SHORT TERM Mobility Program 2016

Report of Adam Petrusek's research with Dr. Marina Manca and other scientists at the CNR ISE

Title of the Research Program: Long-term changes in *Daphnia* diversity under changes in trophic dynamics and climate

Specific Objectives of the Research Program: To evaluate the taxonomic changes in the *Daphnia longispina* complex in Lago Maggiore over the last decades by combining morphology, morphometrics and potentially also genetic analyses of historical samples. In particular, to reconstruct the link between changes in morphology and morphometrics and in parental species and hybrids composition, and impact of different environmental changes, such as predation pressure, trophy and climate, occurring over the long term in Lago Maggiore.

Research completed during the visit: During his visit to ISE, Adam Petrusek focused on selecting and preparing historical samples suitable for the long-term retrospective study on taxonomic composition, phenotypic variation, and size structure of the *Daphnia longispina* complex in Lago Maggiore. Hybridizing species of this species complex are key taxa in pelagic communities in European lakes; in some of these, it has been documented that taxonomic structure of the complex has substantially changed following human-mediated environmental alterations, particularly eutrophication and re-oligotrophication and fish stock changes, by analysis of dormant egg banks (in *Daphnia* ephippia). In Lago Maggiore, one of best and longest studied European lakes, a study reconstructing past population changes from sediment egg banks cannot be conducted, though, as local *Daphnia* apparently did not reproduce sexually in the past.

In case of a missing egg bank, however, we could focus on use of historical plankton samples, which have been collected throughout the 20th century from the lake, and are kept in *Museo del Plancton* at ISE, Verbania. The approach of choice for quantifying *Daphnia* phenotypic changes, and also determining the extent of hybridization, is in this case the geometric morphometrics that allows quantification of even subtle phenotypic changes. For this purpose, we have screened available historical samples that were collected in comparable periods of the year at the same sampling station above the deepest basin of the lake (Ghiffa), and selected those that cover the important periods of environmental changes in Lago Maggiore during the 20th century. The final selection of samples (altogether 24, with over 2700 individuals selected; see table below) cover the period from the late 1940s and early 1950s (representing pre-eutrophication period) to 2012, and two periods of the year: a first *Daphnia* population peak (usually late May), and late summer (usually August) when phenotypic variation should be highest due to, e.g., development of helmets.

Whenever possible, we carefully selected from the samples ca 120 adult *Daphnia* individuals sufficiently preserved for morphometric analyses. These will be in the coming months processed by non-destructive way at the Department of Ecology, Charles University in Prague, and subsequently returned to collections of ISE, Pallanza. Individuals will be digitally photographed from lateral view, their body outline will be characterised by ca 60 equidistantly positioned points, and body size measured. These outlines will be analysed by elliptic Fourier analysis and subsequent multivariate statistics (using R package Momocs), and we will quantify the extent of phenotypic variation within each study period, and the centroid of population position. Together with body size data, these will be confronted with historical information, especially about trophic level and fish harvest in the lake.

Ethanol-preserved samples from the recent years will be used to match the phenotypic variation with the taxonomic position of individuals. Furthermore, we will focus on morphological characteristics that may differentiate between *Daphnia* parental species (incl. *Daphnia cucullata*) and at least hint on presence of hybrids (body shape, rostrum, size and shape of antennular mound, ocellus).

In case the phenotypic patterns suggest interesting events in the evolution of the local *Daphnia* population, we may possibly decide to attempt processing historical samples by recently suggested DNA-based methods that might be suitable even for formalin-preserved samples (analysis of species-specific single nucleotide polymorphisms in short DNA fragments). As such approach would lead to loss of those particular individuals, permission of such analyses will be in advance sought and only undertaken if the potential gain seems very high, and mutual agreement collaborators at ISE, Verbania, is reached.

Apart from studying historical plankton samples, Adam Petrusek discussed various aspects of the historical development of Lago Maggiore environment with relevant colleagues from ISE, in particular Roberta Piscia and Marina Manca (long-term changes zooplankton composition) and Pietro Volta (changes in fish population structure, and their quantity estimated from harvest data). He also performed preliminary analyses of a long-term dataset on *Daphnia* body size and fecundity collected by M. Manca, which will be confronted with the data obtained from the newly selected individuals used for the geometric morphometric analyses. Adam Petrusek also joined the regular sampling at the Ghiffa station to get familiar with the sampling methods used during the long-term lake monitoring.

Products of the Mobility Grant visit:

During the visit, Adam Petrusek gave a seminar at ISE entitled "*Daphnia* evolutionary tales: from canyon-shaped reservoirs to Holy Land", which highlighted some of his past research projects, spanning from studies of hybridization and host-parasite interactions the *D. longispina* complex to paleogenetic analyses of *Daphnia* dormant egg banks in alpine lakes to predator-induced phenotypic plasticity in Mediterranean *Daphnia* from temporary waters.

We expect that analyses of the *Daphnia* samples from Lago Maggiore will result in at least one joint peer-reviewed publication with ISE researchers focusing on the phenotypic and taxonomic changes in local population during the 20th century. Such manuscript would be suitable for the *Journal of Limnology* or other relevant aquatic ecology periodical. A tentative title and authors list is given below.

Petrusek A., Faktorová Z., Piscia R., Volta P., Manca M.: Long-term variation in *Daphnia* diversity and phenotype in Lago Maggiore following environmental changes.

It is not unlikely that the analysis of the resulting dataset will be a base for more than one paper.

Verbania, June 30th, 2016

Marina Manca

Table: Number of *Daphnia* individuals selected for loan from archive samples from Lago Maggiore from collections of ISE, Pallanza. A sample from May 2012 has been already provided for loan to Prague for preliminary assessment of the methodological approach.

Year	Month	individuals
2012	8	120
2008	8	120
2008	5	120
2004	8	120
2004	10	17
2004	5	120
2003	8	120
2003	5	120
2000	8	120
2000	5	120
1997	8	120
1996	5	124
1992	8	120
1992	5	120
1989	5	120
1989	8	120
1986	8	120
1986	5	120
1973	8	120
1973	5	120
1952	6	120
1952	8	154
1949	4 to 6	43
1948	8	94

Marina Manca