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## Impacts of oxygen and temperature stress in *Daphnia* on population dynamics

*Background:* Oxygen or temperature changes lead to impediments of cellular oxygen supply in ectotherms. To restore oxygen homeostasis disturbed by oxygen or temperature stress, the respiratory protein haemoglobin (Hb) plays an important role in *Daphnia*, which has been one aspect of our research (Zeis *et al* 2003: Biol Chem 384**:**1133; Zeis *et al* 2003: Comp Biochem Physiol B 134:243; Paul *et al* 2004: Acta Physiol Scand 182:259 (Review); Lamkemeyer *et al* 2005: Biol Chem 386:1087; Lamkemeyer *et al* 2006: FEBS J 273:3393). In an integrative approach, we also investigate the consequences of Hb variability for the animals' physiology, behaviour and their occurrence within the habitat (Seidl *et al* 2005 J Therm Biol 30:532; Lamkemeyer *et al* 2003 Can J Zool 81:237; Zeis *et al* 2004 Can J Zool 82:1605). In a field study (DFG priority program *Aquashift*), we have characterised clones of the *Daphnia galeata/hyalina* complex in terms of thermal physiology and contribution to the genotype composition along the seasonal changes.

*Aims:* To interpret these data within the framework of population genetics, we wanted to co-operate with experts in this field. From discussion with Prof. De Meester's group working on ecological genomics, the spatial and temporal structure of genetic variation and mechanisms leading to the observed community structure, we expected clues for the interpretation of physiological data and support for the optimization of the design of laboratory selection experiments in the context of temperature acclimation.

*Report:* During my visit in Leuven, I had the opportunity to talk to Luc de Meester and several scientists of his working group (Ellen Decaestecker, Wendy van Doorslaer, Kevin Pauwels, Robby Stoks, and Joost Vanoverbeke). To illustrate my background (biochemistry and physiology), I summarized some results and objectives of the research done in Münster in a small presentation. I was introduced to recent results on thermal adaptation, experimental designs and methods used by Luc de Meester and his team. Consulting experts in the field of population genetics, which I am not familiar with, was very useful. In the discussions, I got many helpful answers to open questions, and the visit may mark the start of a more intensive collaboration, given the complementary expertise of the Leuven and Münster research groups. More specifically, we considered the following items:
1. **Population genetic analysis of field populations in relation to temperature**. After studying acclimation processes to temperature and oxygen changes on Daphnia in the laboratory, we only started in 2005 to include field studies to the research program. In the context of this project in a reservoir, we collected data on the seasonal changes of the genotype composition of the lake Saidenbach *Daphnia* population. They are a by-product of the aim to choose clones for

physiological characterization. To evaluate these results from the view of population genetics, some further calculations and evaluations are necessary. Special problems need to be taken into account, since the population consisted of two parental species as well as hybrids and backcrosses. The discussion with Luc de Meester was very helpful, as he provided valuable advice on the data to include for the calculation of genetic distances and suggested details on the calculation of allele frequencies and the test whether multilocus genotype frequencies are in accordance with expectations under Hardy-Weinberg equilibrium.

2. **Modelling population development under different temperature regimes**. Our physiological characterization of clones isolated from Saidenbach revealed differences of individual fitness as a function of temperature. As global warming will lead to warmer winters, ice coverage of the lakes will be shortened and the temperature increase in spring will be sped up. Our data lead to the estimation that it is not the absolute temperature, but rather the rate of temperature change that will act as a selective force on the zooplankton population. For this reason, I would be interested to perform a simulation with a model established in the Leuven group (J. Vanoverbeke & Luc de Meester), to follow the reproductive success of clones with the observed characteristics under different temperature regimes (slow vs. fast warming) and food conditions. There would be a basic interest of the group, but for the species in question (*Daphnia galeata* and *D. hylina*) I will have to determine several key parameters necessary either from own measurements or from the literature, before a simulation can be carried out. A practical cooperation on this item thus needs to await the gathering of these data, for which it is uncertain whether they can be extracted from literature.

3. **Characterizing clones selected under different temperature regimes**. Both working groups are carrying out competition experiments to analyse the potential of animal groups (*Daphnia* clones or species) to cope with enhanced temperature. In Münster, the experimental design of these studies was slightly different (using adults instead of neonates to start the laboratory populations), which is disadvantageous for the need to guarantee uniform conditions in all setups. So I got useful suggestions on improvements of our experiments here. Wendy van Doorslaer & Luc de Meester have carried out a very interesting experimental evolution culturing genetically diverse *Daphnia magna* populations at different temperatures (20 vs 24 °C). The resulting clones differ in an important life-history parameter (size at maturity), which may be related to oxygen transport within the animals. We agreed to analyse the hemoglobin concentration and induction potential for this respirative protein on several clones from the study, and I took ten clones to Münster for this purpose.

4. **Hemoglobin analysis of infected *Daphnia***. Studies on the role of parasite infections of the Leuven team (Ellen Decaestecker and Luc de Meester) evoked my interest, because one parasite (*Pasteuria ramosa*) causes a red colour of the haemolymph, which might result from haemoglobin expression, since the bacterium itself is colourless. I offered spectrophotometrical measurements as well as gel electrophoreses to be carried out in Münster to identify the origin of the red colour. Here, infected and control animals would have to be provided from Leuven in the future.

Summarizing the benefit of the short visit, I could take home some important suggestions and insights. In my opinion, especially the opportunity to discuss unpublished data of both groups turned out to be fruitful, which is the advantage of a visit instead of a mere study of the group's literature. Both myself and the different researchers I met at Leuven strongly felt the potential for future collaboration because of the complementary strengths in research. We discussed ideas for reciprocal visits for carrying out specific measurements (e.g. haemoglobin characterization and quantification of respiratory activity in Münster; swimming activity in Leuven) in future.

A direct impact of the visit on publications in preparation cannot be estimated yet.