Review of the Ph.D. thesis

Horňák, K., 2006:

Diversity and functioning of bacterioplankton assemblages in the Římov reservoir.

(In English). Faculty of Biological Sciences, University of South Bohemia, České Budějovice, Czech Republic: 62 pp.

The thesis consists of three main chapters:

- **Introduction**, summarising the methodology and describing general background of the authors experiments and conclusions, including the literature.
- **Aims and hypotheses** of the authors research, formulated as:
  1. How selected factors of bottom–up and top–down constrains regulate the phylogenetic structure as well as morphology and biomass of bacterioplankton assemblage in the Římov reservoir?
  2. Are the changes in the phylogenetic composition induced by bottom–up and/or top–down factors correspondingly reflected by changes in metabolic activity of bacterioplankton?
- **Results**, presented as three team publications (one of them is a "submitted" manuscript). In two of them K. Hornak is presented as the first author, in one as a second author:
  1. Šimek, K., Horňák, K., Mašín, M., Christaki, U., Nedoma, J., Wienbauer, M. G.,Dolan, J. R. (2003): Comparing the effects of resource enrichment and grazing on a bacterioplankton community of a meso–eutrophic reservoir. Aquat. Microb. Ecol. 31: 123–135.
  2. Horňák, K., Mašín, M., Jezbera, J., Bettarel, Y., Nedoma, J., Sime–Ngando, T., Šimek, K. (2005): Effects of decreased resource availability, protozoan grazing and viral impact on a structure of bacterioplankton assemblage in a canyon-shaped reservoir. FEMS Microbiol. Ecol. 52: 315-327.
  3. Horňák, K., Jezbera, J., Nedoma, J., Gasol, J. M., Šimek, K. Bacterial leucine incorporation under different levels of resource availability and bacterivory in a freshwater reservoir. (Submitted to FEMS Microbiology Ecology).

Short **Conclusions** close the booklet.

Review:

There should be a problem for a postdoc student at the Hydrobiological Institute and Faculty of Biology of the South Bohemia University to present a thesis worse than "good", nevertheless that case should be evaluated as excellent.

The results are based on a series of exposition/"transplantation" experiments, modelling conditions for solving the relative importance of true "lake" plankton processes (based on "lake" nutrients an primary production) and processes influenced by supply of river material at the transient zone when the river merges the "lake" - the Římov reservoir. It is to be noted that typical Czech canyon- shape reservoirs, which are studied by the team for decades, are an excellent field for this type of studies, having a significant gradient along the longitudinal profile (also a vertical one). The gradient of decreasing "river" TOC and nutrients supply to true lake conditions enables the study of top-down and bottom-up control of both structural and functional characteristics of bacterial assemblages.

There is no reason to repeat results and conclusions here, it was or is a job for editorial boards of very good journals, which accepted the papers.

In is important to see a development of methodology during the time of research, e.g. change to more sensitive CARD-FISH in the Paper 3, change in system of expositions of the dialysis bags, including a parallel with phosphorus addition (and. parallel exposition in bottles), and of course new functional characteristics measurement.

I feel there is an item, out of the aim of the entire work, which is very important or inspiring to people dealing with aquatic bacterial assemblages "species" composition: The FISH (CARD-FISH) methodology used alone, as based on phylogenetic signs/markers, does not give an insight into the structure of an assemblage or community of aquatic bacteria, which are heterotrophic (chemoorganotrophic) in a common lake plankton. First step to the proper use and interpretation of FISH techniques results is to use them to indicate changes during the incubation/change of conditions. Second step is to combine FISH techniques with physiological methods. A nice example in the Paper 3 is the clear demonstration that only one component of generally used β-Proteobacteria (BET) group, the cluster (R-BT) is responsible for metabolic and growth responses of BET as an unit. I think the methodological background of the thesis, or that of the mother laboratory, was exploited in a most effective way. Also the interpretation of results and conclusions correspond with the results measured and calculated.

Conclusions on prevalence of bottom-up control in nutrient/substrates rich conditions (supply) and that of top-down control in low nutrient/substrates supply are valuable and very well documented.

Questions:

There remains an open question on the exchange of water, solutes etc. between the microcosmos and ambient water mass. I think, some estimation of rate of exchange of some types of materials/substances in question should be made, as

1. the description of suspension of dyalisis bags in the reservoir does not seem to provide sufficient movement of water on both sides of the bag wall/membrane., and

2. there should be a doubt which substances are transported/exchanged between bag and water and which ones are produced by processes inside the bag, though influenced by the ambient water.

Conclusions:

I fully recommend the thesis of Karel Horňák to be accepted as a basis for the academic degree of Philosophiae doctor (Ph.D.).

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Review

of the Ph.D. thesis by Karel Horňák: Diversity and functioning of bacterioplankton assemblages in the Římov reservoir. University of South Bohemia, Faculty of Biological Sciences, 62 pp.

Introduction

Submitted Ph.D. thesis focuses on the changes in bacterioplankton community composition (BCC) and metabolic activity under different nutrient levels and both protist grazing and viral lysis. The work is a part of long-term research focused on changes in bacterial community composition along longitudinal profile of reservoirs and the study of the factors that may induce these changes. The results of this thesis follow up with previous observations of Prof. Karel Šimek and his co-workers which revealed that bacterivorous HNF and ciliates can exert significant changes in bacterioplankton community. Thus, this study of Karel Horňák brings new findings that have enabled to test new hypotheses and assumptions postulated by Šimek's team during previous research.

Two main questions were highlighted by author: (i) whether a bacterioplankton community composition is affected more by bottom-up or top-down control and (ii) are these changes also followed by changes in metabolic bacterioplankton activity ?

General evaluation of the thesis

The Ph.D. thesis is composed of short introduction chapter, outlines of the main aims and hypotheses, and three scientific papers with general conclusions ending this thesis. Introduction chapter is well written and author here shortly summarized up to date knowledge on factors influencing dynamics and structure of bacterioplankton as well as modern methods used to determine both the bacterial community composition and bacterial metabolic activities. Presented papers revealed several interesting findings that the author highlighted in the final chapter. This chapter well summarizes major conclusions of the author's research and contributes to much better understanding and reading of the text.

The most important results obtained by the author can be shortly summarized as follows: (i) in spite of actual nutrient level it is likely that bottom-up effects were major structuring forces determining bacterial composition; (ii) different phylogenetic groups of bacteria exhibited markedly different growth capabilities and vulnerability to protist grazing.

Results of Karel Horňák's work are original and bring very interesting and important knowledge that extend our information about dynamic, structure and metabolic behaviour of bacterioplankton under different levels of nutrients and grazing pressure. Moreover, only scarce data exist about bacterial succession in the water environment, as well as linking of bacterial community structure and its metabolic diversity. Thus, Karel's findings appear to be

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very inspiring for the future research not only in the ecology of bacterioplankton but also in the study of benthic biofilm communities.

Of three presented papers or manuscripts, two of them were already published in the international reviewed journals with no zero impact factor, one remaining was submitted to an international journal. Karel Horňák is the first author of the 2 papers. Since majority of presented papers were published, it is obvious that those were carefully read by independent reviewers. Thus, I am sure that results presented in this thesis are undoubtedly of great value for other scientists and no other comments or improvements are necessary.

When reading the results of Karel's work I found several interesting points that need to be explained during a defence of this thesis.

1) Generaly, it is suggested that viral lysis can cause significant losses of bacteria in the water ecosystems. Hovewer, in your paper II you have found only negligible effect on bacterioplankton. Do you have any explanation for such discordance?
2) Leucine uptake was chosed as an indicator of bacterial metabolic activity. Did you measure another parameters of the cell activity? For instance, could we expect that there is any correlation between the rate of leucine uptake and extracellular enzyme activity of the bacterioplankton?

Conclusion
The autor has proved his competence in elaborating and experimental testing of the scientific hypotheses and constructive intepretation of results obtained during his Ph.D. study. I can authorize that submitted thesis meets all requirements for absolvents of the Ph.D. study.

Thus, I highly recommend to accept
Karel Horňák's Ph.D. thesis for defence

Olomouc, April 16th 2006

Martin Rulík, Ph.D.

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30 March 2006

Evaluation of the PhD thesis "Diversity and functioning of bacterioplankton assemblages in the Řimov reservoir" by Karel Horňàk

Karel Horňàk has selected a very timely topic. The link between diversity and ecosystem functions is a hot topic in general ecological theory as losses in diversity might threaten ecosystem functions performed by the biota. Microorganisms in pelagic systems are very likely not threatened but global change can also effect the relative composition and thus, function. Therefore, studies are needed, which investigate the control mechanisms of diversity and function in parallel. This has been achieved by the submitted PhD work.

Resources and predators (including viruses) are the main controlling mechanisms and all of them have been investigated during this PhD thesis (some through collaboration). Resources were the main causes shaping the community structure and and overall growth of bacterioplankton. Predators such as flagellates more specific effects on different phylotypes, e.g. by causing shifts in morphology of cells, which was also linked to phylogeny. This study also shows that morphological investigations have some value in the genomics area.

The most innovative study is manuscript III, which used a combination of fluorescence in situ hybridization (FISH) and micro-autoradiography and applied it to relatively narrow taxonomic groups. I am not aware of another study doing that except for large bacterial groups, such as *Beta-* and *Gammaproteobacteria.* This allows for new avenues of research by opening the black box of bacterioplankton at a level previously only known for eukaryotes. In parallel, resource and predator control were studied. Such studies are building blocks for a autoecology (or 'species' ecology) based on field and experimental studies and not only work with isolates. I hope to see in the future a monograph on the R-BT065 phylotypes!

The R-BT065 cluster is a small subset of the Betproteobacteria but represents a significant fraction of the abundance and activity of bacterioplankton. The combined work of this PhD shows that this rather narrow taxonomic cluster is a key group in the Řimov reservoir. Some reasons for that such as a potentially opportunistic life strategy have been elucidated. I am not aware of many environments, where a key bacterial player has been identified and studied in such detail. Karel Horňàk (as part of Prof. Šimek and his team) has contributed significantly to these findings.

This study has also implications for applied science. Knowing a key bacterial player and its reactions to e.g. P additions, is important for understanding what keeps freshwater 'clean' and this a crucial knowledge for a reservoir.

I was a bit astonished to see only one co-author paper in this thesis. There are more published and submitted and a brief synopsis of those would have been nice, since they were part of the PhD work and this should have been mentioned. In addition, I learned that a an additional first author paper is in preparation. It would have been nice to see even a version not intended to be submitted in the presented form. This is quite common in many countries, since it is typical that when a PhD is finished, the publishing part is not. Maybe, this will do done in the oral presentation.

Karel Horňàk is, as his many co-author papers show, involved in many collaborations and presents his findings frequently at international meetings. He has mastered the conceptual plan of his PhD program, excelled in laboratory, experimental and field work and has proven that he is able to put his findings into an international scientific context. Such are the characteristics of a scientist and so I evaluate him worthy of doctoral dignities.

Markus Weinbauer