Thesis Summary

Microplastic pollution is now recognized as a persistent pressure on marine ecosystems, but its effects on the smallest components of pelagic food webs remain insufficiently understood. While many studies have focused on larger organisms, the smallest microplastic particles overlap in size with bacteria and other microbial prey. This makes them especially relevant for pelagic microbial food webs, where bacteria and heterotrophic nanoflagellates play central roles in carbon transfer, nutrient recycling, and the regulation of microbial community structure. This thesis investigated how bacteria-sized microplastics affect the structure and functioning of pelagic microbial food webs, with particular focus on bacteria, heterotrophic nanoflagellates, and methodological challenges associated with detecting very small particles.

The first part of the thesis addressed a key analytical problem: the reliable detection of small microplastics. Because particles below 5 micrometres are difficult to detect using standard approaches, an optimized Nile Red staining protocol was developed for microplastics smaller than 5 micrometres, with emphasis on particles of 2 micrometres or less. The protocol was tested using polystyrene and polyethylene particles, together with inorganic controls. Under optimized filtration and staining conditions, 1 and 2 micrometre polystyrene particles and 2 micrometre polyethylene particles showed strong and consistent fluorescence signals, while glass particles and biological material did not stain. Formalin fixation, commonly used for preserving microbial samples, improved fluorescence brightness and signal-to-noise ratio without compromising selectivity. This study therefore provided a simple, cost-effective, and practical method for detecting small microplastics in preserved marine samples, forming an important methodological foundation for studying their ecological effects.

The second part of the thesis provided the first experimental assessment of how bacteria-sized polystyrene particles affect natural Baltic microbial food webs. A five-day microcosm experiment tested three concentrations of 1.1 micrometre polystyrene particles and compared them with size-matched glass particles to separate polymer-specific effects from the physical effects of suspended particles. Using bacterial and HNF abundance measurements, respiration estimates, and 16S and 18S rRNA gene amplicon sequencing, this chapter showed that HNF growth declined in a concentration-dependent manner, with the strongest effects in the highest polystyrene and glass treatments. Because similar responses occurred in both plastic and inert particle treatments, the results indicate that the physical presence of particles, rather than polystyrene toxicity alone, was the main driver of reduced HNF growth. Bacterial abundance and respiration were not significantly affected, suggesting that the decline in HNFs was not caused by reduced prey availability. Instead, bacteria-sized particles may have interfered with feeding by reducing prey encounter efficiency, increasing handling costs, or occupying food vacuoles without providing nutritional value.

The community analyses in Chapter 2 showed that eukaryotic communities changed mainly over time, with shifts from initially dominant phototrophic or mixotrophic groups toward heterotrophic taxa, including bacterivorous flagellates. Bacterial communities were comparatively stable. These findings demonstrated that high concentrations of bacteria-sized particles can rapidly suppress key microbial grazers and potentially alter carbon transfer within the microbial loop. However, because this chapter relied mainly on amplicon sequencing, it described taxonomic community shifts but could not resolve genome-level functional potential or identify which bacterial populations might carry genes associated with plastic-related metabolism.

Chapter 3 therefore extended Chapter 2 in two important ways. First, it compared two environmentally relevant polymers, polystyrene and polyethylene, under the same experimental conditions and at the same bacteria: particle ratio, while retaining glass particles as a non-plastic particle control. Second, it added genome-resolved metagenomics to move beyond community composition and examine bacterial population dynamics, metagenome-assembled genomes, and putative genes associated with plastic-processing pathways. In this way, Chapter 3 tested whether the limited community-level responses observed in Chapter 2 were specific to polystyrene or reflected a broader response to bacteria-sized particles, while also asking whether polymer-specific effects could be detected at finer genomic and functional levels.

In Chapter 3, microbial abundance and community composition again changed strongly over time. HNF abundance increased initially and later declined in the plastic treatments, while bacterial abundance showed variable but not clearly treatment-specific dynamics. Both bacterial and eukaryotic community composition were shaped mainly by incubation time rather than particle type. Eukaryotic communities showed temporal restructuring, including an increase in Stramenopiles and bacterivorous genera such as Paraphysomonas and Spumella. Bacterial communities were dominated by Pseudomonadota, Bacteroidota, and Actinobacteriota with temporal shifts among dominant families but no strong polymer-specific separation.

Genome-resolved analyses added a finer layer of interpretation. A total of 118 medium to high quality metagenome-assembled genomes were recovered, many of which could not be assigned to described species, highlighting the unexplored microbial diversity in Baltic coastal waters. Most abundant MAGs did not show strong treatment-specific enrichment. However,

three low-abundance MAGs affiliated with Spirosomaceae and Shewanellaceae were enriched in the polyethylene treatment and contained homologs of genes associated with processing synthetic polymers or related compounds. Across all MAGs, putative genes linked to polyethylene and polystyrene processing were detected, but their abundance patterns were influenced more by time than by polymer treatment. These results suggest that short-term exposure to bacteria-sized PE and PS particles did not cause broad community-level disruption, but PE may selectively favour a narrow subset of bacteria with potential polymer-associated metabolic traits.

Overall, this thesis demonstrates that the ecological effects of bacteria-sized microplastics on pelagic microbial food webs are subtle, size-dependent, and strongly mediated by physical interactions and natural microbial succession. By first establishing a robust methodological basis for detecting the smallest microplastic particles, this work subsequently reveals that bacteria-sized polystyrene and inert particles can suppress heterotrophic nanoflagellate (HNF) growth through physical particle effects. Expanding upon this framework through a comparison of polystyrene and polyethylene, the research utilizes metagenomics to detect finer bacterial and functional responses. Together, these findings show that while short-term exposure to bacteria-sized microplastics may not drastically restructure entire microbial communities, it significantly affects key microbial grazers and reveals narrow, polymer-associated responses among specific bacterial populations. Ultimately, this thesis contributes to a more mechanistic understanding of how small microplastics influence microbial food-web structure, grazing interactions, and carbon flow at the base of marine ecosystems.