UNIVERSITY OF WARSAW

Faculty of Biology
Institute of Evolutionary Biology

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PhD Thesis Acceptance Report
Field of Earth and Environmental Sciences
Scientific Council of the Institute of Oceanology of the Polish Academy of Sciences

Candidate's name and surname: M.Sc. Uroosa
PhD Thesis Title: Effects of Microplastic Pollution on Structure and Functioning of Pelagic Microbial Food Webs
Thesis Supervisor: dr hab. Katarzyna Piwosz, prof. MIR-PIB
Thesis co-supervisor: dr hab. Józef Wiktor, prof. IO PAN
Reviewer: dr hab. Anna Karnkowska, prof. UW

1. The topic of the dissertation

The dissertation addresses a relevant and timely question in marine microbial ecology: how microplastics in the size range of bacterial prey may affect the structure and functioning of pelagic microbial food webs, with a focus on protists and bacteria. The choice of smaller particles is highly valuable because they overlap in size with bacteria and may therefore be encountered by microbial grazers in ways that differ from the effects of larger microplastics. The Baltic Sea provides a relevant study system, and the combination of methodological work with experiments on natural microbial communities gives the thesis a clear overall rationale.

The thesis comprises three complementary studies: (1) optimisation of a Nile Red-based approach for detecting microplastics at or below 2 µm (published paper); (2) an experiment testing concentration-dependent effects of polystyrene particles, with glass spheres as a control (manuscript); and (3) a further experiment comparing polystyrene, polyethylene and glass spheres using community-level and metagenomic approaches (manuscript). Together, these studies address both the analytical challenge of detecting small particles and the ecological question of whether their presence affects microbial communities.

The Nile Red protocol presented in Chapter I is a relatively accessible approach for screening very small particles, with potential utility where more expensive spectroscopic methods are

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not readily available. The main findings of the experimental chapters (II and III) are that the presence of bacteria-sized particles can affect the heterotrophic nanoflagellate (HNF) community; however, changes in the bacterial and eukaryotic communities are generally limited or are strongly shaped by incubation time and experimental context. Similar responses to plastic and glass particles support a role for physical interference rather than a polymer-specific effect.

The thesis demonstrates a clear effort to interpret results mechanistically rather than limiting itself to reporting differences among treatments. In particular, the use of glass particles as a physical control provides a useful way to assess whether observed responses are attributable to the presence of suspended particles in general or to properties specific to microplastics.

2. The candidate's knowledge

The dissertation demonstrates familiarity with several relevant areas, including marine plastic pollution, microplastic detection, microbial food-web ecology, bacterial and protistan community analysis, and molecular approaches to community characterisation. The candidate combines fluorescence-based detection with abundance measurements, respiration measurements, amplicon sequencing and, in the third chapter, metagenomic analysis. This breadth is a strength of the work.

The introduction provides a broad context and shows the candidate's knowledge. However, some aspects of the state of the art could be developed more critically. In particular, the discussion of HNF feeding ecology should more clearly explain the prey-size ranges typically ingested, the extent of selective grazing, and whether grazers discriminate among prey types or particle-like objects of similar size. These points are central to interpreting the proposed mechanism of physical interference. The literature on microbial responses to plastics is not entirely consistent; the thesis should distinguish more clearly between effects on bacteria, effects on grazers, and indirect effects mediated through changes in prey communities. The synthesis chapter provides some integration of the findings and demonstrates the candidate's knowledge of the topic, but it remains relatively brief and largely descriptive, with limited in-depth discussion. A more fully developed synthesis could have demonstrated the candidate's understanding of the broader implications of the results more clearly.

3. Independence of the candidate

The thesis presents a substantial body of experimental and analytical work across three connected studies. This includes the development and optimisation of a detection protocol, the design and execution of experiments with natural microbial communities, measurements of microbial abundance and respiration, and molecular analyses of bacterial and eukaryotic communities. The combination of methodological development and ecological experimentation demonstrates an ability to engage with different stages of a research project. It is highly unlikely that all experiments were performed by one person, which is

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understandable given the intensity of the experimental work. However, as there are no clearly stated roles in Chapters II and III, it is difficult to characterise the candidate's independence precisely. I would therefore appreciate a comment on the division of tasks in those Chapters.

4. Originality of the dissertation

The dissertation's originality lies in its focus on small microplastics that correspond in size to bacterial prey, and in linking a practical detection method with experimental tests of their potential ecological effects.

The experiments comparing plastic particles with glass spheres are a valuable feature of the thesis. They address a mechanistic question that is often difficult to resolve: whether responses are caused by polymer-specific properties or by the physical presence of numerous non-nutritive particles within a size range relevant to bacterial prey. The results support a cautious interpretation: some responses, particularly those involving HNFs, are consistent with physical interference, whereas the broader community response is limited and context dependent.

The thesis is also original in its experimental design, which uses natural communities and tracks them over time, whereas most studies measure the responses of particular species. The effects are likely less visible at the level of the whole community, but they more closely reflect the natural environment and are more informative for understanding how marine environments respond to microplastic pollution.

5. Comments and questions

1. How confident are you that the Nile Red protocol specifically detects microplastics in environmental samples? Only one cell type was tested as a control. What additional controls would you prioritise, particularly for other organic particles and preserved biological material?
2. Figure 3 in Chapter 1 is difficult to interpret because the standard deviations are very large and extend into negative values, which is impossible for the measured quantities. Can you suggest a better way to present these results?
3. How much of the community change might be related to changes in the light regime? How much does dark incubation itself shape the community trajectory, and could it confound the HNF responses attributed to particles?
4. Why was the V6–V8 region of the 18S rRNA gene selected for eukaryotic community analysis? It seems that V4 and V9 are more commonly used.
5. Some taxonomic inconsistencies are present in the text. Pelagodinium is a dinoflagellate; Urotricha is typically a relatively large ciliate, often exceeding 20 µm, and some other reported protists are also larger than 5 µm. How do you explain the sizes of the observed organisms relative to their expected sizes given the filtration step?

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6. Chapter II established a clear concentration-dependent effect. Why did Chapter III use only a single concentration? Could the weaker effects observed there reflect a sub-threshold dose?
7. How do you explain that glass spheres suppressed HNF growth comparably to polystyrene in Chapter II, but behaved much more like the control in Chapter III?
8. In Chapter II, bacterial growth rates increased in most treatments during Phase 2, but not in Glass-Low and PS-High. What is the proposed explanation for this pattern?
9. The experimental treatments were designed with specific sphere-to-bacteria ratios calculated from bacterial abundance at T0. However, bacterial abundance fluctuated considerably during the experiment. Were the actual ratios recalculated at each sampling point?
10. How do you explain the mid-experiment increase in bacterial numbers observed specifically in PS and PE treatments in Chapter III, which has no clear counterpart in Chapter II?

Although I would appreciate hearing the Candidate's responses to the questions raised above, they do not diminish the value of the work. I hereby state that the evaluated doctoral thesis satisfies the requirements for doctoral dissertations set out in Article 187 of the Act of 20 July 2018 on Higher Education and Science, consolidated text: Journal of Laws of 2021, item 478, on academic degrees and scientific titles, as well as degrees and titles in the arts. The doctoral thesis presents a substantial set of new data and their interpretation, which constitutes an original solution to the research problem. The presented thesis provides the basis for a positive evaluation of the Candidate's general knowledge in the field of Earth and Environmental Sciences and her ability to conduct independent research. Accordingly, I apply to the Scientific Council of the Institute of Oceanology of the Polish Academy of Sciences to approve the dissertation and admit M.Sc. Uroosa to the following stages of the doctoral process.

Sincerely,

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