- ECO-ALPSWATER -

Innovative Ecological Assessment and Water Management Strategy for the Protection of Ecosystem Services in Alpine Lakes and Rivers

Priority 3: Liveable Alpine Space. SO3.2 - Enhance the protection, the conservation and the ecological connectivity of Alpine Space

Work Package WPT1 - Coordination: PP6 INRA
Activity A.T1.1 - Deliverable D.T1.1.2.
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D.T1.1.2 Identification and formalization of protocols for eDNA analysis (bacteria, algae and fish)

The protection and preservation of aquatic ecosystems is a major challenge to preserve goods and services provided by these ecosystems. Aquatic biomonitoring is essential for the management and conservation of freshwaters and has become an essential task in most of the countries. However, the traditional methods used for the quality assessment of lakes and rivers require high level of taxonomic expertise and are generally invasive (e.g. electrofishing), time consuming, technically complex and thus expensive to deploy on a large scale. The use of environmental DNA (eDNA) associated to High Throughput Sequencing (HTS), i.e. metabarcoding approach, has recently been identified as a promising approach for freshwater biomonitoring, allowing to provide biodiversity inventories for lakes and rivers using non-invasive methods, with many advantages in terms of speed, comparability and costs. The characterization of the aquatic biodiversity using eDNA metabarcoding, is based on the application of successive protocols to be implemented both on the field and in the laboratory (Figure 1). The main steps are (i) to collect a representative eDNA sample on the study site, and preserve it in an optimal way for downstream molecular analysis ; (ii) to efficiently extract the DNA from environmental samples, considering the different types of DNA (i.e., intracellular or extracellular DNA) that might be of interest for the molecular analysis ; (iii) the selection and amplification of specific DNA region (barcode regions) to target the biological group of interest (e.g. fish) ; this DNA fragment represents a small portion of the total DNA extracted, and is amplified using a well-known method called PCR; (iv) to sequence massively the DNA products obtained after PCR, using High-Throughput Sequencing (HTS) technologies that allow a genetic screening of the “biological” information that is , at this step, transformed into a “numerical” information, i.e. thousands of short DNA reads/sequences composed of the 4 oligonucleotides (G,A,T,C). These DNA reads are then treated by bioinformatics pipelines to obtain taxonomic lists describing the diversity of the targeted biological group (see D.T1.1.3).

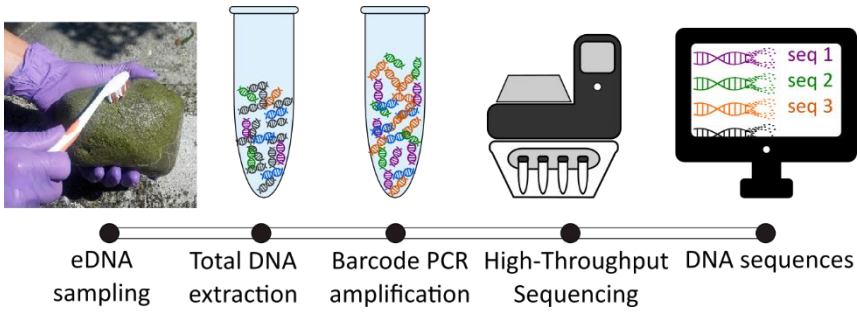

Figure 1 – Major steps of eDNA metabarcoding from field sampling to the production of raw DNA sequences using High-Throughput Sequencing (HTS) technologies.

This general workflow is representative of most of the eDNA metabarcoding studies, however the detailed protocols and methods used can vary according to the freshwater ecosystem (lakes or rivers), the ecological compartment (e.g. biofilm or plankton) and obviously according to the biological community targeted (fish, bacteria, algae, diatoms,…). In the context of the Eco-AlpsWater project, a set of formalized protocols have been formalized within the D.T1.1.2 deliverable. Several technical documents are constitutive of the D.T1.1.2 ; they are organized according to the chronology of the workflow (sampling & preservation, DNA extraction, PCR and library preparation) and they are specific

to the 3 biological compartments, i.e. biofilm, plankton and fish, and to the different biological groups, i.e. bacteria & cyanobacteria, micro-algae & micro-eukaryotes, diatoms, fish), for lakes and rivers. These protocols help to consolidate the approach of standardization of the eDNA workflows for aquatic biomonitoring at the European level, and should facilitate the future the implementation of these tools for routine survey of lakes and rivers.