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REVIEW OF THE DOCTORAL DISSERTATION

Ms. Uroosa

entitled "Effects of microplastic pollution on the structure and functioning of pelagic microbial food webs"

The doctoral dissertation submitted for review by Ms. Uroosa was prepared under the supervision of dr hab. Katarzyna Piwosz, Professor at the National Marine Fisheries Research Institute (MIR-PIB), and dr hab. Józef Wiktor, Professor at the Institute of Oceanology of the Polish Academy of Sciences (IO PAN). This review is based on the resolution adopted by the Scientific Council of the Institute of Oceanology of the Polish Academy of Sciences on 16 September 2026. I have prepared the review in accordance with the applicable provisions of the Act of 20 July 2018, the Law on Higher Education and Science (Journal of Laws of 2024, item 1571, as amended), and based on the copy of the doctoral dissertation provided to me.

In her doctoral dissertation entitled "Effects of microplastic pollution on the structure and functioning of pelagic microbial food webs", Ms. Uroosa addresses the impact of microplastic pollution in aquatic environments on the structure and functioning of pelagic microbial food webs. This topic falls within the broader field of research on the ecological consequences of plastics in aquatic ecosystems, particularly their effects on microorganisms that cycle matter and energy.

The dissertation covers issues related to the detection of small microplastic particles, the assessment of microbial responses to their presence, and the investigation of potential relationships between polymer type and the composition and functional potential of microbial communities. I consider this multifaceted approach justified, as the effects of microplastics in aquatic environments may depend on both the chemical properties of the material and particle size, as well as on the physical presence of particles and the natural variability of the biological communities under investigation.

The dissertation was written in English and follows the structure typical of doctoral theses based on a thematically coherent series of publications. In the first part, the Doctoral Candidate presents abstracts in Polish and English, a table of contents, and an introduction in which she briefly outlines the current state of knowledge on the research topic and the study's main objectives. These objectives focus on understanding the effects of microplastics on the structure and functioning of pelagic microbial food webs. Their achievement involves both the development and optimization of a method for detecting small plastic particles and the experimental assessment of their effects on aquatic microorganisms, together with an analysis of changes in the composition and functional potential of the communities studied. An important element of the adopted approach is the attempt to determine whether the observed changes depend on the type of polymer or result from the mere presence of particles in the environment. I consider these objectives well-justified and scientifically relevant, as they account for the complex nature of interactions between microplastics and aquatic microbial communities. An additional strength is the interconnection between the successive stages of the research and the use of complementary methods that allow the problem to be examined from different perspectives.

The main body of the doctoral dissertation consists of a thematically coherent series of three papers, one of which has already been published in a journal indexed in the Web of Science: "Systematic optimization of Nile Red staining parameters for the reliable detection of microplastics ≤ 2 µm." Frontiers in Marine Science 13:1818345, 2026 (Impact Factor = 3.4). The other two papers, "Concentration-dependent effects of polystyrene microplastics on bacterial and protistan communities: evidence for physical stressor" and "Limited effects of bacteria-sized microplastics on coastal baltic microbial dynamics", are currently under review. Each of the papers listed above is accompanied by supplementary materials containing additional results. The third part of the dissertation comprises a summary in which the Doctoral Candidate synthesizes the research conducted, a bibliography, and statements specifying the contributions to the publications. The Doctoral Candidate is listed as the first author of all the research papers included in the dissertation, which clearly indicates her leading role in the research and her responsibility for carrying out the principal part of the laboratory work.

The dissertation has been prepared with care; however, the Author has not entirely avoided minor grammatical and editorial errors. These include, for example, the duplicated numbering of subsection 3.5 in Chapter III and a grammatical error in the caption of Figure 5 in Chapter II, where

"The grey circles represents" is used instead of "The grey circles represent". These shortcomings are formal in nature and do not affect the overall assessment of the dissertation's scientific value.

The individual parts of the doctoral dissertation form a logical whole. The first stage focuses on optimizing a method for detecting small microplastic particles. In the second stage, the Author analyses the responses of selected groups of microorganisms to exposure to polystyrene particles using a microcosm experiment. The third stage extends the scope of the research by comparing the effects of different polymer types and conducting metagenomic analyses, which enable characterization of microbial community composition and the potential functional traits of selected populations. This structure is scientifically justified. The successive parts of the dissertation are not merely separate studies but address different aspects of a common research problem. A major strength of the work is the integration of methodological, experimental, and molecular approaches.

The literature review provides a foundation for justifying the research topic and the methods employed. The Author addresses an issue of importance to contemporary aquatic ecology, considering both the challenges of detecting the smallest fractions of microplastics and the difficulties of distinguishing effects arising from polymer chemical properties from those caused by the physical presence of particles. Particularly important is the recognition that natural microbial communities change over time and that changes in their composition need not be a direct consequence of exposure to a particular type of plastic.

The first stage of the research focused on optimizing Nile Red staining to detect microplastic particles smaller than 5 μm. The Author demonstrated that a fluorescent signal could be obtained from tested polystyrene particles measuring 1 and 2 μm, and from polyethylene particles measuring 2 μm. Comparable staining was not observed in glass or the biological material examined, while formalin fixation improved signal intensity and the signal-to-background ratio. These results indicate that the developed procedure may be useful for detecting small plastic particles. The significance of this stage is both methodological and practical. The ability to detect small particles may prove useful for developing procedures to monitor microplastics in aquatic environments and for studies where the presence of the smallest particle fractions is important. At the same time, the results obtained do not justify concluding that the method has already been validated for all polymers, particle shapes, and complex environmental matrices. Practical application of the procedure would require further studies using real environmental samples, a broader range of materials, and comparisons with appropriate reference methods. Despite these limitations, the results provide a valuable starting point for further improvements in microplastic detection methods.

The second stage of the dissertation involved a five-day microcosm experiment using Baltic Sea water to assess microbial responses to different concentrations of polystyrene particles approximately 1.1 μm in size. A control treatment containing glass particles was also included. The analyses covered bacterial and heterotrophic nanoflagellate abundances, microbial community respiration, and changes in community composition based on 16S and 18S rRNA gene sequence analyses. I consider the observed reduction in heterotrophic nanoflagellate growth with increasing particle concentrations to be an important result of this part of the study. The similar direction of change in the polystyrene and glass treatments suggests that the observed effect may have resulted, to a considerable extent, from the physical presence of particles rather than exclusively from the polymer's specific chemical properties. At the same time, bacterial abundance and respiration rates did not show significant changes in response to the experimental treatments, while microbial community composition changed primarily over time. These findings indicate that the response of a microbial food web to the presence of microplastics need not be uniform. It may depend on the group of organisms examined and the parameter measured, while natural microbial community succession may play a more important role than the type of polymer used. A strength of this part of the study is the inclusion of a glass-particle control, which enables a more critical assessment of the mechanisms responsible for the observed responses. From a practical perspective, the findings may be useful in designing further studies on the effects of microplastics on aquatic ecosystems. They highlight the need to consider not only the chemical properties of plastics but also the physical characteristics of particles and the natural biological variability of the systems under investigation. They may also provide a basis for formulating hypotheses about the sensitivity of particular microbial groups to particle presence. It should be borne in mind, however, that a five-day experiment primarily allows short-term responses to be assessed and does not establish the long-term ecological consequences.

In the third stage, the Author compared the effects of polystyrene and polyethylene, again including a glass-particle control, and subsequently conducted metagenomic analyses. A total of 118 medium- or high-quality metagenome-assembled genomes were obtained, many of which could not be assigned to previously described species. This finding indicates considerable diversity among the microorganisms studied and reflects the incomplete state of knowledge concerning their taxonomic representation. As in the preceding stage, the overall structure of the microbial communities changed primarily over time, while the effect of polymer type on the composition of the community as a whole was limited. At the same time, several low-abundance genomes assigned to the families Spirosomaceae and Shewanellaceae were enriched in the polyethylene treatment and contained

homologs of genes potentially involved in the processing of synthetic polymers or related compounds. I consider this an interesting finding that identifies a promising direction for further research into the potential functional responses of microorganisms to the presence of plastics. Nevertheless, it requires cautious interpretation. The detection of homologs of particular genes does not constitute direct evidence that these microorganisms actually degrade polyethylene. Confirmation of such activity would require additional investigations. A strength of the research is that it identifies potential candidates for such investigations rather than claiming to have demonstrated the biodegradation process itself. The practical significance of this part of the work, therefore, lies primarily in providing data that may help in selecting microorganisms and genes for further research into biological interactions with plastics. The results may serve as a basis for designing experiments to verify whether the observed enrichment of specific populations in the presence of polyethylene is attributable to actual metabolic activity. At present, however, they do not support conclusions about the direct use of the identified microorganisms for plastic biodegradation or the technological readiness of such applications.

In assessing the dissertation as a whole, it should be emphasized that the Author has successfully integrated methodological, experimental, and molecular approaches to address a common research problem. A major strength of the work is the demonstration that short-term exposure to microplastic particles may selectively affect the functioning of specific components of the microbial food web, whereas temporal changes appear to play a greater role in shaping the overall structure of microbial communities. The study also highlights the importance of accounting for the physical presence of particles when interpreting the effects of microplastics and designing relevant experiments. In my opinion, the dissertation constitutes an original contribution to solving a scientific problem. Its scientific value lies in the multifaceted investigation of microplastic effects on pelagic microbial food webs, combining the optimization of a method for detecting small plastic particles with experimental analyses of microbial responses and metagenomic characterization. By distinguishing effects associated with the physical presence of particles from those potentially attributable to polymer-specific properties, the dissertation provides a more nuanced understanding of microbial responses to microplastic exposure. Its findings also identify directions for further research into the functional potential of the microorganisms studied. Importantly, the Author critically interprets the results without drawing premature conclusions about the ability of the identified microorganisms to biodegrade plastics. The practical value of the results should be assessed primarily in methodological and scientific terms. The development of a procedure for detecting small plastic

particles may advance analytical methodology. At the same time, the ecological experiments may inform the design of future studies investigating the effects of microplastics on aquatic environments. The metagenomic data provide a basis for further investigation of microbial populations and their potential functional capabilities. However, the findings do not establish a direct technological application or provide a validated method for polymer biodegradation. This distinction does not diminish the value of the dissertation but rather defines the scope of the conclusions justified by the research.

The content of the dissertation demonstrates that the Author possesses theoretical knowledge of microplastics, the ecology of aquatic microorganisms, and the application of molecular methods in ecological research. Her ability to plan and conduct multistage research, employ a range of analytical methods, and interpret results while accounting for the limitations of the approaches used demonstrates her preparedness to conduct independent scientific research. The Author also deserves credit for recognizing the complexity of the relationships investigated and the need to distinguish genetic potential from demonstrated biological activity.

My review of the doctoral dissertation has raised several questions and suggestions, presented below, which may serve as a starting point for discussion during the doctoral defense.

1. What are the main limitations of the Nile Red staining method for detecting microplastics in real environmental samples, and what additional tests would be required to confirm its effectiveness across a broader range of polymers and particles of different sizes and shapes?
2. How do the results obtained in the polystyrene and glass treatments help distinguish the effects of the physical presence of particles from those associated with the specific chemical properties of microplastics? What experimental design would make it possible to separate these effects more precisely?
3. What additional experimental evidence would be necessary to confirm that microorganisms whose genomes contain homologs of genes associated with the processing of synthetic polymers or related compounds actually participate in polyethylene biodegradation?

Final conclusion

The doctoral dissertation submitted by Ms. Uroosa has been prepared as a thematically coherent series of publications. It constitutes a valuable and original scientific contribution to research on the effects of microplastics on pelagic microbial food webs. The results provide new knowledge of methodological and scientific significance and may serve as a basis for further research in marine

ecology and environmental microbiology. The number of comments and suggestions I have raised is small and does not affect my overall very good assessment of the dissertation. I hereby conclude that the doctoral dissertation submitted for review by Ms Uroosa meets all the requirements for doctoral dissertations specified in Article 187(1–4) of the Act of 20 July 2018, the Law on Higher Education and Science (Journal of Laws of 2024, item 1571, as amended). I therefore recommend that the Scientific Council of the Institute of Oceanology of the Polish Academy of Sciences admit Ms. Uroosa to the subsequent stages of the proceedings for the award of the doctoral degree.

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