematically describe possible transport processes on and in marine sediments. The influence of physical properties of the MP particles (e.g. size, shape, density) on transport velocity and burial depth should be investigated. With regard to their activity on the seabed, invertebrates can be divided by different types of bioturbation, whose individual burial performance of particles can differ considerably in some cases. In laboratory experiments the influence of different representatives of selected bioturbation types on the distribution of MP should therefore continue to be investigated with the aim of identifying potential key species or groups for particularly deep, rapid or above all permanent burial of plastic particles.

WP4. Stability of microbial colonization and activity. To simulate the distribution and enrichment of certain microbial populations and their activities on MP *in situ*, knowledge of their taxonomic and functional stability is essential. This is particularly interesting for the MP that is most likely also populated by pathogenic microorganisms. In order to assess the resistance of biofilms, some MP particles taken from a sewage treatment plant and from the faeces of key organisms should therefore continue to be stored in the laboratory without further environmental influences and be analysed over a longer period of time.

WP5. Transport behaviour of MP. The aim of AP5 was to link the concentrations, distribution, sources and transport behaviour of MP in the Baltic Sea. Thesis was that in particular beaches would be a sink (washed ashore debris) of microplastics. This area therefore was supposed to be examined in greater detail using new and less specific methods. Thus, plan was to develop monitoring approaches from which, in addition to abundance, emission sources and accumulation areas could be estimated. Furthermore, a model had to be developed which depicts the physical processes in the Warnow estuary and thus enables conclusions to be drawn about the transport behaviour of microplastics.

Finally, it was MikrOMIK's declared goal to consolidate this network through further projects and cooperation’s in order to investigate ecologically relevant questions regarding MP, microbial functions and activities in the Baltic Sea.

Development of the tasks carried out, including changes from the original concept, scientific failures and problems in project organisation or technical implementation

At the time of compilation of the final report, 26 publications, 25 Bachelor/Master/PhD theses and 6 follow-up projects from MikrOMIK were produced; further manuscripts have been submitted to scientific journals. This is a remarkable achievement for a network project that dealt with a topic that was quite new for most partners. Consequently, this also meant that established methods, protocols or experiences could often not be used. For this reason, a great deal of development work had to be carried out in the first 12 months in particular. Individual targets had to be modified or adjusted. These are explained below for the individual WPs:

WP1. Basic investigation of the composition of MP in the Baltic Sea. At the beginning of the project, no literature or experience in the international scientific community could be drawn on how to extract MP most effectively from environmental samples. Unexpected problems occurred, which delayed sample preparation and led to fewer processed samples in the project than planned. In particular, the separation of MP and organic material, which is indispensable for spectroscopic analysis, caused difficulties. This was also the case for other national and international MP projects with which MikrOMIK has been exchanging information from the very beginning. However, the difficulties meant that it was not possible to examine such a large number of samples as would have been necessary for the entire Baltic Sea region. Thus, MikrOMIK focused mostly on the estuary of the Warnow and the beaches of its catchment area: In July 2014, water and sediment samples were taken at 10 stations in the Warnow estuary area along a transect (from the lock in Rostock up to 2 km after the estuary in the open Baltic Sea) and further samples from the entire Baltic Sea area. The latter were initially not examined for time reasons. Sediment and sediment trap samples from the Gotland and Arkona basins could be processed, each covering a sedimentation period of about 10 months. A plunging tube method used for sampling on the beaches proved unsuitable, as only a few plastic particles were found with a sampling volume of 6 litres. In addition, it turned out that the processing of the sediment samples, with which plastic particles (< 2 mm) were examined, was very time consuming. This was mainly due to the large amount of organic matter in the samples, which was extracted together with the MP during density separation with the Microplastic Sediment Separator (MPSS). The organic material made enzymatic digestion of the sample necessary. Otherwise a screening of MP < 2 mm was not possible.

The MPSS (Imhof et al., 2012) was generally used for extraction of MP from sediment samples. The samples were divided into two size fractions: Fraction 1 covered the sizes 5 mm - 500 µm, fraction 2 500 - 10 µm. Particles and fibres of fraction 1 were isolated manually by visual inspection; fraction 2 was filtered and subjected to an enzymatic purification procedure (Biniasch, 2014, 2016).

Despite the challenges briefly described above, several qualification papers on the properties of MP in the Warnow estuary were successfully completed and a manuscript written (Enders et al. 2019, in revision). The methodological findings gained in the course of the project were also the basis for the subsequent projects BONUS MICROPOLL, BMBF-FONA MicroCatch_Balt, BMBF-FONA PLASTRAT, UBA - joint project: marine litter and ERA.Net RUS - joint project BalticLitter. Important for WP1 was an intensive cooperation with the University of Bayreuth, the ICBM, and the AWI regarding the further development of methods for sampling, extraction, purification, and identification of microplastics, which continues until today and has been practically consolidated in the FONA projects MicroCatch_Balt and PLAWES.

WP2. Method optimizations and properties of MP. It quickly became clear that the identification of MP on those filters that are commonly used for transmission FTIR imaging did not allow qualitatively sufficient identification, as the analysis was disturbed by too high background noise. Therefore, an IR-transparent silicon (Si) filter for the analysis of filtered MP samples using transmission FTIR imaging was developed and produced in cooperation with the Fraunhofer Institute for Reliability and Microintegration (IZM). The advantages and applicability of this filter were tested using MP model samples and subsequently on real environmental samples. The two methods Raman and FTIR spectroscopy were critically compared and verified using real environmental samples from the MikrOMIK project. This was done for isolated single particles > 500 µm as well as for filtered samples < 400 µm (Käppler et al., 2015, 2016). Furthermore, FTIR and pyrolysis gas chromatography mass spectrometry (py-GCMS) were compared with each other in cooperation of IPF and ICBM for MP from Warnow sediments and the possibilities of comparability were published (Käppler et al., 2018).

The first purified MP samples from the Warnow were examined by ATR- FTIR- microscopy (ATR: attenuated total reflection) and partly by Raman spectroscopy. Contrary to the original intention, it was not possible to examine the entire sample filter for time and resource reasons. Therefore, randomly selected parts of the filter were analysed.

Two systems have been established for the determination of sinking rates of polymer particles of different densities and sizes. For larger MP in the range > 0.3 mm < 4 mm Atterberg cylinders were used and the sinking of the plastic particles within a defined range (2x10 cm) was measured with a timekeeper. For small MP (<0.3 mm), a shadowgraph setup established by Glockzin et al. (2014) was used and adapted to the above question. The sinking of microscopically small plastic particles in a photometric cell, which was located in another vessel with water in order to achieve a more stable ambient temperature, was recorded with a high-resolution CCD camera. The sinking rate was determined by means of image analysis (Glockzin et al., 2014).

To test how UV light affects the properties of MP, a setup with a metal vapour lamp with UV component, which is close to that of natural sunlight, was installed. The lamp proved to be very suitable for simulating natural conditions as far as possible. However, in order to achieve visible effects that go beyond yellowing of the particles during the project run time, other methods are necessary, such as treatment in a weathering chamber (UV test chamber). To investigate the effect of mechanical stress on MP in the surf zone along the coasts, plastic particles were filled into sealed jars together with natural seawater and beach sand and kept in motion on a laboratory shaker for 6 months. Already after one month of shaking, clear signs could be detected by REM analysis. In the summer of 2016, an incubation experiment began in which MP was exposed for 14 weeks in the Warnow and in Heiligendamm. In Heiligendamm, the incubation had to be restarted after two weeks, as attached ropes were torn during a storm. After regular sampling, MP particles were recorded under the light and scanning electron microscope and their sinking velocity measured (Kowalski et al., 2016; Kaiser et al., 2017).

WP3. Biofilms on MP. The Warnow field experiment, in which MP pellets were exposed at different stations with different anthropogenic influences compared to a natural material (wood), was carried out without any problems. The methods proposed in the application, here Illumina MiSeq sequencing, were applied as suggested. Initially there were difficulties with DNA extraction from the MP samples, as the DNA yield was much lower than expected. However, by optimizing the extraction protocol, this problem was solved. The composition of the biofilms formed was finally characterized using 16S (Oberbeckmann et al., 2018) and 18S rRNA amplicon analysis (Kettner et al., 2017, 2019). Further functional analyses using metagenomics have not yet been completed, as the simultaneous processing of prokaryotic and eukaryotic data represents a major bioinformatic challenge. Two additional experiments were carried out, not in the Baltic Sea ecosystem, but as field and laboratory experiments with water samples from freshwaters in Germany and Italy (Arias-Andres et al., 2018b; Eckert et al., 2018). Here, the effect of bacterial communities from wastewater treatment plant effluent on freshwater bacteria communities under increasing MP concentrations was investigated and the hypothesis tested whether MP favours horizontal gene transfer between bacteria.

In order to analyse the metaproteome of the MP associated microbial communities, an extraction method was first developed that enabled the efficient enrichment of the overall proteome of the biofilms. The protein extracts were then digested, purified and measured by mass spectrometry in very high resolution. Although more than 400000 spectra were recorded per sample, the identification rates were below our expectations. The reason for this was presumably that highly abundant eukaryotic proteins overlaid less abundant bacterial proteins and thus showed lower protein diversity than was actually present in the samples. In addition, the metagenome database contained many short ORFs that represent only a fraction of the protein to which they belong. Therefore, many of the proteins present in the samples could not be identified. The strong fragmentation of peptide sequences in the database made valid taxonomic and functional assignment difficult. Among the identified proteins there are many hypothetical proteins and proteins without annotation in the databases (COG/KOG; TIGRFAM). Nevertheless, metaproteome analyses allowed a comprehensive overview of the taxonomic and functional composition of biofilms that would not have been possible with other methods (Oberbeckmann, Schweder, et al., in prep.).

To investigate the physiology and demands of bacterial communities on plastics and wood, a substrate test was prepared using an immunocytochemical method based on the incorporation of thymidine analogue 5-bromo-2-deoxyuridine (BrdU) into the genomic DNA of growing bacterial cells. The *in situ* biofilms of the Warnow field experiment were used for this purpose. In the substrate test, 31 substances were tested, including organic acids, mono- and disaccharides and carbon-nitrogen compounds. The samples from the BrdU experiment were then used for BrdU immunocapture to identify key organisms of specific substrate uses. The succession of bacterial communities and the associated identification of the key biofilm forming bacterial species were investigated using a surface- transfer- experiment. Plastic surfaces were prepared in selected media and inoculated with water from the *in situ* biofilm sites and transferred several times after distinct incubation periods. The bacterial communities on all surfaces were sequenced. Several strains of *in situ* and *in vitro* biofilms of plastic could be isolated and identified, but a detailed description of the isolates is still pending.

With regard to bioturbation, a method for extracting MP from sediments was developed at the beginning of the project. The density separation of sediment and plastic in rolling tanks proved to be simple and very efficient. Major problems were encountered in the further processing of the extracted particles, which often remained in the vessels due to their high adhesiveness and significantly affected the efficiency of the method used in some cases. Further measurements such as the use of a denser separating agent (sodium polytungstate) or the use of specially developed vessels and attachments also remained unsuccessful. The grinding of the plastic used in many subprojects (polystyrene, PS 143 E, BASF) at the IPF Dresden proved to be technically complex and could not produce defined size fractions, but only a mixture of many different size classes. Subsequent sieving of the particles was tested, but due to strong attraction forces between the particles or with other surfaces, no satisfying result was achieved. In cooperation with Micromod Partikeltechnologie GmbH, a fluorescent coating of PS with various dyes (rhodamine, fluorescein) was tested, but the dyes used did not prove to be durable enough for use in long term bioturbation experiments. Due to the aforementioned problems in handling MP, PS 143 E (particle diameter 1000 µm) and other polymers in different size classes (Trofil PA 6, 500 µm, Monofil-Technik GmbH; fluorescent polyethylene, 130 µm, Cospheric) were used for the experiments. The use of macroscopically visible or fluorescent MP allowed the application of already established techniques for sampling and particle quantification, as they already exist for bioturbation experiments with luminophores (fluorescent sediment particles). Protocols for sample processing and computer-aided particle counting based on photographs could be created and successfully used, among other things, for qualification work. Due to the increasing number of literature treating the interaction of filtering organisms, especially bivalvia, with MP, two representatives of polychaeta were used for the laboratory experiments (*Arenicola marina*; *Hediste diversicolor*), as very little data on the interaction with MP is available for this group of organisms, but high transport rates are to be expected due to the lifestyle of the animals. The laboratory tests themselves were carried out without major problems (Gebhardt et al., 2018).

WP4. Resistance to microbial colonization and activity. Studies on the influence of *A. marina* (Kesy et al., 2016) and *M. edulis* (Kesy et al., 2017) on biofilm formation on MP particles were carried out and published without any problems; an analysis of copepods was no longer possible for time reasons.

WP5. Transport behaviour of MP. In the project it became clear early on that the methodically complex and time-consuming preparation and analysis of MP from environmental samples would not allow a comprehensive supply of data suitable for simulation. In order to allow a comprehensive assessment of the load of the Warnow estuary and its banks with small plastic fractions, simple supplementary monitoring methods for plastic fractions >2 mm in size for beaches were developed, tested and used. With the frame method (area of 9 m²) more particles were found, but this method did not cover the whole beach, so that the sand rake method was further developed. The sand rake method proved to be the most suitable because the largest area (from the water line to the dune foot) could be sampled in a very short time. In addition, the highest number of waste particles was found here, which is important for determining the source of contamination. The 10 m² flood accumulation zone- method was developed and used to determine the pollution of the beaches from the sea side (Haseler et al., 2018).

MP modelling was performed with the General Estuarine Transport Model (GETM, www.getm.eu). The model calculates, based on the incompressible Navier-Stokes equations, time series of the 3D flow fields and physical state variables such as salinity and temperature. The former was used to calculate the position of Lagranges tracers representing the MP particles. Contrary to the plan, a larger area around the Warnow estuary was integrated into the model area in order to better map the transport through the river plume. In the course of this, the spatial resolution was also increased to 20 m in order to be able to model small-scale deposits of MP. The vertical discretization with 25 following layers proved to be sufficient.

Presentation of the results achieved and discussion with regard to the relevant state of research, possible application perspectives and potential follow-up projects

MikrOMIK has generated and already published trend-setting results for all three planned milestones. In the following, the results are assigned to the three milestones aimed for:

(I) Initial analysis of the distribution, as well as potential sources and sinks of microplastics in the Baltic Sea.

The data required for the study of potential sources, distribution and sinks could be collected after considerable optimization of the processing of samples with sodium polytungstate (Biniasch, 2014, 2016; Stollberg, 2016) and spectroscopic identification. MikrOMIK has developed & validated new products that have already been published and have received international attention. This concerns, for example, the reliable identification of MP via transmission FTIR imaging, a spectroscopic method by means of which filtered MP samples can be automatically analysed directly on the filter without visual presorting. This requires an IR-transparent filter; however, the aluminium oxide fabric filter (anodisc) usually used before MikrOMIK shows a very strong self-absorption in the wave number range of 1250 - 600 cm^{-1}, which prevented the detection of characteristic polymer bands in this spectral range. In addition, the Anodisc is slightly brittle and not completely planar, which can lead to focus problems in FTIR and Raman microscopy. Therefore, a new filter material for IR-microscopic methods in transmission (e.g. imaging) was developed and established. For this purpose, continuous pores were introduced into a Si wafer in cooperation with Fraunhofer IZM by lithographic etching. The resulting Si filters have a pore diameter of 10 µm, are mechanically stable and allow the filtration of aqueous MP samples. It is IR-transparent in the entire mid infrared range (4000 - 400 cm^{-1}) and thus makes it possible to clearly distinguish between different polymers on the basis of characteristic bands in the fingerprint range. This filter is also suitable as a measuring substrate for Raman microscopy. The spectroscopic suitability (FTIR in transmission and Raman) of the Si filters was successfully tested using MP model particles for both single and imaging measurements and used for the filtration and analysis of real MP samples from the MikrOMIK project (Käppler et al., 2015).

Chemical-analytical methods such as FTIR and Raman spectroscopy or py-GCMS are indispensable for a reliable identification of MP. These have already been used in several studies (Oberbeckmann et al., 2015). However, no comparison of the different examination methods has taken place so far. Therefore, the identification results of isolated MP particles and fibres (> 500 µm) obtained by ATR-FTIR and Raman microscopy were critically compared and verified. It became clear that both spectroscopic methods are suitable for the identification of MP in marine samples. However, especially with coloured particles, it is necessary to use both methods for complete and reliable identification. In a second step, MP samples < 400 µm were examined using FTIR imaging in transmission and Raman imaging and compared with respect to the number, size and polymer type of the identified MP particles. In addition, the spectra quality, measurement time and the handling were included in the comparison. Using Raman imaging, significantly more (approx. 35 %) MP particles, especially very small particles (< 20 µm), could be detected. However, compared to FTIR imaging, the measurement time was significantly longer for the same measurement area. The cooperation of IPF and ICBM allowed a comparison between ATR-FTIR and py-GCMS. In a blind study, the respective identification results of isolated particles > 500 µm and fibres of both processes were compared with each other. It turned out that in most cases both methods led to the same result with regard to the classification of "plastic" vs. "non-plastic". Thus, within the framework of the MikrOMIK project, different chemical-analytical methods for MP identification were compared and validated for the first time. The detailed results of the individual investigations can be found in the corresponding publications (Käppler et al., 2016, Käppler et al., 2018).

The new developments described above were the basis for validated data collection on MP from beach, sediment and water in the Baltic Sea and Warnow. For further validated simulation or modelling of the input, dispersion and sedimentation of MP, the passive transport of microplastics must be supplemented with the dynamic buoyancy due to the density of MP and the high variability of the density of Warnow water. The reason for this is that sinking rates of MP particles vary greatly in brackish water systems such as in estuaries. But here as well, MikrOMIK has already been able to generate important new insights. For the first time, sinking rates of polymer particles of different densities (PS, polyamide (PA), poly-methyl methacrylate (PMMA), polyethylene terephthalate (PET), polyoxymethylene (POM) and polyvinyl chloride (PVC), 1050-1560 kg m^{-3}) and varying in size (size ranges: <0.3 mm and 0.3-3.6 mm) and shape in water with different salinity (Sal 0, 15, 36 with densities 998, 1010 and 1026 kg m^{-3}) was measured (Kowalski et al., 2016). As expected, an increase in sink rates could be observed with increasing particle density and size. In addition, however, the particle shape had a large influence, as strong deviations from the spherical shape and an additional flattening of the particles led to a significant reduction in sinking speeds. As a result, significant differences between the experimentally measured and calculated data based on theoretical formulas for the sink rate of spherical bodies could occur. In the meantime, it has been experimentally proven that the dependence of the sinking velocity on the MP particle size can be relatively well described by a quadratic linear regression (Kaiser et al., 2019).These results refute the assumption that theoretical calculations based on the sinking behaviour of perfect spheres are sufficient to estimate the sinking speed of microplastics.

However, the results are only valid for new, not already weathered particles. If the particles remain in the environment for longer, they are exposed to various weathering processes, such as biological weathering (biofouling), which could change their physical properties. The results of a corresponding incubation experiment showed that the biological colonisation of MP has a serious influence on the sinking behaviour of the particles (Kaiser et al., 2017). MP with a density higher than that of the ambient water was sinking faster due to biofilm formation. MP, which is not expected to sink due to low density, can be caused to sink, especially by the colonisation of macroinvertebrates. This increase of MP sedimentation due to biofouling has so far been used several times as a possible explanation for an obvious discrepancy between plastic input and plastic deposits in sea surface water (Cozar et al., 2014, Eriksen et al., 2014). It was also used to explain the presence of low-density MP on the seabed. However, these conclusions have so far been based on theoretical approaches supported at best by observations of significantly larger plastic objects. However, the size of the object plays a decisive role, as it has a major influence on biofilm formation. Due to these facts, the effect of natural biofilms on the MP sinking rate (Kaiser et al., 2017) needs implementation in model calculations.

The last important factor to estimate the distribution of MP in aquatic environments is the knowledge of their transport processes in sediments, often catalysed by higher marine organisms. In this context, laboratory experiments for both *A. marina* and *H. diversicolor* have shown the burial of MP of different size and composition as a result of the bioturbation activity of the animals. It should be pointed out that for both species, belonging to different types of bioturbation, different transport processes and efficiencies were observed:

For the conveyor belt-feeder *A. marina*, burial and simultaneous upward transport of MP particles to the sediment surface is already known from scientific literature (Valdemarsen et al., 2011; Green et al., 2016; Van Cauwenberghe, 2015). The hypothesis that the size-dependent particle feeding of *A. marina* causes larger MP to enter the sediment, but that it is not taken up by the organisms there and thus remains permanently in the sediment, has now been confirmed experimentally. Thus, the bioturbation of *A. marina* represents a way to bring MP ≥ 500 µm in a short time comparatively deep into the sediment and to leave it there for long periods of time. Due to the characteristic feeding mechanism of the polychaetes, a typical accumulation horizon in 10 - 14 cm sediment depth was found for this MP. With regard to the burial speed, no difference could be detected between large MP (ø 1000 - 500 µm) and smaller luminophores (ø 130 µm), despite different sizes and densities of both particle types. The possible difference in the transport of sediment particles and MP was also a main focus in the experiments carried out with *H. diversicolor*. The particle transport of luminophores and plastic particles of equal or larger size was processed in a mechanistic bioturbation model and quantified using appropriate transport coefficients. With regard to the burial behaviour, no difference between the particle types could be determined here either. With the gallery-diffuser *H. diversicolor*, the main particle transport does not take place by feeding but by accidental entrainment of the particles into the burrow. Due to the characteristic behaviour of the polychaetes during the search for food, a large part of the MP particles on the sediment surface were transported to different depths in the sediment in a short time. Typical accumulation zones were horizontal or vertical sections of the structure. Particles become attached to or fall in the burrow. It has also been shown that the bioturbation of sediment in general, such as that of MP in particular, can be stimulated by increasing the food supply.

These results underline the role of marine sediments as a potential sink for MP. Since 80% (Duis & Coors, 2016) of the MP particles introduced into the oceans can reach the seafloor, it can be assumed that bioturbation processes play an important role in the further distribution of these particles on the seabed. On the one hand, such buried particles may be more difficult to access for marine organisms, on the other hand, the accumulation in the sediment under exclusion of light and oxygen further delays the decomposition of polymers that are difficult to degrade. This can lead to the long-term preservation of MP in the marine environment. The proof of the bioturbation of MP completes the knowledge about the transport of these particles in the oceans and can help to explain the discrepancy between the amount of MP introduced into the oceans and the amount found with previously available methods. In addition, the findings demonstrate the need to take deeper sediment areas into account when evaluating the MP load of marine sediments. Existing figures on MP concentrations of sediments could therefore underestimate the actual MP load.

A difference in transport behaviour between MP and sediment particles could not be determined in any experiment, regardless of the shape or density of the MP. The assumption that the lower MP density compared to natural sediment particles causes different transport behaviour could not be confirmed by the modelled transport coefficients. With regard to possible interactions with marine fauna, the particle size alone seems to determine the further whereabouts of the particles and thus decide, for example, whether they are buried or eaten. The transport of MP in the sediment is similar to that of sediment particles or luminophores. The individual bioturbation performance is already known for many marine species in the form of specific transport coefficients. The potential of a species or community of marine invertebrates to bury MP can therefore be derived from existing data.

Based on the validations described above, MP ≥ 500 µm was spectroscopically quantified and identified for estuary sediments of the Warnow. The MP distribution was related to hydrodynamic parameters and the effect of local sources (Enders et al., 2019, in revision). Significant correlations between the occurrence of specific sediment grain size classes and MP fractions were found, with the result that MP distribution patterns can largely be determined from simple sedimentological data. The mean grain size (d50) was obviously the most accurate proxy for high density (HD) MP ($r = -0.9$, $p < 0.001$). Particulate polymers with low density (LD) in the investigated size range showed a more complex transport deposition behaviour and require further investigations. The MP-sediment relationship in transport behaviour showed an average size difference of one order of magnitude, which compensates for the differences in density with the size. Comparison of complementary data from other studies (references included in Enders et al., 2019) revealed an influence of the spatial-time connectivity of the investigated system on the degree of adaptation. Spatial occurrences of type-specific high-density MP were correlated with their emission location and served as a local source indicator. Using a generalized linear model (GLM), MP point sources, such as sewage treatment plants and ports, could be identified. The importance of an integrated analysis of the sediment grain size for the elimination of hydrodynamic variability could be extrapolated to the effect that anthropogenic influences can be determined over space and time. The long distance transport of MP to the Baltic Sea basins Arkona Basin and Gotland Deep is considered possible based on the data for fibrous MP, but the origin of other MP particles seems to be a sea-side entry (ships etc.). MP sedimentation rates (37 m^{-2} y^{-1}; 0 m^{-2} y^{-1}) were determined for the central Baltic Sea and showed coherence with the underlying sediment compartments. The conclusion is that the investigated sediments of the Warnow and the central Baltic Sea can be considered as a potentially permanent geographical sink for MP.

It should be noted that a large proportion of the identified particles were paint particles, similar to Imhof et al. (2016), which were mainly isolated from samples near the fishing port and along the Alter Strom in Warnemünde. In addition, considerable amounts of PS spheres were detected at various stations, most of which originated from ion exchangers. Other polymer types identified were polypropylene (PP), polyethylene (PE), polyvinyl acetate, ethylene vinyl acetate (EVA), PA, PMMA, polyacrylonitrile, and PVC.

Beach sampling in the Warnemünde area revealed particle counts of the MP fraction >2mm from Ø 2.6 litter pieces/m² (sand rake method) up to 65 litter pieces/m² (flood accumulation zone- method). These data were sufficient to estimate the degree of pollution of various beaches. The advantage of the flood accumulation zone- method was that it was particularly important to learn about the pollution of beaches after storm events deriving from the sea. Furthermore, a contamination event could be recorded by the flood accumulation zone-method: On 17.03.2015 thousands of microplastic pellets were washed up on the beach of the IGA-Park; on 1 m² of the flood accumulation zone approx. 2,500 pellets were found. Apart from that, the Matrix Scoring method has shown that tourism and recreation seekers are mainly responsible for the pollution of the beaches. The sand rake method, with which 50 - 60 m² of beach can be sampled within 2 hours, showed that micro and meso litter on Baltic Sea beaches represents a problem which is not addressed with the standard macro litter monitoring (OSPAR method 100 m) for macro litter. Beach users are mainly responsible for the pollution of German Baltic Sea beaches and intensively used beaches are pollution hot spots. With the sand rake method and the flood accumulation zone- method two inexpensive and reliable methods were developed, which are suitable to reflect the pollution of the beaches with recovery rates of about 60%. They can be used on all sandy beaches, regardless of whether a regular beach cleaning is carried out or not. The large micro- and meso-litter fractions are almost not detected during beach cleaning, which is why a continuous mechanical decomposition and a constant accumulation in the environment can be assumed. Thus, it is also possible to examine beaches that are heavily used by tourists although regularly cleaned.

Due to the high spatial resolution of the samples described above, physical processes, the coastline and bathymetry in the Warnow estuary could be better mapped in the GETM model. The physical processes include primarily the late-rale circulation, which causes a stronger exchange flow. This reduces the influence of wind on the transport. The more accurate bathymetry is particularly relevant to assess the influence of the planned deepening of the Warnow fairway in another model study. The model is suitable for further particle tracing studies, such as the transport of biological tracers (e.g. *E. coli* bacteria), tracers to estimate the age of water (age-tracer) and tracers to determine the origin of water (marking of water packages).

(II) Determination of the role of microplastics as a carrier of specific microbial populations and their functions.

Microbial communities on MP have received little scientific attention so far, and little is known about the vector potential of MP for pathogenic microorganisms. Therefore, an *in situ* experiment was carried out to investigate the microbial colonization of MP along an environmental gradient. PE, PS, and wood pellets were incubated for 14 days at 7 stations with different anthropogenic influences in the river Warnow (Rostock), on the Baltic coast, and in a sewage treatment plant. The biofilm communities were identified by 16S rRNA and 18S rRNA high-throughput sequencing and compared with the corresponding water communities (free-living and particle-attached). Special attention was paid to potentially pathogenic organisms. It could be shown that only in waters with low nutrient concentration and increased salinity plastic-specific bacterial biofilm communities developed (Oberbeckmann et al., 2018), which underlines the importance of nutrient-poor MP accumulation zones, such as the Sargasso Sea. In comparison to natural substrates, potentially pathogenic taxa were not enriched on MP; no indications of particular pathogenic factors have been found at the metagenomic level either. However, certain bacteria on MP could be detected in the wastewater treatment plant, which are often associated with antibiotic resistance. This indicates MP as a potential hotspot for horizontal gene transfer. An extension of the experimental approach to the salinity gradient of the southern coastal regions of the Baltic Sea confirmed these results in principle, but also revealed that *Vibrio* is generally to be regarded as a primary coloniser of particle surfaces (Kesy et al., 2019, in revision). A first study on bacterial communities on colour particles from the sediment of the Warnow identified a significant accumulation of *Desulfatitalea tepidiphilia*, but the reasons for this need further investigation (Tagg et al., 2019).

In a laboratory experiment, bacterial communities from a meso-oligotrophic lake were mixed with an *E. coli* culture containing a plasmid for resistance to the antibiotic trimethoprim (Arias-Andres et al., 2018b). It was observed how this plasmid spread through horizontal gene transfer within the bacterial community if (case a) these bacteria came from a MP surface (the MP was previously incubated in the lake) and if (case b) these bacteria came from the surrounding lake water. This showed that the gene transfer was significant and increased by a multiple when the bacteria grew on MP. Bacteria of different phylogenetic groups were able to take up the plasmid. In the surrounding seawater, however, the plasmid spread more rarely or more slowly. The results suggest that the presence of microplastics in aquatic systems favours gene transfer between the attached bacteria and microplastics could thus increase the risk of the spread of antibiotic resistance. Since potential pathogens for MP could already be detected from the environment (Kirstein et al., 2016) and additional antibiotic resistance would pose a particular risk to humans, further studies are urgently needed to first determine the risk potential and then find suitable countermeasures. Despite good purification performance, sewage treatment plants, for example, discharge both MP and microorganisms, including antibiotic-resistant bacteria, into lakes, rivers or seas (Mintenig et al., 2017; Di Cesare et al., 2016). In a chemostat experiment (for continuous cultivation) microorganism communities - mixed in equal parts from sewage plant effluent and seawater - were cultivated over a period of 15 days (Eckert et al., 2018). The MP number rose from 0 in the first chemmostat to 1600 in the ninth. At the end of the experiment, the occurrence of integrase I was quantified. Integrase I is often used as a marker for anthropogenic influences, as it occurs for example in combination with antibiotic and heavy metal resistance. The occurrence of integrase I among MP-associated microorganisms also increased with increasing MP concentration. Especially at higher MP concentrations the MP-associated communities were at the end more similar to the original communities from the wastewater treatment plant effluent than those from the lake. This was the case although the culture medium consisted of sterile seawater. This means that high MP concentrations promote the attachment and survival of bacteria from wastewater treatment plant effluents and that these bacteria increasingly carry genes that are typical of anthropogenic environmental influences.

Over 500 different eukaryotes, including over 100 different fungi, were detected on the MP samples of the Warnow *in situ* experiment. The communities were composed of organisms of different trophic levels, from primary producers to consumers to decomposers (Kettner et al., 2017; 2019). Dominant groups of organisms were chloroplastida, holozoa including metazoa, fungi and eukaryotes of the "SAR" group (stramenopiles, alveolata and rhizaria). The hypothesis was confirmed that the MP-associated eukaryotes communities differ significantly from those on wood and those in the surrounding water. A difference between the respective communities on PE and PS could not be statistically proven. The composition of the eukaryotes communities also varied from station to station. This supports results of previous studies (Hoellein et al., 2014; Oberbeckmann, 2016), but we were able to prove this substrate and location dependence for the first time in the ecosystem and catchment area of the Baltic Sea. Since previous studies were preferably dedicated to bacteria, our experiments are among the few that provide detailed information about the eukaryotes and fungal communities on MP. Since fungi can also degrade hardly degradable substances thanks to their enzyme systems, they are potential plastic degraders (Krueger et al., 2015). Overall, the MP communities showed a significantly lower biodiversity than those on wood and in water. Dinoflagellates of the genus *Pfiesteria* were abundant on the MP samples at two stations. Two species of this genus are potential fish pathogens and can cause toxic algal blooms (Burkholder and Marshall, 2012). Network analyses, which included both, prokaryotes and eukaryotes, showed that many organisms were positively correlated. It reinforces the assumption that the MP-associated organisms interact with each other at the same and at different trophic levels. Possible interactions would be, for example, the joint degradation of complex nutrient sources, symbiosis, endobiosis, parasitism, gene exchange or predator-prey relationships.

Metaproteome analyses of MP-associated biofilms revealed that the spectra of eukaryotic proteins were surprisingly highly abundant in all of them, especially near the sewage treatment plant (PE: 84 % of all identified spectra; PS: 92 %). In contrast, protein spectra of prokaryotic origin dominate in wood biofilms at all sites (each > 90 %). An increased relative abundance of protein spectra of potentially harmful taxa within the plastic biofilms could not be shown. Although protein spectra of the genus *Vibrio* were detected in some plastic samples, they were higher abundant in the corresponding wood samples. Initially, the protein functions were annotated using the cluster of orthologous groups database (COG or KOG for eukaryotic proteins). With regard to the distribution of functions of bacterial proteins, hardly any significant differences between the sites and plastics were found. Spectra for energy production and conversion within the metabolism were most abundant in all MP samples, especially at the site near the wastewater treatment plant. In addition, comparatively high spectra were accounted for by carbohydrate metabolism proteins, above all by PE at the boat terraces sampling site (4.73%) and by the transport and metabolism of inorganic ions (5.27% on PE and 3.79% on PS at the boat terraces and 3.14% on PS at the Heiligendamm sampling site). Also for bacterial proteins for storing and processing genetic information, translation proteins made up the majority of the spectra. During the analysis of the samples for proteins for the degradation of xenobiotics (KEGG database), a specific "2-haloacid dehalogenase" from *Methylobacterium* sp. NC0032 was detected, which is involved in the degradation of chloroalkanes, chloroalkenes, chlorocyclohexanes and chlorobenzenes. All other spectra associated with this metabolic pathway are unspecific enzymes that can play a role in several metabolic pathways. Similarly, no specific proteins have been found to be resistant to antibiotics. This is also the case with quorum sensing. Due to some misleading annotations in the COG database (histone H3/H4 is one of the most abundant proteins in Erythrobacteraceae) and a more accurate description of protein functions, the analysis of protein sequences using the "TIGRFAM" database is currently in progress. Initial results indicate that in the transport processes *TonB*-dependent transport mechanisms play a particularly important role. However, proteins for the exchange of genetic information (subcategory conjugation or transformation) are underrepresented. Regardless of the database selection, the proportion of hypothetical proteins or proteins without annotation is high in all samples, which is common in marine metaproteome studies.

The substrate use of microorganisms on MP was quantitatively determined by BrdU immunocytochemistry. It became clear that specific substrate preferences of the colonizing bacteria could be observed for each surface. The use of a substrate as well as the proportion of bacteria using the substrate could be determined. In this way, not only specific usage patterns for each surface could be identified, but also differences in the substrates converted by all biofilms. Biofilms isolated from HDPE showed significant use of the substrates acetate, benzoate, butyrate, formate, propionate, salicylic acid, urea, uric acid, fucose, glucose and lactose. Biofilms isolated from PS showed a significant use of the substrates acetate, butyrate, formate, propionate, salicylic acid, urea and glucose. Biofilms isolated from wood showed a significant use of the substrates acetate, butyrate, formate, propionate, fucose, glucose, mannitol and trehalose. Thus, different usage patterns could be determined. At the same time, it was shown that for commonly used substrates, such as acetate, the number of active bacteria varies (HDPE: ~48%, PS: ~12%, wood: ~41%). A Kruskal-Wallis test showed that there was no significant difference in substrate use between plastic biofilms, but biofilms from both plastic surfaces were significantly different from wood in their substrate use. To investigate the succession of bacterial communities on plastic, plastic stripes (HDPE and PS) of the same quality as the pellets used for *in situ* experiments were transferred in several steps in an *in vitro* experiment. Each transfer resulted in the exchange of the medium and the replacement of an old plastic strip with a new one. This experiment has increased the selection pressure on the community. Only bacteria that were able to change between planktonic life and adhesion could be found on the surfaces after several transfers. Water samples from the sites of the *in situ* experiments were used as primary inoculates in order to make a comparison with the *in situ* communities. DNA was extracted from 271 plastic stripes and prepared for sequencing. The 16S rRNA genes (V3-V4 region) were sequenced for all DNA samples from the *in vitro* biofilm experiment.

Parallel to the substrate experiments and *in vitro* biofilm experiments, bacterial strains were isolated from the biofilms produced *in situ* and *in vitro*. Several methods were used, including the use of media of different nutrient composition and salinity, as well as dilution series and direct plating of biofilm samples. It was possible to isolate 1325 bacterial strains. The 16S rRNA genes of all isolated strains were sequenced at least partially and the closest relatives were determined using BLAST. Of the isolated strains, ~7% showed close relationship to pathogenic and potentially pathogenic organisms that could be assigned using TRBA466. Most of the isolated bacteria were alpha and gamma proteobacteria, followed by flavobacteria. Based on the information from the 16S-rRNA sequences, more than 90% of the isolated strains belong to at least one new species. Bacteria other than wood could be isolated from plastic. However, the surface had less effect on the isolation success than the nutrient composition and concentration of the media, as well as the origin (location) of the biofilm.

(III) The assessment of health risks for Baltic Sea countries by microplastics as a vector of pathogenic microorganisms

In situ 16S and 18S rRNA gene and metagenome analyses of biofilms on MP, wood, and environmental particles along the environmental gradient in the area of the Warnow did not reveal any unusual accumulation of pathogenic microorganisms or their functions on synthetic polymers. Therefore, the question was addressed whether the bacterial community on MP is permanently altered during intestinal transit through the key marine organisms *Arenicola marina* and *Mytilus edulis* and whether potentially pathogenic intestinal bacteria can establish themselves on MP. It was found that the bacterial communities on PS, glass and in the natural faeces of *A. marina* were more similar after passing through the digestive tract than before, populated with some typical sediment organisms. The communities also changed on the excreted PS and glass particles after 24 hours of incubation in Baltic Sea water. No accumulation of potentially pathogenic bacteria on PS could be detected neither after passing through the digestive tract of *A. marina* nor in postincubations. However, a substrate-specific colonization by the oceanospirillum *Amphritea* sp. was observed, which could only be detected in the PS approaches, and there in very high relative abundances. In PA- and chitin-associated biofilm communities no accumulation of potentially pathogenic bacteria was detected after passing through the digestive tract of *M. edulis*. There were hardly any differences between the bacterial communities on PA, chitin, natural particles and faeces. Also in this case a high relative abundance could be detected by a close relative of the oceanospirillum *Neptunomonas* sp. on PA.

Compared to natural particles, it can be summarized that for the MP used in MikrOMIK no increased potential as a vector for pathogenic microorganisms could be determined. A comparison with internationally published literature confirms this conclusion (Oberbeckmann & Labrenz, 2019). Consequently, the health risks for Baltic Sea countries due to MP as a vector of pathogenic microorganisms can be considered to be low. However, there are taxa that specifically colonize plastics, and gene transfer or genes that are typical for anthropogenic environmental influences can also be significantly increased to MP. In addition, in the ecosystems studied by MikrOMIK, MP have been found in high concentrations, with unforeseeable consequences for the aquatic food web. Further research is needed here. Consequently, follow-up projects have already started from MikrOMIK, via a successful stabilisation of the network. These projects investigate the properties of MP from a Baltic Sea catchment area, via the estuary into the entire Baltic Sea (incl. ecotoxicology and monitoring approaches):

- BONUS MICROPOLL, July 2017-June 2020 (Project Management: IOW; Partner IPF, AWI). Objective: Multi-stage assessment of MP and related pollutants in the Baltic Sea.

- BMBF-FONA MicroCatch_Balt, August 2017-July 2020 (Project Management: IOW; Partner IPF). Objective: Analysis of MP sources and sinks from the Warnow river basin to the Baltic Sea. The project is very close connected with the BMBF-FONA project PLAWES (project management: University of Bayreuth, AWI. Partner ICBM), which covers the catchment area of the Weser.

- BMBF-FONA PLASTRAT, September 2017 - August 2020 (Project management: Bundeswehr University Munich; Partners: IOW, IPF). Goal: Development of solution strategies to reduce the input of urban plastic into limnic systems

- UBA - Joint project: marine waste (coordination: AquaEcology; partner: IOW). Objective: Follow-up assessment and establishment of long-term monitoring of the pollution of various marine areas and biota by marine waste.

- ERA.Net RUS -Joint project: BalticLitter (coordination: Shirshov Institute of Oceanology of Russian Academy of Sciences (Atlantic Branch), Kaliningrad; partner: IOW). Objective: Development and testing of monitoring methods for marine waste with focus on the inner coastal waters of the Baltic Sea.

- In addition, teaching materials for school lessons were produced via the "PlasticSchool" project (https://plasticschool.de) funded by the state of Mecklenburg-Western Pomerania.

Statement as to whether the results of the project are economically exploitable and whether such exploitation takes place or is to be expected; Information on possible patents or industrial cooperation

MikrOMIK has established cooperation with IZM for the development and production of Si filters for MP analysis. The Si filter has already been requested by several interested parties. The incubators from the incubation experiment or their concept is used further and also applied in other projects/countries. Otherwise, no economic usability or patents are assumed.

Contributions from potential cooperation partners at national and international level who have been involved in the results of the project

In addition to the project members, the following scientists contributed directly to the project: Keilor Rojas-Jimenez, Ester Maria Eckert, Andrea Di Cesare, Diego Fontaneto, Gianluca Corno and Uli Klümper (see authors in the list of publications). The working group mass spectrometry (Dörte Becher) of the Institute of Microbiology at the University of Greifswald has contributed mass spectrometric measurements to the project.

Marie Therese Kettner was financially supported by COST-European Cooperation in Science and Technology, COST Action ES1403: New and emerging challenges and opportunities in wastewater reuse (NEREUS) for a one-month research stay in Italy (for Experiment B).

Through a cooperation of G. Schernewski with the University of Klaipeda, Lithuania, comparative studies on Lithuanian beaches were made possible.

At the IPF, model particles from the polymers PE and PS were produced and used for various subprojects within MikrOMIK (incubation experiments, substrates for bacterial growth, etc.). Cylindrical pellets (1x1x1 mm^3) and rectangular rods (127 x 12.7 x 0.8 mm^3) were produced by injection moulding. In addition, the PE and PS granules (5x5x5 mm^3) were grounded into fragments of different sizes. A special challenge was the further comminution of plastic particles to a size <5mm without changing the properties of the plastic. Ultimately, the comminution of industrial pellets using ultracentrifugal and cryogenic mills proved to be suitable. The crushing was carried out by BTU Cottbus.

In cooperation with Micromod Partikeltechnologie GmbH, a fluorescent coating of the ground PS with the dyes rhodamine and fluorescein was tested.

High-throughput sequencing was carried out at the Institute for Medical Microbiology, Virology and Hygiene (University of Rostock, WG Kreikemeyer).

Qualification work completed in the course of the project

<u>Promotion</u>

1. Arias-Andrés, Maria (expected to finish in mid-2019): Microbial gene exchange on microplastic particles. University of Potsdam.

2. Gebhardt, Christopher (expected to finish in mid-2019): The influence of bioturbation on the transport and fate of plastic particles on the seabed. University of Rostock.

3. Käppler, Andrea (2018): Characterization of microplastcs in marine samples: Possibilities and limitations of FTIR and Raman spectroscopy. Technische Universität Dresden.

4. Kesy, Katharina (expected to finish by the end of 2019): Influence of higher organisms (*Copepods*, *Mytilus, Arenicola*, *Homo sapiens*) on the composition of microbial biofilms on microplastic. University of Rostock.

5. Kettner, Marie Therese (2018): Microbial colonization of microplastic particles in aquatic systems. University of Potsdam.

<u>Diploma</u>

1. Mathias Pfützner (2016): Further development of sample preparation of marine samples for identification and quantification of microplastic. Technische Universität Dresden.

Master

1. Michael Beckers (2017): Bioturbation of plastic particles by *Hediste diversicolor* (O.F. Müller, 1776). University of Rostock. This work was awarded on 07.12.2017 with the Faculty Prize for the best work of the MNF in the Faculty of Biology of this year.

2. Florian Krause (2017). Phylogenetic composition of microbial biofilms on paint particles isolated from sediment of the "Old Stream" (Warnow River, Germany). University of Rostock

3. Daniel Bartosik (2017): Metaproteome analyses of microplastic associated biofilms from the Baltic Sea. University of Greifswald.

4. Oliver Biniasch (2016): Identification of microplastics isolated from sediment traps of the Arkona Basin and the Gotland Deep. University of Rostock.

5. Mirco Haseler (2015): Marine Litter monitoring along sandy beaches of the Baltic Sea. Ostfalia University of Applied Sciences.

6. Claudia Weder (2015): Marine litter on German Baltic Sea beaches: New methodological approaches, quantification and ecological and practical consequences. University Zittau/Görlitz.

7. Franziska Klaeger (2014). Structure, composition and stability of biofilm communities on plastic particles after passing through the digestive tract of *Mytilus edulis* and *Arenicola marina*. University of Rostock.

8. Alexander Hentzsch (2013). Composition of microbial biofilms on microplastic particles after passage through the digestion tract of *Mytilus edulis*. University of Rostock.

9. Katharina Kesy (2013). Composition of microbial biofilms on microplastic particles after passage through the digestion tract of *Arenicola marina*. University of Rostock.

Bachelor

1. Nicole Stollberg (2016). Identification of microplastics > 500 µm in Warnow sediments. Universität Rostock.

2. Philipp Konrad Schätzle (2016): Transport of plastic particles through *Arenicola marina*. University of Rostock.

3. Leonie Bushbeck (2016): A monitoring strategy for large micro- and meso- plastic waste at Baltic beaches Brandenburgische Technische Universität

4. Felix Müller (2015). Quantification of *Amphritea* sp. in microplastic biofilms using real-time PCR. Universität Rostock.

5. Anne Breznikar (2015). Quantification of "Black particles" in different areas of the Baltic Sea. Universität Rostock.

6. Reichardt, Aurelia M. (2015): Sinking velocity of new and aged microplastic particles. Universität Rostock.

7. Florian Krause (2014). Composition of brackish microbial biofilms on microplastic. Universität Rostock.

8. Julius Degenhardt (2014). Stability of brackish microbial biofilms on microplastic particles. Universität Rostock.

9. Oliver Biniasch (2014). Identification of microplastic originating from sediments traps of the Arkona basin. Universität Rostock.

10. Stephanie Mothes (2014). Structure and composition of biofilm communities on plastic particles after passing through the digestive tract of *Mytilus edulis*. Universität Rostock

List of publications from the project

1. Oberbeckmann, S., Labrenz, M. (2019). Marine microbial assemblages on microplastics: diversity, adaptation, and role in degradation. *Annu Rev Mar Sci*, in press
2. Tagg, A. S., Oberbeckmann, S., Fischer, D., Kreikemeyer, B., Labrenz, M. (2019). Paint particles are a distinct and variable substrate for marine bacteria. *Mar Poll Bull*, in press
3. Kaiser, D., Estelmann, A., Kowalski, N., Glockzin, M., Waniek, J. J. (2019). Sinking velocity of sub-millimeter microplastic. *Mar Poll Bull*, 139, 214-220
4. Kettner, M. T., Oberbeckmann, S., Labrenz, M., Grossart, H.-P. (2019). The eukaryotic life on microplastics in brackish ecosystems. *Front Microbiol*, https://doi.org/10.3389/fmicb.2019.00538.
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Measures to secure the research data produced in the project and its availability

Meta and laboratory data from the project are stored with the individual partners and the IOW database IOWDB. The nucleotide and protein sequences resulting from the project will be publicly deposited for publication at NCBI (see the individual publications for more details).

Press releases, media reports, publicity activities

The declared goal of MikrOMIK was to provide the basis for statements on current socio-ecological problems in the Baltic Sea region. This was achieved by investigating MP as a carrier of certain microbial functions, populations and, if necessary, infection possibilities. This was done because a further increase in plastic emissions into the sea, climate change and the steady growth of tourism in the Baltic Sea region increase the potential for infection risks in coastal areas. MikrOMIK was intended to provide a scientifically sound basis for assessing the hazard potential and socio-economic weight of MP for fishing industry and tourism on the German Baltic Sea coast. This network should be available beyond the project framework as a competent contact for the spread of pathogenic microorganisms in the marine environment and also for urgent problems.
This goal was achieved through press releases, answers to numerous media inquiries and other publicity activities of MikrOMIK experts. Between 04/2014 and 06/2018, more than 100 media reports in all media sectors (print media, online media, radio, TV) were recorded nationwide, focusing on microplastics and its influence on microbial communities in the sea and referring to MikrOMIK research. Among the best known media were ARTE TV, WDR Fernsehen, Deutsche Welle, Spiegel online, Süddeutsche Zeitung, Stuttgarter Zeitung and Hörzu. Thus, the MikrOMIK scientists are not only in science but also - at least in Germany - in the general public seen as one of the leading experts in this field. Some examples of notifications and activities are listed below:

Press releases:

- **1.4.2014:** Krankheitserreger Huckepack? – Neues Leibniz-Netzwerk MikrOMIK widmet sich dem Gefährdungspotential von Mikroplastik (https://bit.ly/2yteJhu)
- **17.8.2015:** Von Mikroplastik und Mikroben: IOW leitet Ostsee-Expedition des Forschungsschiffs POSEIDON (https://bit.ly/2K1OoZj)
- **1.8.2016:** Gewicht ist nicht alles: Partikelform beeinflusst Sinkgeschwindigkeit von Mikroplastik (https://bit.ly/2thkcSG)
- **14.3.2017:** Forschung macht Schule: Die „PlasticSchool" geht an den Start (https://bit.ly/2nBf8nB)
- **21.2.2018:** Neue IOW-Studie: Birgt Mikroplastik zusätzliche Gefahren durch Besiedlung mit schädlichen Bakterien? (https://bit.ly/2HwHhXM)

Activities (selected):

- Supervision of the youth research project "The invisible danger: microplastics - the effects of our waste using *Hediste diversicolor* as an example (https://bit.ly/2K92Guq). In cooperation with the BilSE-Institut (Bildungs-Service für Europa), the doctoral students Christopher Gebhardt (University of Rostock) and Martin Albrecht (University of Rostock, Institut für Biowissenschaften, WG Angewandte Ökologie und Phykologie) supervised a group of three students of the Innerstädtisches Gymnasium Rostock, who dealt with the effect of MP on microalgae and a possible MP transport by *H. di-*

versicolor. The results of the project were presented in the Youth Research Competition 2017. The project was awarded as follows:

- Landessieger Mecklenburg-Western Pomerania 2017 in the field of biology
- Special Prize of the Ernst A. C. Lange Foundation, Bremen (Jugend-Forscht-Bundes-wettbewerb 2017, Erlangen)

· Exploitation of MikrOMIK results in the development of the IOW student laboratory MariSchool (https://marischool.de)

· Presentation of MikrOMIK results in the context of the journalist workshop "RADO - Ran an die Ostsee" (https://ran-an-die-ostsee.de)

· Presentation of MikrOMIK findings at public events such as the Baltic Sea Days 2014 and 2016 (analogous to the upcoming Baltic Sea Day 2018 https://www.ostseetag.info) and the MV Day 2018 (http://www.mvtag2018.de)

· Presentation of MikrOMIK findings in special event formats such as "Leibniz im Bundestag 2017 (analogous to last year's event https://bit.ly/2M4Bl9Z) and the I.C.E Expedition 2017 by Arved Fuchs (https://bit.ly/2gPV7eq)

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