**Masters Project 2022-2023**

**Starts in Sept 2022**

**(duration: 9 - 12 months)**

**Project title:** Using cell culture of endemic freshwater fish to study the effects of climate change

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**Laboratory/Institution, location:** Evolutionary Genetics (EG), Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências da Universidade de Lisboa.

**Abstract:** Climate change is threating biodiversity at an unprecedent rate. Thus, developing non-lethal methods to study the effect of environmental changes at the molecular level is of crucial importance to avoid the sacrifice of individuals and its impact on population size of species, in some cases, highly endangered by these changes. Freshwater organisms can be particularly vulnerable to small changes in water conditions, as they depend strictly on the environment to maintain homeostasis and, due to their limited ability to migrate, can become exposed to harsh conditions. *Squalius* genus is represented in Portuguese rivers by four known fish species distributed across an environmental latitudinal cline. Previous studies to assess responses of *S. carolitertii* and *S. torgalensis* to scenarios of thermal stress revealed that changes in gene expression can be involved in the response to environmental change. In fish, cell culture has been widely used during the last decade to study the effects of pollutants on several biochemical pathways. However, its use for studying the effects of temperature at molecular level is still under investigation. In this project, the student will work with cell cultures derived from dorsal fins of the two mentioned freshwater species which will expose to different thermal conditions and measure its effect on the expression of target genes. In particular the student will:

1. Establish cell lines from both species, confirm the origin of the cell lines through the amplification of cytochrome c oxidase I gene and and the absence of contaminations using bacterial and fungal universal genes
2. Expose the cell lines to different environmental conditions (control and increased temperature).
3. Quantify levels of gene expression of target genes and compare this with the results obtained with tissues derived from whole organism exposed to the same conditions.

This project will provide the student with transferable skills in cell culture laboratory (e.g., establishment of cell lines, cell counting and viability assays) and genetics laboratory (use of genetic markers for cell line characterization, PCR, electrophoresis, gene sequencing, qPCR).