- 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.T1.1.1. – 1
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D.T1.1.1 State of the art of methods for the analysis of environmental DNA in lakes and rivers

Freshwaters in lakes and rivers provide goods and services of critical importance to human societies everywhere; the protection and preservation of these aquatic ecosystems is therefore a major challenge. Aquatic biomonitoring now underpins much of the management and conservation of freshwaters and has become an essential task in Europe as a consequence of strong anthropogenic pressures affecting the health of lakes and rivers. An effective evaluation of the quality/status of aquatic ecosystems requires comprehensive data on various freshwater organisms (from micro-algae to fish) used as indicators of the ecosystem health. The required biodiversity metrics are obtained by collecting bioindicator organisms, which are identified at the species level to constitute taxonomic lists and subsequent quality indices. Those approaches 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 temporal and spatial scale. High-throughput genetic screening methods such as environmental DNA (eDNA) metabarcoding have been recently proposed as a solution to these shortcomings. Such new generation biomonitoring has many advantages over the traditional approach in terms of speed, comparability and costs, offering the possibility to monitor aquatic biodiversity with a non-invasive and rather easy-to-standardize approach.

eDNA is the DNA collected from environmental samples (here water, or biofilms), including both DNA found in living cells (e.g. bacteria, micro-algae) and DNA released in the environment by all types of organisms (e.g. fish). From eDNA samples, short DNA regions called “barcodes” can be amplified, sequenced using High-Throughput Sequencing (HTS) technologies and compared to a reference library allowing to identify taxa initially present in the sampled water/biofilms. Though eDNA metabarcoding has been recognized as highly promising for next generation biomonitoring, the associated methodologies are not standardized so far and each step of the eDNA workflow needs to be normalized and validated at the European level before the implementation for routine survey of lakes and rivers.

One of the first aim of the Eco-AlpsWater project is to formalize standard eDNA protocols for both bacteria, micro-algae and fish, and to implement those protocols at the alpine scale for pilot lakes and rivers. However, before proposing such standardized protocols for the implementation of the eDNA metabarcoding approach, the first deliverable of WP1 (D.T1.1.1) provides a state of the art of the existing methods. As highlighted by the figure 1 (extracted from the D.T1.1.1), the state of the art we have compiled here includes information for each step of the eDNA workflow, i.e. sampling, DNA extraction and laboratory preparation (including selections of barcodes), as well as bioinformatic treatments allowing to produce taxonomic list. From the synthesis provided in this technical document the Eco-AlpsWater consortium has made some methodological choices that help to formalize appropriate eDNA strategies transferable for freshwater routine monitoring. The main questions addressed in this bibliographic synthesis (DT1.1.1) are (i) when, how and where to sample for eDNA (ii) how to concentrate and preserve eDNA (iii) what is the most appropriate DNA extraction method to apply (iv) what are the barcodes to use according to the objective of biomonitoring (v) what are the sequencing technologies and bioinformatics pipeline to select for obtaining robust taxonomic inventories for the studied biological groups.
The deliverable directly feeds the step two of this work package, i.e. the formalization of protocols for eDNA biomonitoring.

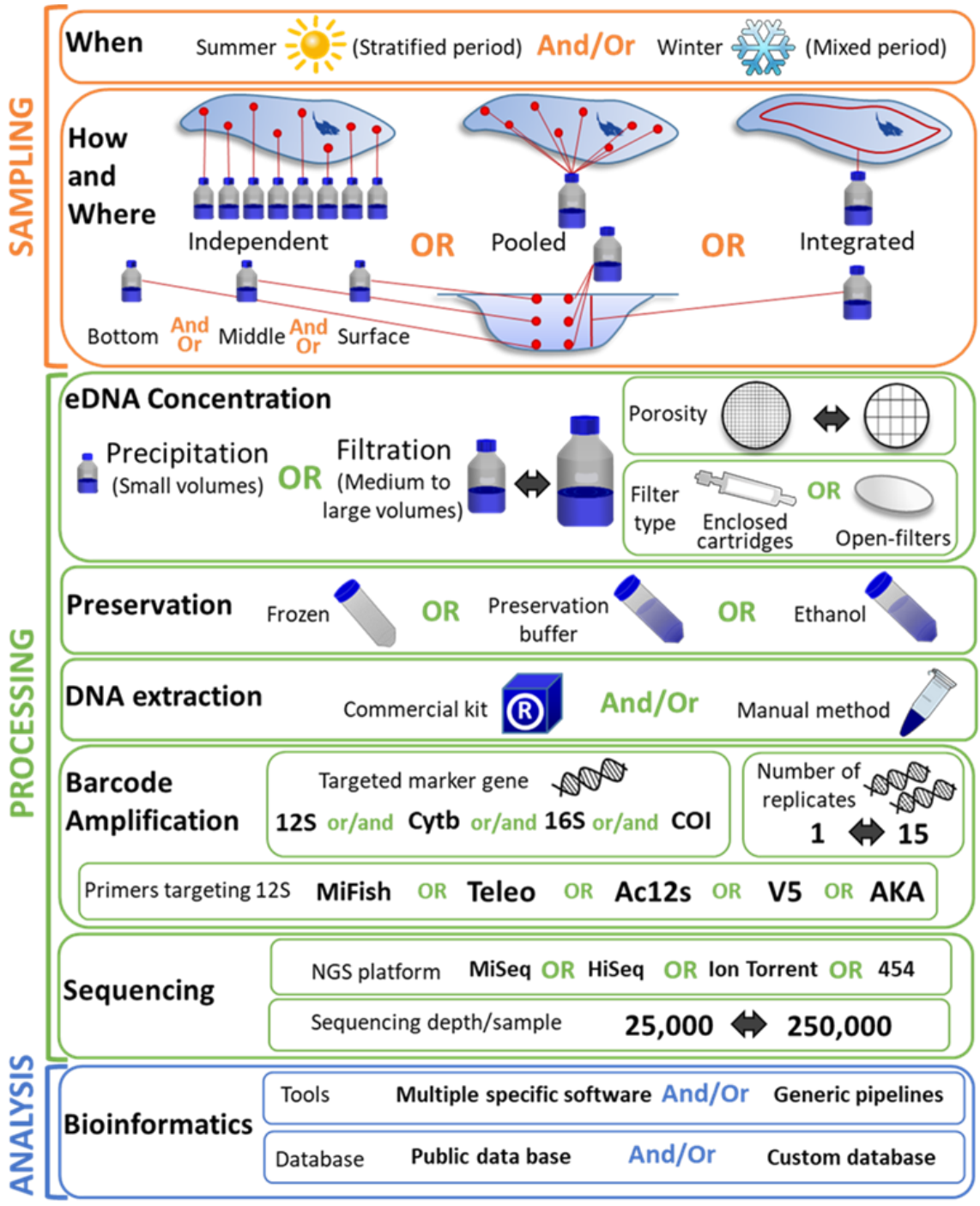

Figure 1 – Synthetic representation of the major steps and methods applied in the literature for fish eDNA metabarcoding in freshwater ecosystems (lakes and rivers) presented in the D.T1.1.1 deliverable.