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WYDZIAŁ INŻYNIERII LĄDOWEJ I ŚRODOWISKA

UCZELNIA BADAWCZA
INICJATYWA DOSKONAŁOŚCI

Gdańsk, 20.10.2026

REVIEW OF THE DOCTORAL DISSERTATION

Doctoral candidate	Uroosa
Dissertation title	Effects of Microplastic Pollution on Structure and Functioning of Pelagic Microbial Food Webs
Discipline	Earth and Environmental Sciences
Institution	Institute of Oceanology, Polish Academy of Sciences (IOPAN) in collaboration with International Environmental Doctoral School, University of Silesia in Katowice
Supervisor	dr hab. Katarzyna Piwosz, prof. MIR-PIB
Auxiliary supervisor	dr hab. Józef Wiktor, prof. IO PAN
Reviewer	Prof. Aneta Łuczkiewicz, PhD, DSc, Eng., Faculty of Civil and Environmental Engineering, Gdańsk University of Technology, Narutowicza 11/12, 80-233 Gdańsk, Poland

Formal basis and scope of the review

This review of the doctoral dissertation submitted by Uroosa, MSc**,** entitled *Effects of Microplastic Pollution on Structure and Functioning of Pelagic Microbial Food Webs*, has been prepared at the request of the Scientific Council of the Institute of Oceanology, Polish Academy of Sciences, in Sopot.

The dissertation is written in English and is structured around three thematically related research papers. The submitted thesis comprises 164 pages and includes a general introduction, three research chapters with accompanying supplementary materials, an overall summary, and references.

Chapter I reproduces the article by Uroosa and Piwosz (2026), published in *Frontiers in Marine Science, 13, 1818345 (doi: 10.3389/fmars.2026.1818345).* The journal has a 2025 Journal Impact Factor of 3.4 and a Q1 ranking in Journal Citation Reports**,** as reported by the publisher. Chapters II and III are presented as research manuscripts, under review in Frontiers of Marine Science and in preparation, respectively.

The review evaluates the scientific merit, methodological rigour, and formal presentation of the dissertation, including the originality of the research, the robustness of the evidence, and the validity of the conclusions. It also assesses the extent to which the dissertation meets the requirements for the award of a doctoral degree in the discipline of Earth and Environmental Sciences**.**

Scientific relevance and justification of the research topic

The scientific relevance of the dissertation lies in addressing an important ecological question: to what extent do microbial responses to bacteria-sized microplastics reflect polymer-specific properties rather than the general effects of suspended, non-nutritive particles? To investigate this question, the Author combines

analytical method development with controlled experiments on microbial assemblages collected from the coastal Gulf of Gdańsk, focusing on selected components of pelagic microbial food webs.

The topic is scientifically well justified because the smallest microplastics overlap in size with microbial prey, yet remain difficult to detect and characterise reliably. At the same time, bacteria and their protistan consumers mediate organic matter transformation, nutrient regeneration, and the transfer of microbial biomass to higher trophic levels. Investigating their responses therefore extends the assessment of microplastic pollution beyond effects on individual organisms towards processes relevant to ecosystem functioning.

The use of natural coastal assemblages is particularly valuable because responses observed in single-species cultures do not necessarily predict those of interacting communities. Previous Baltic mesocosm studies have reported limited or variable responses, reinforcing the need to identify the conditions under which particle exposure produces measurable biological effects. The dissertation addresses this uncertainty through controlled experiments.

Review of the international literature

The literature review provides a relevant interdisciplinary background, connecting microplastic detection, microbial ecology, protistan feeding, and bacterial metabolic potential. It includes both foundational studies and recent community-level investigations, and recognises that published findings are not uniform. The Author acknowledges differences between evidence from single-species cultures, particle-associated biofilms, and natural planktonic assemblages. A more systematic comparison of particle properties, exposure conditions, and community composition would further strengthen the interpretation of contrasting findings.

Main strengths of the dissertation

1. **A relevant research question and a coherent overall concept**

The dissertation addresses the interaction between bacteria-sized microplastics and pelagic microbial communities. Its progression from particle-detection methodology to concentration-dependent responses and comparisons between particle materials provides a logical framework linking an analytical challenge with ecological questions.

2. **An informative comparative experimental design**

The use of natural Baltic microbial assemblages, together with plastic and non-plastic particle treatments, is a particular strength. Including silica microspheres provides a useful basis for examining whether observed responses are associated with polymer properties or with the presence of suspended particles more generally.

3. **A practical contribution to the detection of small microplastics**

Chapter I addresses several operational aspects of Nile Red staining, including solvent–filter compatibility, incubation time, sample preservation, and carry-over contamination. The resulting procedure offers a useful starting point for screening small polystyrene (PS) and polyethylene (PE) particles in preserved samples, while identifying conditions that require further validation.

4. **Complementary methods and substantial genomic resolution**

The combination of microscopy, community-respiration measurements in Chapter II, amplicon sequencing, and genome-resolved metagenomics allows the research questions to be examined at different biological levels. In particular, the reconstruction of 118 non-redundant bacterial metagenome-assembled genomes

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(MAGs) extends the analysis beyond broad taxonomic patterns and provides material for investigating population-specific responses.

5. Identification of population-specific responses that warrant further investigation

Chapter III identifies three bacterial MAGs with higher relative abundance in the PE treatment than in the control, despite limited changes at the overall community level. This is a valuable result because it directs further research towards specific populations rather than assuming a uniform microbial response to plastic exposure. The Author appropriately acknowledges that these findings do not demonstrate plastic degradation.

6. The candidate's declared contribution to the research

The authorship declaration identifies Uroosa as the principal contributor to the experimental work, data analysis, interpretation of the results, and preparation of the manuscripts. The study-specific contribution statements provide a more detailed account of her responsibilities across the three research chapters. For Chapter I, published in *Frontiers in Marine Science*, her responsibilities included conceptualisation, data curation, formal analysis, investigation, validation, visualisation, and preparation of the original draft. Her overall contribution to this study is declared as approximately 90%. For Chapter II, reported as a manuscript under review in *Frontiers in Marine Science*, her declared responsibilities included conceptualisation, methodology, formal analysis, investigation, visualisation, and preparation of the original draft, with an estimated overall contribution of 70%. For Chapter III, reported as a manuscript in preparation, her declared responsibilities included conceptualisation, methodology, formal analysis, investigation, validation, visualisation, and preparation of the original draft, with an estimated overall contribution of 50%. Taken together, these declarations indicate that the candidate's involvement extended beyond experimental execution to the conceptual development, analysis, and scientific presentation of the research. In my assessment, the scope of these responsibilities supports recognition of her leading role across the three studies and her ability to conduct independent scientific work within a collaborative research setting.

Weaker points and critical remarks

1. Incomplete validation of staining selectivity in environmental matrices

Chapter I includes a biological control based on one formalin-fixed algal species, *Chlamydomonas vectensis*, and additional observations on seawater containing added PS microspheres. These tests provide useful evidence under the conditions examined, but do not establish selectivity across the diversity of natural organic material present in marine samples. Background fluorescence and carry-over contamination were also investigated; however, these assessments do not replace quantitative validation of matrix-dependent interference. Although the Author acknowledges the need for further environmental validation, this limitation should be reflected consistently in statements concerning the exclusion of biological interference. The broader environmental applicability of this procedure would require further quantitative validation, including assessment of particle recovery, matrix-dependent background fluorescence, and false-positive and false-negative detection rates.

2. Inconsistencies in methodological reporting that affect reproducibility

Chapter I reports final concentrations of 10 µg mL^{-1} Nile Red and 5% acetone (p. 21), whereas the volumes and stock concentration specified in Supplementary File 1 (p. 28) yield nominal final concentrations of 20 µg mL^{-1} and 10% acetone in a 5 mL staining mixture. The Author should clarify which formulation

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was used in the optimisation experiments. In Chapter III, the particle concentrations reported as final values in Section 2.1 (p. 92), approximately 5×10^9 particles mL^{-1}, are inconsistent with the targeted 1:1 particle:bacteria ratio and the initial bacterial abundances shown in Figure 2b, which are of the order of 10^6 cells mL^{-1}. Stock concentrations, addition volumes, and resulting final concentrations should be reported separately. These discrepancies require clarification for reproducibility, but do not, by themselves, demonstrate that the experimental dosing was incorrect.

3. Disagreement between the description of results and the corresponding figure

Chapter III describes an initial increase in heterotrophic nanoflagellate (HNF) abundance between T1 and T3 (p. 101), whereas Figure 2a (p. 102) shows a decline over that interval. The same description is repeated in the thesis summary (p. 141). The text, figure labels, should therefore be checked against the underlying data and reconciled.

4. Insufficient transparency in the statistical description

For analyses spanning multiple time points, the methods should clarify how the non-independence of repeated samples from the same experimental bottle was handled. Reporting effect sizes and uncertainty would strengthen the interpretation. In particular, non-significant differences should not, by themselves, be interpreted as evidence of equivalence or community resilience.

5. Mechanistic interpretations and limits of the measurements

Similar responses to PS and silica support the hypothesis of a general particle-associated effect, but do not demonstrate impaired feeding or exclude a chemical contribution. Likewise, bacterial abundance alone does not fully characterise prey availability or nutritional quality. Since ingestion, clearance, particle retention, and trophic carbon transfer were not directly measured, impaired feeding and altered carbon transfer should be presented as possible mechanisms or consequences, rather than demonstrated outcomes. The Author recognises this limitation in the discussion, but the qualification is not maintained consistently in the Chapter II abstract and the overall summary.

6. **Limited grounds for extrapolation to intact food webs and long-term environmental exposure**

The experiments used short incubations in darkness, size-fractionated assemblages, and a restricted range of model particles. These conditions are appropriate for controlled comparisons but do not reproduce the full complexity of pelagic food webs. Differences in fractionation between Chapters II and III also limit direct comparison. Conclusions about ecosystem resilience or responses to current and future pollution should therefore remain explicitly bounded by the experimental conditions.

<u>Synthesis of findings and scope of the conclusions</u>

The final summary would benefit from a more explicit assessment of which research hypotheses were supported, partly supported, or remained unresolved. In particular, the description of ecological effects as "size-dependent" (p. 142) requires qualification. Although different particle sizes were used across the dissertation, Chapter I addressed analytical detection, while Chapters II and III primarily compared particle concentrations and materials, without isolating particle size as an independent experimental factor. The results therefore document responses to particles within the bacterial size range, rather than establish an independent relationship between particle size and ecological effect. Size dependence should be presented as a question for further investigation, not as a demonstrated conclusion of the thesis.

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Final conclusion

The dissertation addresses an important research problem and provides original evidence concerning the responses of Baltic microbial assemblages to bacteria-sized particles. I appreciate the substantial experimental and analytical work undertaken and the candidate's command of complementary microbiological and molecular methods. Uroosa leading contribution to the conception, execution, and interpretation of the studies, as described in the contribution statements, further supports my assessment of her ability to conduct independent scientific research within a collaborative team.

On the basis of the assessment presented above, I conclude that the doctoral dissertation submitted by Uroosa, MSc, entitled Effects of Microplastic Pollution on Structure and Functioning of Pelagic Microbial Food Webs, meets the requirements set out in Article 187(1) and (2) of the Polish Act of 20 July 2018, Law on Higher Education and Science (consolidated text: Journal of Laws of 2024, item 1571, as amended). I therefore recommend that the Candidate be admitted to the public defence of her dissertation in the proceedings for the award of a doctoral degree in Earth and Environmental Sciences at the Institute of Oceanology, Polish Academy of Sciences.

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Signed by / Podpisano przez:

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