



Kilchberg, 3 May 2018

Evaluation report on the PhD thesis of Mgr. Vesna Grujić

Thesis title: Differential freshwater flagellate community response to bacterial prey with a focus on planktonic *Betaproteobacteria*

Topic of the study

Mgr. Vesna Grujić presents the fascinating world of microbial predator-prey interactions, displayed by freshwater bacteria and flagellated protists. It is the big strength of the candidate to focus on more than one aspect of this interaction. Mgr. Vesna Grujić regarded the taxonomic affiliation of both, prey and predators, and she presented in detail phenological aspects of the microbial community. These results were analyzed in the framework of environmental parameters and the question how these microbial interactions may impact carbon and nutrient flows in freshwater systems. As stated by Mgr. Vesna Grujić, we are now indeed on the way to open the 'black-box' of the functional guild 'heterotrophic nanoflagellates'.

Organization of the thesis

The thesis (112 pages), written in English, is organized in an introduction, four short general chapters (presenting the hypotheses, main results, conclusions of the work, references), and the main chapter consisting of three published manuscripts.

Chapter 1: Introduction

This well-structured chapter gives a fine overview of relevant literature on the topic and on the methodological workflow of the studies. It is good to see that the young candidate had also a look in older literature and conceptual ideas which led to current theses and knowledge.

The introduction gives a nice overview on two major bacterial genera in freshwater systems, which also served as selected prey items in the experiments. My only point of criticism concerns not the content but the depth of paragraph 1.3. ('Black-box' of HNF). The candidate mentions briefly possible taxonomic affiliations of flagellates within the functional term 'heterotrophic nanoflagellates'. For a protistologist, it is clear that flagellated protists are found within all supergroups of the polyphyletic group 'unicellular eukaryotes = protists'. However, some microbial ecologists might not be aware of the 'modern' phylogeny of protists (this fact is even reflected in some new textbooks wherein HNF are still treated as a kind of taxonomic entity). Here, the candidate could have shown some up to date overview on flagellated protists, e.g., the graphical summary (Fig. 1) in Jeuck & Arndt (2013). Probably, this aspect could be presented by the candidate in the defense of the PhD thesis.

Jeuck A, Arndt H (2013) A Short Guide to Common Heterotrophic Flagellates of Freshwater Habitats Based on the Morphology of Living Organisms. *Protist* 164:842-860



Chapters 2-5

These chapters summarize the most important hypotheses, results and points of discussion in a very condensed way. Mgr. Vesna Grujić presents an excellent combination of various techniques to get a holistic picture of the studied microbial interactions.

Chapter 6. Attached publications

Publication #1: This study was published in *Applied Environmental Microbiology* with Mgr. Vesna Grujić as first author.

This study deals with aspects of prey quality for developments of flagellated predators. The paper is based on an elegant design by offering three morphologically different *Limnohabitans* strains to natural flagellate communities. It is a very detailed study based on real organisms and real habitats – this contrasts many studies which are mainly based on lab experiments. The authors present major growth characteristics (GGE, doubling time, lag-phase) of flagellates as reaction on prey types. Interestingly, these effects were also influenced by the time-point of investigation (season) and trophic status of the studied lakes. In sum, this manuscript gave already good indications that the entity 'heterotrophic nanoflagellates' harbors a great diversity of flagellated protists which differently react on offered prey sources.

Publication #2: This study was published in *Limnology and Oceanography* with Mgr. Vesna Grujić as co-author (second place in the list of co-authors).

This publication will soon develop to a standard article for all microbial ecologists working on interactions between bacteria and flagellated protists. It is impressive that the authors always used ecologically relevant bacterial isolates for their experiments. This workflow currently reflects the highest standard for the field of research. It is fascinating that phylogenetically very close bacterial isolates may affect growth dynamics of flagellates in very different ways. Second, it is important to see, that Actinobacteria (Luna 2 cluster) are indeed rather inadequate food items for most flagellates. Finally, Figure 6 is an ideal summary to be presented in lectures for students and in relevant text books.

Publication #3: This study is published in *The ISME Journal* with Mgr. Vesna Grujić as first author.

This publication gives the workflow how we will open the 'black-box' of HNF in future and it is shown that we may discover numerous still unknown microbial interactions. It is the big strength of the manuscript to combine 'classical' morphotype based methods with molecular techniques. However, the authors give important caveats for the interpretation of mass sequencing data. High throughput sequencing (HTS) is the tool to elucidate the diversity in freshwater systems. Nevertheless, it is definitely an insufficient tool for the quantification of any protistan group in lakes. Thus, single cell methods (e.g. FISH) are needed in combination with HTS techniques for a detailed analysis of flagellated protists. This workflow is presented in the current manuscript in an ideal way for some groups (cryptophytes, Katablepharids).

Hopefully, we will have these tools (e.g. specific FISH probes) for various abundant and frequent flagellates (e.g., chrysophytes, choanoflagellates, etc) in near future.



Synthesis of my evaluation

The thesis presents a real consistent work on interactions between major freshwater bacterial representatives and their protistan flagellated predators. The three published manuscripts form a nice follow-up series of increasing knowledge on these interactions. All manuscripts are published in journals of high reputation and, with the last manuscript in the ISME Journal, Mgr. Vesna Grujčić even published in the top journal of the field.

The applied methodological approaches are definitely reflecting the future workflow for studying flagellate grazing on bacteria. As Mgr. Vesna Grujčić could clearly show in her work, there is need to apply specific FISH probes for an in-depth analysis of freshwater flagellates. And the combination with fluorescently labeled bacteria or FISH of ingested bacteria indeed allows for detailed ecological studies. Hopefully, the candidate will continue her work on this topic in future and further open the 'black-box' of 'heterotrophic nanoflagellates'.

The candidate has already a very broad knowledge on several aspects in the field of aquatic microbial ecology. It is obvious that she carefully looked for relevant literature – from the past to the present. This results in an overall and wide view on topics and allows the candidate to link phylogeny with ecology. Finally, Mgr. Vesna Grujčić gave already six presentations of her work at international congresses which is very ambitious for a PhD candidate. It was a real pleasure to read this thesis.

I recommend that the PhD thesis by Mgr. Vesna Grujčić should be **fully accepted in the present form**.

Yours sincerely,

A handwritten signature in black ink, reading 'Dr. Thomas Posch'.

Prof. Dr. Thomas Posch
University of Zurich, Limnological Station

season different from Simek et al. (2013). Is this the only reason for the difference between Simek et al. (2013) and her study?

- Page 95 to 96: In Table 3, grazing pressure by CRY1 clade is low. Does she think CRY1 clade would be important bacterivores? We also examined grazing by CRY1 in Lake Biwa, and we could not find any coupled oscillation between CRY1 abundance and high HNF bacterivory. This suggests CRY1 grazing is also of less importance in HNF bacterivory of Lake Biwa.
- The approach she has been taking is similar to the one of Simek et al. (2013). Through her dissertation study, she has found the tight linkages between bacterial community shifts and succeeding HNF community shifts, which optimize prey utilization and matter cycling from various bacteria to the microbial food chain. So, what is the next step for her? Is she interested in another food linkage(s) within microbial food webs? Does she have a new approach to study it?

3 May 2018

中野伸一

Shin-ichi Nakano

Professor of Center for Ecological Research, Kyoto University

Evaluation on dissertation study by Ms. Vesna Grujčić

The present dissertation study by Ms. Vesna Grujčić has examined which protistan groups would be important bacterivores on some dominant bacterial species in the freshwater systems where she has been studying. She determined the growth potential of natural HNF communities and an isolated flagellate fed on a broad spectrum of relevant planktonic bacterial groups. For natural HNF communities, their responses to particular prey over different plankton successional phases have so far not been demonstrated. To have a law as general as possible, using many experimental systems, she has taken the unique approach where the authors incubated natural/isolated flagellates on bacterial strains in water samples collected from the lakes during different phytoplankton succession phases. Her and colleagues' approach where addition of bacterial strains originated from the same systems as the bacterivores is important, and not many studies are available so far. In addition, she used a novel method to confirm the presence of the prey bacteria in HNF food vacuoles. Finally, her finding that Cryptophyta protists would be the most important consumers of bacteria is very important for planktonic ecology in freshwater systems, because Cryptophyta is one of dominant protists throughout a year in many freshwaters.

Considering the above, I am enthusiastically supporting her dissertation study and agreeing with her receiving doctoral degree.

I am also providing some specific comments on her dissertation below:

- Page 11, second para: how does Ms. Grujčić think about C:N:P ratio of bacterial prey as food for HNFs? When handling, HNFs cannot tell which bacterial species they are capturing, and this is also the case for C:N:P ratio of bacterial prey.
- Page 16, line 10: nutritive quality: Again C:N:P?
- Simek et al. (2013), ISME-J: Even if in Simek et al. (2013), what “food quality” means is not explained.

Those three questions/comments are closely relating each other.

- Page 20, second para: “katablepharys would prey on small HNF?”, Ms. Grujčić needs to give more explanation or data, if circumstantial.
- Page 94, bottom: In Simek et al. (2013), those authors did not detect any cryptomonads (Fig. 5). Her explanation for this is that she collected samples in the