Åbo Akademi University

Prof. Dr. Magdalena Bełdowska
Scientific Council for Earth and Environmental Sciences
Faculty of Oceanography and Geography
University of Gdańsk

Review of the PhD thesis of Ms. Zofia Maria KONARZEWSKA

As a reviewer appointed by the Scientific Council for Earth and Environmental Sciences, University of Gdańsk, I have reviewed the doctoral dissertation entitled **Allelopathy in different phenotypes of picoplanktonic cyanobacteria *Synechococcus* sp.: A key feature determining the structure of phytoplakton communities in the Baltic Sea** by Ms. Zofia Maria Konarzewska. The scientific field of the thesis is marine biology, more specifically phytoplankton physiology and ecology. It has been supervised by Dr. Aldo Barreiro Felpeo (University of Porto) and Dr. Sylwia Śliwińska-Wilczewska (University of Gdańsk). I would like to present the following statement about Ms. Konarzewska's PhD thesis:

1. Organization of the PhD thesis

The structure of the doctoral thesis follows the usual practice with the following sections: Cover page, Table of Contents, Graphical Abstract, Abstracts in English and Polish, Lists of Primary Publications by the Candidate, Justification for Scientific Research, Hypotheses and Aims, Methodology and Research Design, Reprints/manuscripts of five scientific works constituting the thesis, Verification of Tested hypotheses, Conclusions, Funding Information, Acknowledgments and References. The length of the dissertation is 200 pages including the page count of the five primary publications.

The Justification for Scientific Research ("Introduction") section is compact, three pages giving a fairly short review of the picocyanobacterial genus *Synechococcus*: its habitat, phenotypes, physiology and ecology. Later, allelopathic phenomena and experiments related to them are introduced. The section covers well enough the later scientific literature in the field and mentions a number of major contributions to the topic. Most of the mentioned scientific papers focusing on *Synechococcus* originate from the last ten years while the introduction to plankton ecology, allelopathy and experiments relating to these two topics span a longer time period. There are naturally many more references to the relevant papers in the five primary publications constituting the PhD thesis. It is generally felt that the author is familiar with her field of research. The Justification is lacking illustrations and tables but such are used later in the text. The Justification section ends with a statement of the objective of the candidate's research: it is "through a combination of laboratory experiments and ecological modeling to investigate the potential influence of allelopathy from different *Synechococcus* phenotypes as a driver of phytoplankton community structure."

Three Research Hypotheses are set and they comprise the following:

1. Abiotic factors promoting the growth of *Synechococcus* enhance the allelopathic activity.
2. Allelopathy exhibited by different *Synechococcus* phenotypes affect co-occurring phytoplankton species and the effects vary depending on the *Synechococcus* phenotype and target species.
3. Allelopathy by *Synechococcus* has an influence on the diversity of the plankton community.

Four general steps to test the hypotheses are presented after the hypotheses.

The Methodology and Research Design section contains six pages. This section gives an overview of the involved methodology including the studied organisms and their culture conditions, some chemical and pigment analyses, short-term allelopathy assays, determination of cell abundances, a model of interspecific competition, long-term competition experiments and a few statistical analyses employed. The full details are appropriately given in the primary publications. The methods are presented with references to own and others' work. The used methods require intermediate-advanced skills in e.g. general algology, plankton physiology and plankton ecology as well as in chemical/optical laboratory analyses and statistical treatment of the results. The candidate gives evidence of mastering the methods by providing relevant information in a logical manner.

The Results and Discussion section which is called Verifications of Tested Hypotheses in the dissertation comprises 15 pages and has been divided into chapters each dealing with one of the three hypotheses. These chapters offer both results and discussion and suggest some (ecological) implications. The overall discussion and comparison with the relevant literature is rather thin and cannot be fully explained by the lack of similar work on *Synechococcus* from other authors. Discussion about future research directions and next research steps to be taken is minimal.

The thesis further contains Conclusions which are summarized into four statements of rather general nature (see Section 3 in this report). The Reference section lists 94 references.

2. Evaluation of the individual papers included in the thesis

The work underlying the PhD thesis has been presented in three published/accepted papers and in two papers which are either submitted or ready to be submitted. The published/accepted papers have been printed in international journals of good standing, high visibility, and appropriate scope/readership:

1. Konarzewska, Z., Śliwińska-Wilczewska, S., Barreiro Felpeto, A., Latała, A., 2022. Effects of light intensity, temperature, and salinity in allelopathic interactions between coexisting *Synechococcus* sp. phenotypes. Marine Environmental Research, 179, 105671. IF = 3.0; 100 MNiSW. STATUS: PUBLISHED

2. Konarzewska, Z., Śliwińska-Wilczewska, S., Barreiro Felpeto, A., Latała, A., 2025. Impacts of Nutrient Dynamics on Three Picocyanobacterial Populations: Laboratory Experiments and Seasonal Surveys in the Baltic Sea Region. Estuarine, Coastal and Shelf Science. IF = 2.6; 100 MNiSW. STATUS: ACCEPTED AFTER PhD THESIS SUBMISSION, INFORMATION FOUND ON THE JOURNAL WEB PAGE

3. Konarzewska, Z., Śliwińska-Wilczewska, S., Barreiro Felpeto, A., Vasconcelos, V., Latała, A., 2020. Assessment of the allelochemical activity and biochemical profile of different phenotypes of picocyanobacteria from the genus *Synechococcus*. Marine Drugs, 18(4), 179. IF = 4.9; 100 MNiSW. STATUS: PUBLISHED

4. Konarzewska, Z., Barreiro Felpeto, A., Śliwińska-Wilczewska, S., Vasconcelos, V., Latała, A., 2025. Take this Waltz: allelopathy induces oscillatory coexistence in marine phytoplankton. Aimed at: The American Naturalist. IF = 2.4; 100 MNiSW. STATUS: UNKNOWN TO THE REVIEWER

5. Konarzewska, Z., Barreiro Felpeto, A., Śliwińska-Wilczewska, S., Morais, J., Vasconcelos, V., Latała, A., 2025. Allelopathy of *Synechococcus* sp. Phenotypes influences the structure of coexisting phytoplankton communities driven to equilibrium. Marine Ecology Progress Series. IF = 2.2; 70 MNiSW. STATUS: SUBMITTED

A rule sometimes applied in Finland for the original papers in a PhD thesis is that the sum of the impact factors of the papers should exceed 10. In Ms. Konarzewska's case the sum of the impact factors of the published/accepted papers exceeds this.

While there are four to six authors in the publications (which is not unusual in life sciences) the contribution of Ms. Konarzewska appears central in the papers as evidenced by first author positions in all the included papers. The signed statements of co-authorship estimate Ms. Konarzewska's contribution to 50-70% of the work in the papers. Her contributions cover central parts of the scientific investigation and have been detailed in the co-authorship statements and the primary papers. Ms. Konarzewska's role relates to (Paper 1, 70%) conceptualization, investigation and writing; (Paper 2, 60%) conceptualization, investigation, methodology, data curation, formal analysis and writing; (Paper 3, 60%) conceptualization, data curation, formal analysis, investigation, methodology and writing; and (Paper 4, 50%) conceptualization, investigation, methodology, data curation, formal analysis and writing; and (Paper 5, 50%) conceptualization, investigation, methodology, data curation, formal analysis and writing. It is evident that the quantitative criteria set for a doctoral candidate are satisfied by the scientific project, the original papers and the doctoral dissertation of Ms. Konarzewska. It is unknown to the reviewer how much Ms. Konarzewska has been involved in the funding acquisition but she lists herself (p. 193) as project manager for three University of Gdańsk (UG)-funded projects and principal/co-principal investigator for two UG-funded projects. However, this is a secondary fact not relevant for the evalution of the thesis.

I have no major reservations towards the reported scientific contents of the five individual papers, partially published/accepted in peer-reviewed journals, partially still in submitted/preparation stage. The methodology has been described in adequate details, there are appropriate statistical analyses, the supplementary materials are rich in details and the conclusions drawn are generally justified. Three of the included papers have been peer-reviewed and their details are to be regarded as duly checked by the referees and editors.

I consider Papers 1 and 3 as the core contents of the PhD thesis while Papers 2 and 5 are important in introducing some field observations and natural phytoplankton community to the research. Paper 4 complements the experimental work by modelling some laboratory observations. The main findings of the original papers are following:

Paper 1 and Paper 2 relating to Hypothesis 1: Abiotic factors that enhance the growth of *Synechococcus,* especially higher temperature and higher light intensity (but less consistently also good nutrient availability and optimal salinity) increased the allelopathic potential of *Synechococcus* phenotypes

Paper 3 relating to Hypothesis 2: Type 3a *Synechococcus* (brown strain) had the strongest inhibitory effect on the co-occurring phytoplankton, especially on diatoms and green algae. Some first information on the possibly involved allelopathic phytochemicals is given in the paper.

Papers 4 and 5 relating to Hypothesis 3: Allelopathic influence of *Synechococcus* has an effect on the dynamics and structure of experimental phytoplankton communities and is thought to influence the Baltic Sea phytoplankton communities in a similar way

3. Coherence of the individual papers towards verifying the hypotheses and conclusions reached

The individual papers are connected and support each other in solving the bigger research question as detailed in the hypotheses. The doctoral dissertation forms a logical summary of the reported research.

The candidate's research has made the following conclusions possible:

a) Abiotic factors that promote growth or align with the optimal niche conditions of *Synechococcus* sp. phenotypes enhance their allelopathic activity. REVIEWER COMMENT: This is interesting as the growth-promoting conditions themselves favour *Synechococcus* and logically there is thus less need to exert "chemical warfare" against other species. However, those conditions which favour *Synechococcus* are also bound to favour many other phytoplankton species and there is competition between the species.

b) The allelopathic effect varies depending on the *Synechococcus* phenotype and the specific phytoplankton species targeted. REVIEWER COMMENT: A main result is that *Synechococcus* is widely allelopathic. It is logical that the target species differ in their response to allelopathic effects by *Synechococcus* but the fact that there are differences between *Synechococcus* phenotypes requires further investigation. Possibly there are also strain-specific differences within the *Synechococcus* phenotypes. It remains to be investigated whether the metabolites involved in *Synechococcus* allelopathy are primary or secondary metabolites and what their structure is. Contents of the cyanotoxin microcystin in *Synechococcus* was tested in Paper 2 but its involvement in the allelopathy is not claimed by the author. Other phytochemicals were identified but not quantified in Paper 3.

c) Allelopathy, as an isolated factor, has the potential to facilitate and stabilize phytoplankton coexistence. REVIEWER COMMENT: It is in the very nature of allelopathy to modify the structure of the co-occurring organism communities. As the effects of can be either beneficial or detrimental some target organisms will suffer, some will be favoured.

d) The functional relationship between allelopathy and phytoplankton community diversity exhibits a peak at intermediate levels of allelopathic activity. REVIEWER COMMENT: This is quite logical as allelopathic chemicals can either stimulate or suppress other organisms.

It can be concluded that the main aims related to the PhD project have been satisfactorily met.

4. Some critical observations and points to address in future research

One aspect that is always relevant when working with microorganisms is whether the employed laboratory cultures, this time originating from the Culture Collection of Baltic Algae (CCBA) at the University of Gdańsk, are axenic or not. According to information on the web page of CCBA (https://ccba.ug.edu.pl/pages/en/home.php) "the cultures are unialgal, mostly non-axenic." This fact leaves some room for questions about the origin of the allelopathic properties in *Synechococcus*.

In my opinion, the latter part (underlined below) of the title of the PhD thesis (Allelopathy in different phenotypes of picoplanktonic cyanobacteria *Synechococcus* sp.: A key feature determining the structure of phytoplakton communities in the Baltic Sea) is speculative and an over-statement. Whether or not the

allelopathy exhibited by *Synechococcus* really is a key feature shaping the phytoplankton communities in the Baltic has not been shown by the reported data in a convincing manner even there is some evidence pointing to that. Most of the work is based on laboratory experiments with a limited set of target organisms. The field survey reported in Paper 2 covers only a small semi-coastal area of the Baltic Sea and further relates to findings in one year only. Also, there are probably many other allelopathic actors further shaping the phytoplankton communities. I believe that the Polish title (... *mechanizm decydujący o strukturze fitoplanktonu w Morzu Bałtyckim*) is more appropriate.

It can also be asked whether there are strain/phenotype-specific differences in the target phytoplankton when it comes to the allelopathic pressure caused by *Synechococcus*. In the PhD thesis each target organism within Cyanophyta, Chlorophyta and Heterokontophyta is presented by a single strain taken from CCBA (with the exception of *Synechococcus* and the natural phytoplankton community in Paper 5). I understand, however, that it would be a never-ending Sisyphos task to test all possible species and strain combinations with regard to the allelopathic interactions. The work simply has to be started with a more limited set of organisms and the amount of work done by the candidate is enough for a dissertation.

Further, there is the question of the structure and concentrations of phytochemicals responsible for the allelopathic actions of *Synechococcus*. Papers 2 and 3 started the work to identify these substances but no conclusive results were reached. The authors suggested the involvement of compounds with either antimicrobial or cell-damaging properties. This type of work may be partially beyond the scope of the expertise of the candidate but it needs attention in the future and is also a possible topic for a new project.

5. Recommendation

As detailed above I have examined the doctoral dissertation by Ms. Zofia Maria Konarzewska. I conclude that the scientific quality of the doctoral dissertation fulfills internationally accepted academic standards and the criteria established by the Faculty of Oceanography and Geography, University of Gdańsk. The published/accepted original papers have been disseminated in medium-ranked peer-reviewed journals showing the international acceptance of Ms. Konarzewska's research. **I respectfully propose the acceptance of the work of Ms. Zofia Maria Konarzewska as PhD thesis.**

Ja, niżej podpisany stwierdzam, że recenzowana rozprawa doktorska mgr ***Zofii Konarzewskiej*** *spełnia warunki określone w art. 190 ust. 2 i 3 Ustawy z 20 lipca 2018 roku Prawo o szkolnictwie wyższym i nauce, w zw. z § 26 Załącznika do uchwały Senatu UG nr 121/19 z 27 maja 2021 roku, ze zm. i wnioskuję do Rady Dyscypliny Nauki o Ziemi i Środowisku Uniwersytetu Gdańskiego o dopuszczenie mgr Zofii Konarzewskiej do dalszych etapów postępowania doktorskiego.*

Turku, 28 May 2025

Yours sincerely,

Jussi Meriluoto, PhD

Docent in Analytical Biochemistry, Cluster of Biochemistry and Cell Biology, Åbo Akademi University, Turku, Finland
Visiting professor in Hydrobiology, Department of Biology and Ecology, University of Novi Sad, Serbia
tel. +358-45-7871 6120, email jussi.meriluoto@abo.fi