# ESF ThermAdapt – Scientific report

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*Project Title:* Linking evolution and ecology in the context of Global Warming

*Host name & Institute:* Prof. Brian Moss, Freshwater & Marine Research Group, Liverpool University (UK)

## Purpose of the visit

In this project, our main aim was to quantify whether and to what extent thermal genetic adaptation may feed back on community composition by influencing competitive strength of resident populations and establishment success of immigrants, using zooplankton communities as a model system. We capitalized on a large scale experimental evolution set-up in which we quantify genetic adaptation of a keystone zooplankter, the water flea *Daphnia magna*, to an increase in temperature. During the study visit, which we could carry out thanks to the ESF support, we quantified the degree to which genetic adaptation impacts species composition and the establishment success of southern immigrants. We hypothesized that adaptive evolutionary responses may act as a buffer against species shifts in communities. Changes in environmental conditions (e.g. increasing temperature) often result in species replacements, because some resident species are not able to cope sufficiently with the environmental changes, and face reduced competitive strength compared to other resident species or immigrants. A rapid micro-evolutionary response to environmental changes may prevent or reduce the extent of such shifts in species composition. The experiments we carried out directly test this idea.

## Description of the work carried out during the visit

During this study visit, we built further on the large-scale mesocosm experiment that was initiated in October 2005 at the University of Liverpool (UK). The *Daphnia magna* populations in these mesocosms, inoculated as genetically identical populations in 2005, have been exposed for almost two years to different temperatures. In order to quantify the ecological impact of micro-evolution of this keystone zooplankton species on the communities inhabiting these mesocosms, we carried out transplant experiments between mesocosm populations exposed to different temperature regimes. In addition, we carried out competition experiments with southern *Daphnia magna* clones to assess the impact of micro-evolution on establishment success of immigrant genotypes. In this way, we capture two key elements in our effort to assess the ecological response of communities to climate change. In addition to actually carrying out the experiments, the project involved an extensive logistic effort, as it required building and deploying as many as 72 enclosures in the mesocosms.

*Experiment 1 - Transplant experiment*

In the **first experiment**, we investigated changes in zooplankton community composition related to genetic adaptation to temperature. To this purpose, 20L enclosures were installed in a subset of the high temperature mesocosms (Figure 1). We studied the ecological impact of evolution in three keystone zooplankton species, namely, *Daphnia magna*, *Daphnia pulex* and *Simocephalus vetulus*.

*Fig. 1: 20L enclosures installed in a high temperature mesocosm*

The experiment consisted of three parts: part 1 deals with local English *D. magna* clones, part 2 with local English *D. magna*, *D. pulex* and *S. vetulus* clones, and part 3 with local English *D. magna* and southern French and Spanish *D. magna* clones.

**Experiment 1A**: In the first part of the experiment (exp. 1A), we used the zooplankter *D. magna*. We installed 18 enclosures divided over two high temperature mesocosms. These enclosures were inoculated with the resident zooplankton community of the respective mesocosms. After removing all resident *D. magna* individuals from this inoculum, they were replaced by (a) *D. magna* isolated from two "low" temperature mesocosms, (b) *D. magna* isolated from another high temperature mesocosm (as a control for transfer among mesocosms), and (c) *D. magna* isolated from the "home" mesoscosm (as a control for manipulation). Note that treatment (a) is installed in both mesocosms, treatment (b) is only installed in the second mesocosm and treatment (c) is only installed in the first mesocosm (Figure 2). One of the high temperature mesocosms contained a resident *D. magna* population. The second mesocosm was used for correcting for the effect of adaptation to "other" mesocosms. There were three replicate enclosures per treatment. A detailed design is outlined in the scheme below (Figure 2).

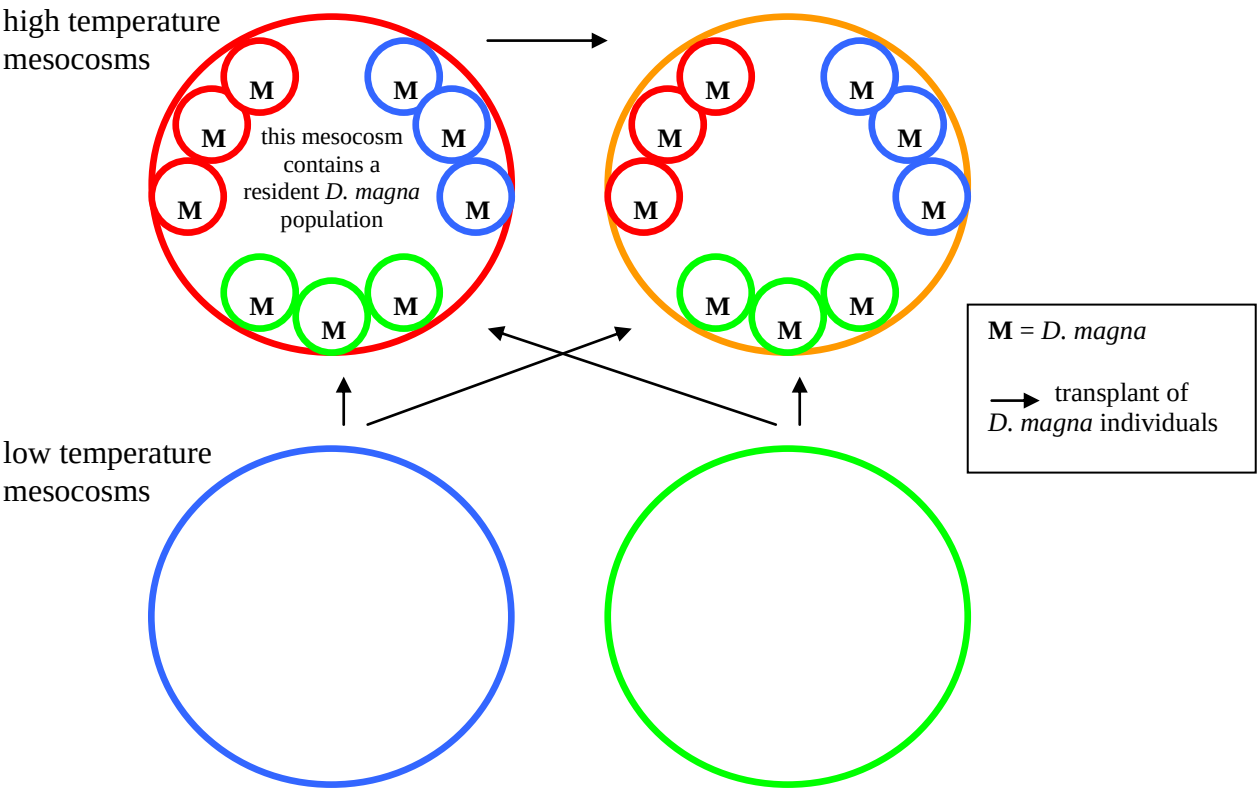


*Fig. 2: Scheme of transplant experiment with D. magna individuals (exp. 1A). All enclosures contained resident zooplankton communities of the high temperature mesocosms. The experimental manipulation consisted of replacing the D. magna individuals with other individuals of the same species but derived from different source mesocosms, or of adding D. magna individuals from different sources (different treatments) to communities that did not contain D. magna at the start (one of the two high temperature mesocosms).*

**Experiment 1B**: In the second part of our transplant experiment (exp. 1B), we worked with two additional keystone zooplankton species, namely, *D. pulex* and *S. vetulus*. We installed 18 enclosures divided over three high temperature mesocosms. These enclosures were inoculated with the resident keystone zooplankton species of the respective mesocosms. After removing all resident *D. magna*, *D. pulex*, *S. vetulus* individuals, respectively, they were replaced by (a) *D. magna*, *D. pulex*, *S. vetulus*, respectively, isolated from two "low" temperature mesocosms, and (b) *D. magna*, *D. pulex*, *S. vetulus* isolated from the "home" mesoscosm. There were two replicate enclosures per treatment. Note that the enclosures inoculated with *D. magna* are the same as used in experiment 1A. A detailed design is outlined in the scheme below (Figure 3).

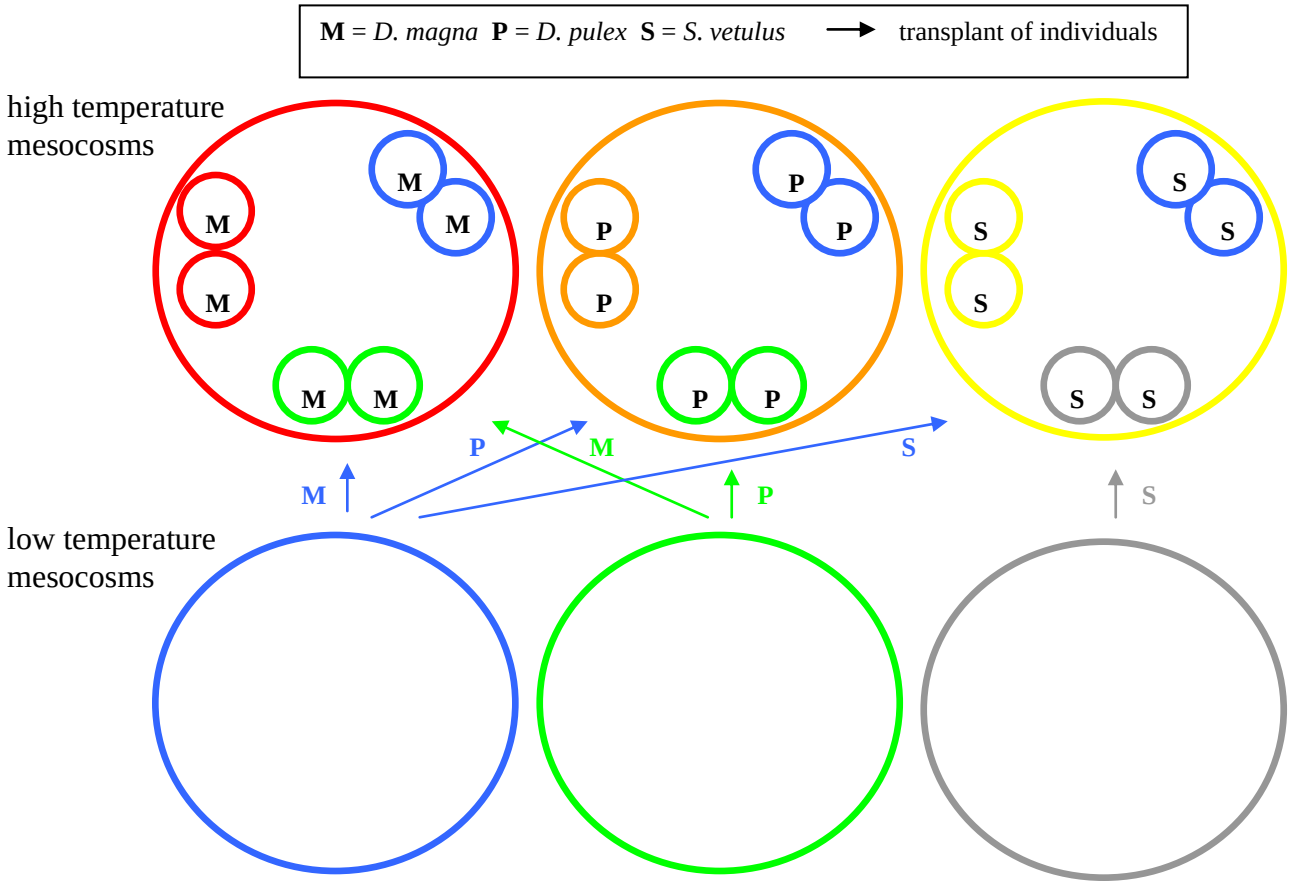


*Fig. 3: Scheme of transplant experiment with D. magna, D. pulex and S. vetulus individuals (exp. 1B)*

**Experiment 1C**: In the third part of our experiment (exp. 1C), we included southern immigrant clones of *D. magna* to quantify possible shifts in species composition upon warming resulting from a reduced competitive strength compared to warm adapted southern immigrants. We installed 15 enclosures in one high temperature mesocosm. These enclosures were inoculated with the resident keystone zooplankton community of the experimental mesocosm. After removing all resident *D. magna* individuals, they were replaced by (a) *D. magna* clones isolated from two "low" temperature mesocosms, (b) *D. magna* from the "home" mesoscosm, (c) southern *D. magna* genotypes hatched from sediments of three French (Camargue, isolated in March 2007) populations and (d) southern *D. magna* genotypes hatched from sediments of three Spanish (Doñana region, isolated in February 2007) populations. For the southern genotypes we used each time (France – Spain) a mixture of 15 clones (3 populations with each time 5 clones). There were three replicate enclosures per treatment. Note that treatment (a) and (b) are a subset of Experiment 1A. A detailed design is outlined in the scheme below (Figure 4).

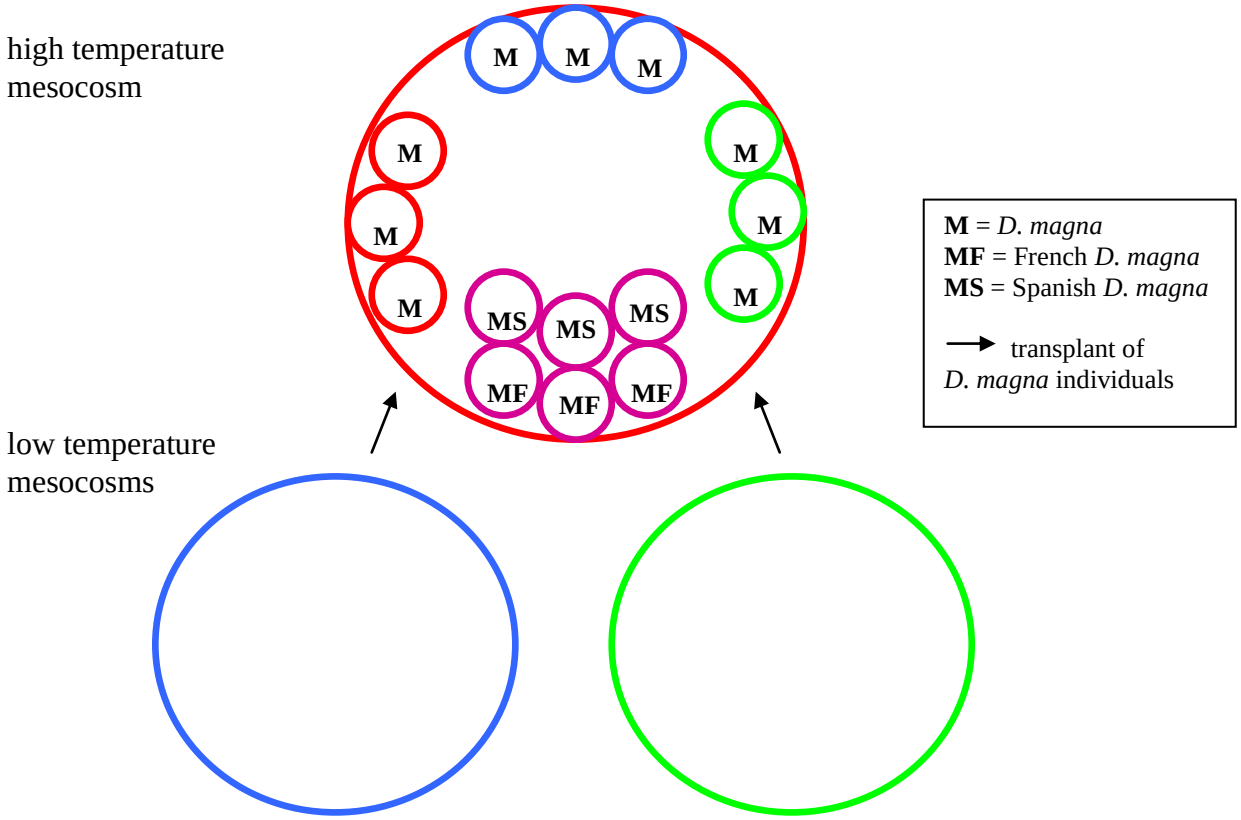


*Fig. 4: Scheme of transplant experiment with mesocosm D. magna and southern D. magna individuals (exp. 1C)*

After one month, we investigated shifts in species composition to quantify the ecological impact of genetic adaptation to high temperature by sampling all 36 enclosures and storing the content on ethanol for further analyses. This analysis will involve identification and counting of all individuals in the samples (subsampled if >>300 individuals).

*Experiment 2 - Competition experiment*

In the **second experiment**, we quantified whether warm adapted southern *D. magna* genotypes from France and Spain can invade enclosures (7L) installed in a high temperature mesocosm in the presence of resident *D. magna* that have or have not been allowed to genetically adapt to the high temperature conditions (Figure 5). We thus directly test the hypothesis that genetic adaptation of resident populations may impact establishment success of invading genotypes.

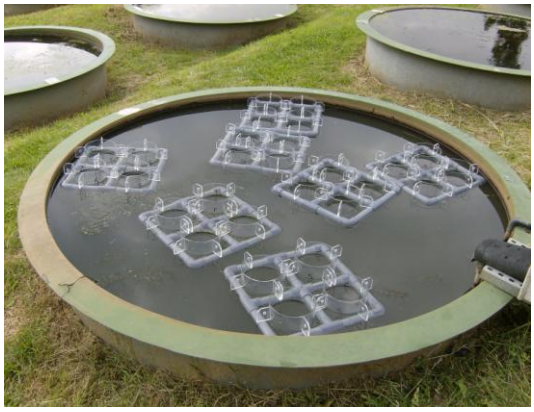

*Fig. 5: 7L enclosures installed in a high temperature mesocosm*

All 7 L enclosures were inoculated with the resident zooplankton community of the experimental mesocosms. After removing all resident *D. magna* individuals, we inoculated *D. magna* in specified numbers and mixtures of genotypes to monitor competition among genotypes. We used (a) *D. magna* clones isolated from two "low" temperature mesocosms, (b) *D. magna* from the "home" mesoscosm, (c) southern *D. magna* genotypes from three French populations (mixture of 5 clones per population, total n=15) and (d) southern *D. magna* from three Spanish populations (mixture of 15 clones) in our competition experiments. We inoculated immigrant and resident genotypes using a gradient of frequencies: (a) 1/1: in this treatment we inoculated an equal number of resident and immigrant clones. This will allow us to quantify the relative fitness in relation to competition of both groups of clones. (b) 1/10 and (c) ca. 1/100: in these treatments we simulated immigration of a few southern genotypes into a crowded resident population. There were two replicate enclosures per treatment. A detailed design is outlined in the scheme below (Figure 6).

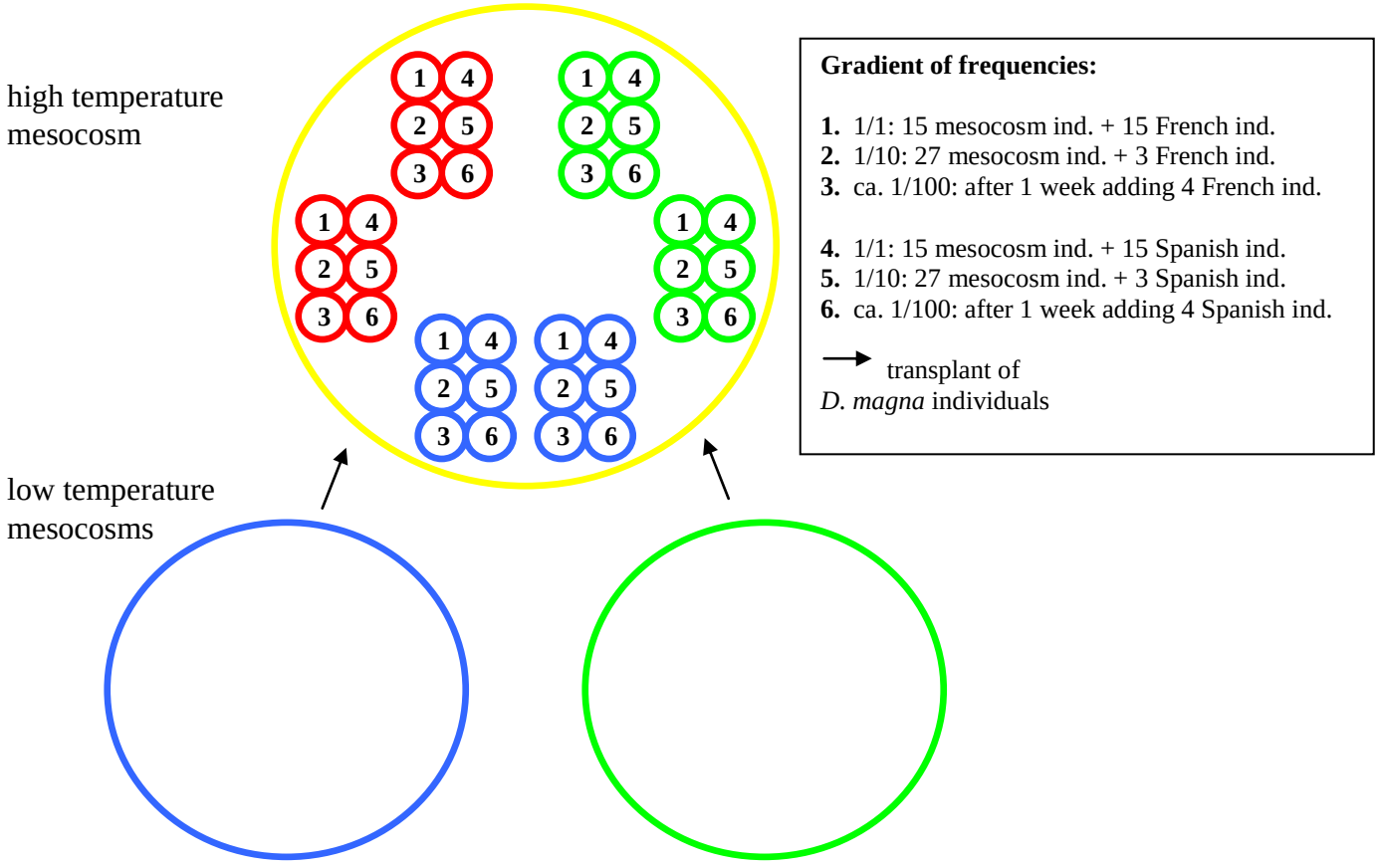


*Fig. 6: Scheme of competition experiment with mesocosm D. magna and southern D. magna individuals*

At the end of the experiment, all enclosures were completely sampled and fixed in absolute ethanol to reconstruct clonal frequencies. All clones used in this competition experiment were

screened for genetic variation at allozyme and microsatellite loci. The French and Spanish clones were screened prior to the experiment and we established diagnostic loci that will allow us to reconstruct clonal frequencies in the samples. In this way, we can quantify relative fitness of the clones in our experiment as well as establishment success of southern immigrant clones in competition with clones that were isolated from populations that were allowed to genetically adapt for almost two years to increased water temperature, and with clones that were not allowed to adapt genetically (i.e. isolated from the ambient temperature mesocosms).

In both the first and the second experiments, densities of the original zooplankton community from the 'home' mesocosm were mimicked. Both experiments together involved in total 72 experimental units (36 20L enclosures in Experiment 1 and 36 7L enclosures in Experiment 2). Before the actual experiment, we allowed all transplanted animals to acclimatize for approximately two weeks to the temperature in the target mesocosm by inoculating them in empty enclosures. This physiological acclimatization is crucial for us to be able to attribute the observed treatment effects in the light of genetic adaptation, and thus relate our observations to the potential impact of micro-evolution on ecological processes.

At the end of the experiments, we collected ca. 20 individuals (if possible) of the three keystone zooplankton species from a subset of mesocosms to perform 1. life table experiments to quantify the micro-evolutionary response to global warming in a semi-natural setting and 2. competition experiments at the interspecific level and/or with southern clones under standardized laboratory conditions in Belgium.

## Description of the main results obtained

Because it takes time for the shift in species abundance and species composition to become apparent, both experiments needed to run for one month (i.e. until the end of the study visit), and only analyses on the final samples are likely to be meaningful. As a result, processing of samples could not be started during the study visit itself and is still ongoing at the time of writing of this report.

*Experiment 1 – Transplant experiment*

In the first experiment, we aimed at testing the hypothesis that adaptive evolutionary responses may act as a buffer against species shifts in communities.

The transplanted individuals had the opportunity to adapt genetically to the temperature of their source mesocosm for approximately 1.5 year. If micro-evolution has an ecologically relevant impact, we should see a change in community composition in the treatment with individuals transplanted from low temperature mesocosms compared to the treatment with individuals isolated from mesocosms with the same (high) temperature as in the experimental mesocosms. The individuals adapted to the low temperature regime are expected to be less good in competing with the resident zooplankton community than the individuals that have been allowed to genetically adapt to the higher temperature.

After one month, we sampled the enclosures by pouring the total content (20L) of each of the 36 enclosure through a zooplankton net (mesh size 50 μm). The samples were

stored on ethanol (70%). The zooplankton individuals will be identified and the community composition in all enclosures will be determined during the course of the coming months (analysis of 36 communities; September-December 2007). The data will then be analyzed using multivariate approaches as well as ANOVA using CANOCO, STATISTICA and SAS.

*Experiment 2 – Competition experiment*

In the second experiment, we aimed at testing the hypothesis that local genetic adaptation creates a benefit in competitive ability in interaction with southern immigrants (mimicking dispersal associated with climate change), thus buffering against establishment of immigrant genotypes.

After one month, we sampled the enclosures by pouring the total content (7L) of each of the 36 enclosure through a zooplankton net (mesh size 50 μm). A subset of the animals was brought alive to Belgium and stored at –80°C for genetic analysis using allozymes; the rest of the samples was stored on ethanol (100%), so that they can be analysed using microsatellite loci. Tables 1 and 2 show the genotype frequencies at four allozyme loci of clones used in the competition experiments.

*Table 1: The genotype at four allozyme loci of all southern immigrant clones (three French populations (numbered 1-3) and three Spanish populations (numbered 4-6)) used in the competition experiment (GPI, glucose-6-phosphate isomerase, EC 5.3.1.9; MPI, mannose phosphate isomerase, EC 5.3.1.8; AAT, aspartaat aminotransferase, EC 2.6.1.1; and MDH, malate dehydrogenase, EC 1.1.1.37). The codes give the population number followed by the clone number. Alleles are coded according to their phoretic speed ($S^{2-}$, $S^{-}$, S, M, F and F).*

| | French *D. magna* clones | | | | | Spanish *D. magna* clones | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **GPI** | **MPI** | **AAT** | **MDH** | | **GPI** | **MPI** | **AAT** | **MDH** |
| **Pop 1.1** | FF | $S^{2-}S$ | SF | SM | **Pop 4.1** | FF | FF | SS | SM |
| **Pop 1.2** | FF | $S^{2-}S$ | SF | SM | **Pop 4.2** | FF | $FF^{+}$ | FF | MM |
| **Pop 1.3** | FF | $S^{2-}F$ | FF | MM | **Pop 4.3** | SF | SF | FF | SM |
| **Pop 1.4** | FF | $S^{2-}S$ | FF | MM | **Pop 4.4** | SF | SS | SF | SM |
| **Pop 1.5** | FF | SS | SF | MM | **Pop 4.5** | SF | SS | FF | SS |
| | | | | | | | | | |
| **Pop 2.1** | FF | SS | SF | SM | **Pop 5.1** | FF | $SF^{+}$ | FF | MM |
| **Pop 2.2** | FF | SF | SS | MM | **Pop 5.2** | FF | FF | SF | MM |
| **Pop 2.3** | $S^{-}F$ | SF | SS | MM | **Pop 5.3** | FF | FF | FF | MM |
| **Pop 2.4** | SF | SS | SF | SM | **Pop 5.4** | FF | SF | SS | MM |
| **Pop 2.5** | $S^{2-}F$ | SS | SF | MM | **Pop 5.5** | SF | $FF^{+}$ | FF | SM |
| | | | | | | | | | |
| **Pop 3.1** | FF | SS | FF | MM | **Pop 6.1** | FF | FF | FF | MM |
| **Pop 3.2** | FF | $S^{2-}S$ | FF | MM | **Pop 6.2** | SF | FF | SF | MM |
| **Pop 3.3** | FF | SS | SF | SM | **Pop 6.3** | FF | SF | FF | MM |
| **Pop 3.4** | FF | $S^{2-}S^{2-}$ | SF | MM | **Pop 6.4** | FF | SF | FF | MM |
| **Pop 3.5** | FF | SF | SF | SM | **Pop 6.5** | FF | FF | SF | MM |

*Table 2: The genotype at four allozyme loci of a subset of 20 of the mesocosm clones used in the competition experiment (GPI, EC 5.3.1.9; MPI, EC 5.3.1.8; AAT, 2.6.1.1; and MDH, EC 1.1.1.37). Alleles are coded according to their phoretic speed ($S^-$, S, M and F).*

| | GPI | MPI | AAT | MDH | | | GPI | MPI | AAT | MDH |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | FF | $S^-S^-$ | SF | MF | | **11** | SF | $S^-S^-$ | FF | MM |
| **2** | SF | $S^-S^-$ | FF | SM | | **12** | SF | $S^-S^-$ | SF | SF |
| **3** | SF | $S^-S^-$ | SF | SF | | **13** | SF | $S^-S^-$ | SF | SM |
| **4** | SF | $S^-S^-$ | SF | SF | | **14** | SS | $S^-S^-$ | FF | SM |
| **5** | SF | $S^-S^-$ | SF | SM | | **15** | SF | $S^-S^-$ | SF | SM |
| **6** | SF | $S^-S^-$ | SF | MF | | **16** | SF | $S^-S^-$ | SF | SM |
| **7** | SF | $S^-S^-$ | FF | MM | | **17** | SF | $S^-S^-$ | FF | SF |
| **8** | SF | $S^-S^-$ | FF | MM | | **18** | SF | $S^-S^-$ | FF | SF |
| **9** | SF | $S^-S^-$ | SF | SM | | **19** | SF | $S^-S^-$ | FF | MM |
| **10** | SF | $S^-S^-$ | FF | MM | | **20** | FF | $S^-S^-$ | SF | MF |

As Tables 1 and 2 show, the mesocosm and immigrant clones can be distinguished using the allozyme marker MPI. A subset of 30 animals per enclosure will be screened for their genotype using cellulose acetate gel electrophoresis (following Hebert and Beaton 1989) to quantify the relative abundance of resident and immigrant genotypes in the enclosures. If only one clone is detected, a larger number of individuals will be screened to assess whether the other clone went extinct or is still detectable at very low frequencies. The data will be analyzed using STATISTICA and SAS.

## Future collaboration with host institution *(if applicable)*

The mesocosm experiment that is currently run at the University of Liverpool will be terminated in September 2007. We will have intense contact with the research group of Brian Moss with respect to the final harvest and sampling. In addition to isolating living animals for further study, the dormant eggs produced in the different mesocosms will be a valuable resource for future work, as they contain sexual eggs produced by populations that were exposed for one to two years to different temperature regimes.

Obviously, the resulting scientific publications from this work will be co-authored by the research group of Liverpool. During the process of data analysis, interpretation and writing, there will be regular contact and exchange of ideas.

## Projected publications/articles resulting or to result from your grant

We expect two papers to result from this project. These will correspond to experiments 1 and 2. Both papers will explore the ecological relevance of micro-evolution. The first paper will focus on the quantification of the ecological impact of micro-evolution, in this study genetic adaptation to temperature change, on community composition. The second paper will focus on the degree to which local genetic adaptation may impact establishment success of southern immigrant genotypes. This second paper therefore explicitly explores a scenario that is very relevant in a climate change context: to what extent are local populations impacted by

immigration of southern genotypes, and to what extent does local genetic adaptation of resident populations influences this impact of immigrant genotypes. Depending on the results of the first experiment, it may be that the data may be dealt with in several publications (cfr. experiments on three different species, and different questions tackled in experiments 1A-1B-1C).

## Other comments *(if any)*

*Experimental design*

The design of the experiments was slightly modified compared to the application. This was due to practical reasons, and largely due to extinction of *D. magna* in some of the heated mesocosms. We therefore had to adapt our transplantation scheme somewhat, without impacting the core of the design or the research questions. Because other species had become dominant in some of the mesocosms, we extended experiment 1 with experimental units dealing with two other large-bodied zooplankton species, *D. pulex* and *S. vetulus*.

*Illness*

Because I became seriously ill during my stay abroad, which prompted urgent hospitalization, I was not able to finish myself the experiments, which I started up in June. As a result, I returned back to Belgium three weeks earlier than intended in the proposal. However, several Belgian colleagues (PhD students Sarah Rousseaux, Mieke Jansen, Cathy Duvivier and Bastiaan Jansen) of the KULeuven research team came to Liverpool to help with the experiments and carry out the final harvests. In this way, we were able to complete the planned experiments successfully. Although I returned earlier than anticipated, I herewith ask permission to keep the budget that was allocated to my project – the money (and more) was used to be able to organize the backup of helping hands when I was hospitalized. Without this help, the effort invested in preparing and setting up the experiments would have been wasted, as I was unable to carry out the final sampling and terminate the experiment. As can probably be appreciated from the above report, the experiments involved a lot of effort, and were complex in harvesting.