

Exploration of the development, functioning and role of circadian rhythm in early life stages of *Perca fluviatilis*

Circadian rhythm have guided evolution for millions of years and animals - also fish - have evolved under natural light fluctuations. Naturally, in the temperate zone, the main important parameter related with light condition is photoperiod (the period of daily illumination received by an organism) allowing the fish to 'perceive' the period of a day as well as period of a year. Therefore, the **general aim of this project is to verify at which developmental stage the embryos or larvae of Eurasian perch, *Perca fluviatilis*, acquiring 'sense of time' (SoT - defined here as the physiological/biological response to daily rhythm induced by rhythmic changes in the light conditions) and how this ability undergoes future dynamics (during the larval and/or juvenile periods).** Additionally, the project will allow to answer the question 'whether' and 'to what extent' modified photoperiod and/or light spectrum, affects circadian rhythm of early life stages of the species studied. The research that are planned in this project will allow also to answer 'if' and 'to what extent' the fish may adapt to the modified light conditions. Current knowledge in this matter is very limited for most freshwater fish species (especially fish embryos and larvae) and for Eurasian perch is still insufficient.

The main research questions of the project will be addressed by two-phases: initial and experimental. The aim of the initial phase will be identify when Eurasian perch embryos or larvae acquire their 'sense of time', therefore their circadian rhythm starts to function properly. For this purpose, the embryos and larvae will be incubated and reared in 'control' photoperiod 14 Light:10 Dark (14L:10D) and natural light spectrum (white) potentially mimicking natural lighting conditions during natural spawning season in the wild. At certain moments, embryo and larvae will be sample for evaluation of zootechnical parameters (for example: diameter of the egg, larvae body weight or body length, survival) as well as for further transcriptomics and proteomics studies. Transcriptomics is a method allowing simultaneous analysis of expression of tens of thousands of genes at the same time, what will allow in current project to identify processes being under photoperiod and light control and to find and describe potentially new, additional 'clock markers' (genes) being candidates for determining the circadian rhythm. Whereas, using proteomic techniques, we will be able to 'reach a level higher than genes' and find protein markers responsible for the course of the circadian rhythm in the early development stages of the Eurasian perch. During the experimental phase, where findings of initial phase will be utilized, it is expected to acquire knowledge about changes in functioning of circadian rhythm and 'sense of time' in embryos, larvae and juveniles. For these purpose in first experiment embryos, larvae and juveniles will be reared in three different photoperiods: 24L:0D, 12L:12D and 6L:18D but in one 'control' light spectrum (white), whereas in second experiment three different light spectra (white, red and blue), but one 'control' photoperiod (14L:10D) will be used. Very important feature of these experiments will be splitting of each experimental group at hatching and after completion of larval period to three different subgroups exposed to other two photoperiods or light regimes (different than originally). Such experimental approach will allow to verify the adaptability of different early life stages (larvae or juveniles) to the again-modified light conditions. Such an experimental approach will allow to verify (1) the effect of different photoperiod or light spectrum on the course of the early life history of the fish and their performance, as well as (2) adaptability of larvae and juveniles to shifts in photoperiods or light spectra. At the selected moments of the experiments the samples for evaluating the zootechnical parameters and for molecular (qPCR) analysis will be collected.

Therefore, the project, by multi-dimensional research activities will **fill the important gaps in the development and functioning of circadian rhythm in early life stages of Eurasian perch.** It will allow to **understand the effect of modified light conditions (photoperiod and light spectrum) on the overall biological quality of the larvae and their adaptability.** In conclusion, expected results of this project provide, within a long-term perspective, key information which could have a significant importance in protection of freshwater ecosystems against light pollution (understood as presence of anthropogenic and artificial light in the night environment) as well as may, determine future research priorities in the field and to determine the basic knowledge being potentially useful for the hatchery managers intending growing fish for either commercial aquaculture purposes of restocking operations, of not only Eurasian perch, but also other percids and freshwater fish species.