

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

Project Eco-AlpsWater

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**Recommendations to strengthen the dialogue between AS water
management sectors, and adoption of new strategies at EU level**

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1. Introduction

This deliverable of the project Eco-AlpsWater provides recommendations to strengthen the dialogue between AS water management sectors, and adoption of new biomonitoring strategies at EU level.

Alpine Space Area is particularly vulnerable due to its extreme geo-sociological characteristics, and so are the integrated water bodies such as rivers and lakes. Alpine territories have to develop strategies for natural risk management, energy strategies and today a necessary adaptation to climate change, which is the focus of the EUSALP strategies.

The risks for Alpine Waters are observed with legal monitoring such as the River Basin Management Plans (RBMP) and with specific surveys, which include regional aspects and ecosystem services. The state of the art of the regulatory framework is summarized in the Water Governance in the Alpine Region (Bastiani et al. 2018), a report of the Interreg project AlpGov.

Metabarcoding of eDNA is one of the tools of choice of the 21st century for fundamental research and the future of large-scale biodiversity monitoring programs, thanks to its cost-effectiveness and relatively easy implementation.

According to the discussions within the project consortium and within several project meetings and teleconferences the following topics are regarded:

- Improvement of biodiversity knowledge
- Modern taxonomy approaches including quality assurance
- Search for neobiota and climate change signals
- Supporting assessment by Water Framework Directive (WFD)
- Suggestions for integration into WFD
- Communication of limits in the application of present HTS technologies
- Adoption of new strategies at EU level

2. Improvement of biodiversity knowledge

The eDNA approach allows large scale biodiversity surveys at an unprecedented level, thanks to its cost-effectiveness and relatively easy implementation. EU Strategy Alpine Region (EUSALP) members of Action Groups (AG) 6 and 7 have evaluated the potential to include the metabarcoding approach for freshwaters during the international Eco-AlpsWater project meetings.

Gian Antonio Battistel (FEM and EUSALP AG6 member) referred at 20th May 2021 on the EAW teleconference the specific role played by the Alpine Convention (AC) as follows:

“The Contracting Parties are the States such as Austria, France, Germany, Italy, Liechtenstein, Switzerland, Slovenia and Monaco. AC. The AC aims at the protection and sustainable development of the Alpine mountain area. The AC is a unique, legally binding sustainability instrument that aims at safeguarding the sensitive Alpine (it means only Alps mountain area) ecosystem”.

Water management is needed for drinking water and households, irrigation, sports and hydropower. There are conflicts over the use of aquatic ecosystems and water as a major factor in natural disasters, such as floods, permafrost melting, water scarcity and droughts.

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The AC (AC framework-convention) defines several objectives and its secretariat that coordinates the EUSALP AG 6. AC mentions water as one of 12 main Alpine issues and sets out the objective to “*preserve or re-establish healthy water systems, in particular by keeping lakes and rivers free of pollution, by applying natural hydraulic engineering techniques and by using water power, which serves the interests of both the indigenous population and the environment alike.*”

“To date, the remaining three EU macro-regional strategies Baltic Sea Region (EUSBSR), Danube Region (EUSDR) and (Adriatic-Ionic EuroRegion (EUSAIR) have their own water strategies. These are policy frameworks which allow countries located in the same region to jointly tackle and find solutions to problems or to better use the potential they have in common.”

Overall, there are relevant activities within these strategies, which have clear links to biological monitoring for water management. For example, in the Danube region strategy a Management Plan for the Danube Delta Biosphere Reserve will be completed and also a plan for a viable populations of Danube sturgeon species. In all cases where large scale biodiversity surveys are needed at the macro-regional level, the metabarcoding approach delineated in the EAW project can be of interest in future activities. The adoption of modern, affordable, and replicable monitoring surveys is essential not only to preserve aquatic ecosystem biodiversity and natural heritage (which have intrinsic values), but also to preserve ecosystem services and therefore ensure a conscious and safe use of water used for drinking and for tourist and bathing use.

Global warming increasingly and severely menaces the preservation of aquatic natural heritage, which is shrinking the habitat range of many stenothermal cold-water species, and expanding the geographical range of stenothermal warm-water species (Tunney et al., 2014; Van Zuiden et al., 2016). Similar effects, but due to anthropogenic transport of organisms from different ecoregions, were established or suspected for many aquatic organisms (Bacela-Spychalska et al., 2013; Heiler et al., 2013; Wittman, 2010). In such cases, an early detection of introduced organisms is essential to identify the causes and stressors, and countermeasures. Such activities require however the adoption of approaches, such as HTS/metabarcoding, that can be replicated over large spatial and temporal scales, as in the case of the detection and spread of the quagga mussel (*Dreissena bugensis*) in European waters (Ventura et al., 2017). In the EAW consortium, these techniques allowed the identification of several species that were not recorded by using the traditional light microscopy techniques. This is the case (among several examples) of the cyanobacterium *Cylindrospermopsis raciborskii*, which was detected for the first time in a small lake south of Lake Garda (Lake Frassino). This species is able to produce cylindrospermopsin, which is toxic to liver and kidney tissues (Fastner et al., 2003; Meriluoto et al., 2017), so that the ability to detect even small abundances of this species is essential for a correct outlining of water management plans. The spread of this species in Europe, which is thought of tropical origin (Antunes et al., 2015), was linked principally to the effects of global warming (Suklenik et al., 2015; Kokociński et al., 2017). Besides these cases, which have potential health implications, these techniques allowed us to obtain information on the bacterial communities across the alpine space regions for the first time. The amount of data is very high (over 50000 genotypes), and thus it will require adequate time for a systematic analysis.

The safe use of water for drinking or bathing purposes requires a specialized biomonitoring, which is mainly addressed towards the identification and quantification of bacterial pathogens and cyanobacteria. In the first case, the potential use of HTS to identify pathogens is still under scrutiny. In the case of cyanobacteria, the use of novel methods in the EAW consortium allowed to clarify the distribution of several species. For example, the presence of *Planktothrix rubescens* (hepatotoxic) was confirmed throughout the Alpine Space

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area, and *Tychonema bourrellyi* (neurotoxic), which seems still mostly circumscribed to the pelagic waters of the southern Alps, where it was recognized for the first time (Shams et al., 2015). In Bavarian biofilms, a benthic-occurring *Tychonema* genotype (neurotoxic) was detected with low signal in other locations, than that in which it was originally described for Bavaria at the first time (Bauer et al., 2020; e.g. River Lech system).

3. Modern taxonomy approaches including quality assurance

Stakeholders make use of the metabarcoding results and the gained taxa inventories to check the quality of the records monitored by their traditional national methods (light microscopy or fishing).

The traditional biological monitoring data can be checked for

- Completeness of taxa inventories
- Species determination
- Up-to-date nomenclature

An example for improved quality assurance by species determination with HTS is the discovered wide geographic distribution of an invasive diatom species *Achnantheidium delmontii*. It was first found in French rivers (Pérès et al., 2012), but is newly confirmed in Bavaria by metabarcoding and by a SEM analysis proof carried out in the EAW project (Goos, 2021).

Overall, large gaps of taxa inventories were discovered when comparing taxa lists obtained from metabarcoding to traditional lists. The traditional methods are based on limited morphological traits, but the full diversity becomes visible only by applying genetic methods in parallel.

The traditional species determination is time-consuming and partly subjective (Lee et al., 2014), so the more objective metabarcoding is an important contribution to the quality assurance, especially to distinguish between closely related taxa.

4. Search for neobiota and climate change signals

The introduction and successive establishment and invasion of alien species are the cause of severe detrimental impacts on biodiversity, human health, and ecosystem services (IPBES, 2019). Reports on the global economic costs over the last 50 years, estimate that invasive alien species are responsible for at least 1288 trillion US-Dollars in damages, a number that is progressively rising over time (Zenni et al., 2021). The early identification of neobiota is essential to identify organisms in the first phases of introduction, before establishment and invasion. The remedies vary depending on the organisms, and can rely on direct manipulation of populations or on the recovery of pristine environmental conditions. Nevertheless, the correct identification of neobiota requires procedures that are applied on large spatial scales and repeated over time. These characteristics qualify metabarcoding as a suitable approach to be used in wide biodiversity surveys. EAW considered only a selection of organisms (bacteria/cyanobacteria, microalgae and fish; see section 2), but other protocols are increasingly available for other organisms not considered in this project, e.g. macrozoobenthos and macrophytes (Jeunes et al., 2021; Feist et al., 2021).

5. Supporting assessment by Water Framework Directive (WFD)

The EU Member States provide River Basin Management Plans (RBMPs) about all executed activities every six years to report about the status of water bodies and the success of measures. The required activities and methodologies are precisely described in the WFD but not in the RBMP. Particularly the RBMPs describe the execution of the WFD and the success for example in coming closer to the desired ecological status. RBMPs provide information on the amount of water bodies in high, good, moderate, poor or bad status and which or how many measures are planned or have been carried out. On the other side, the details of methods and instructions are described in separate papers/protocols or websites as well as in national reports. A list of the accepted biological assessment methods is provided in the technical reports of the intercalibration process.

Here it becomes obvious, that the RBMPs are not the documents to directly link the Eco-AlpsWater metabarcoding methodology to the requirements of the WFD, but the national biological assessment methods of the Member States, here the five EU-countries and in addition the Swiss approach (WPO). All of these assessment methods require taxa inventory lists, which have to be compiled by specific collection and detection methods, to which the metabarcoding approach proposed by the project Eco-AlpsWater project can contribute significantly.

The future prospects for implementation of the EAW innovative monitoring approaches into WFD/WPO are (see D.T.4.2.2):

- Combination of traditional and eDNA approaches allows biodiversity assessment at an unprecedented level.
- Cost efficient eDNA approaches are perfectly suited for large-scale, continuous monitoring, providing the ability to detect changes in the ecosystem at an early stage and to react accordingly.
- Development of eDNA metrics, especially for questions exceeding WFD/WPO, e.g. climate change or control of effects of measures to improve the status.

At present, the contribution of HTS approaches is mainly focused towards the completion and integration of taxonomic lists of biological quality elements, to be used for the determination of biological indices, developed in inter-countries intercalibration processes. Nevertheless, in perspective, new metrics provided by innovative eDNA methods could be used to complement indicators for the study of ecosystem functions and services. However, this is an open and exciting research field that can rely on the use of all the biodiversity dimensions that cannot be assessed by the traditional methods. This includes, for example, the determination of organisms that are difficult or impossible to determine using isolation and cultivation methods, or of organisms that, although rapid results are needed for biomonitoring, require excessively long analysis times (such as bacteria, pico-cyanobacteria, small micro-eukaryotes). Perspectives for the development of new metrics have been widely discussed in the deliverable D.T 1.4.

6. Suggestions for integration into WFD

To obtain complete taxa inventories from freshwater samples, the metabarcoding approach of environmental DNA combined with next-generation sequencing needs to be further improved, as the methodology is not equally developed for all target organism groups.

While the eDNA methods for benthic diatoms (Eco-AlpsWater) and macroinvertebrates (see results from project DNAqua-Net) are already highly standardized and produce high-resolution inventories with some gaps for regionally distributed taxa, they have still innovative and more preliminary character for phytoplankton and for fish. The use of additional markers and primers for more accurate general taxonomic coverage or determination of specific groups (phytoplankton) is expected to increase the depth of determination.

In CIS (work program 2019-21) the Working Group on Ecological Status (ECOSTAT) worked on the task to exchange information regarding several fields in ecological status assessment and eDNA was explicitly mentioned in use of innovative methods in classification.

Apart from a refinement with additional gene markers, the most critical bottleneck is however represented by the incompleteness of the taxonomic reference databases, which prevents the complete classification of the sequences obtained with the eDNA analyses.

Indices and assessment methods purely based on eDNA/DNA are in development, but currently they need the validation by traditional methods carried out in parallel. Beside a present-absent record, the abundance of organisms are a normative requirement in WFD to assess the status of bio-components. Although it is possible to normalize the number of sequences into abundance for particular groups to obtain a quantitative proxy, this procedure can be difficult for taxa groups with varying numbers of DNA copies per organism.

Further projects to improve EAW methods and to analyse all EAW results in depth would ensure the establishment of these methods and create a standard for ecological monitoring.

An evaluation of the applicability for WDF/WPO is provided in D-T.4.2.2 "Recommendations for the inclusion of innovative monitoring approaches in water quality assessment and manag. (WFD/WPO) (1)".

7. Communication of limits in the application of present HTS technologies

The correct evaluation of results have to take into account the limits that characterise the adoption of present HTS technologies, which are summarised here:

- Reference taxonomic databases are widely incomplete; in the analyses carried out in the entire EAW consortium, and depending on the algal taxonomic rank analysed, approximately 35% to 60% of the microalgae species determined by light microscopy are not represented with 16S or 18S rRNA sequences in the GenBank taxonomic reference database (NCBI) (analysis in progress). Curated and checked reference databases, including sequences from cultivated species in a common eco-region

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(e.g., excluding marine or arctic taxa for Alpine freshwaters), are still the golden standard, but are not yet available for all biological groups.

- Phylogenetically related species may have very similar DNA sequences in the target DNA regions analysed by HTS; this issue is exacerbated by the short reads (up to 300 bp) that can be obtained with the present and most used technologies (Illumina MiSeq). Other technologies exist, but due to higher costs, the sequencing depth and bioinformatics analyses required for adequate taxonomic coverage are not easily affordable for day-to-day biomonitoring surveys. Despite this, technology is advancing very fast, and we can expect substantial improvements in the next generation aquatic biomonitoring approaches.

Due to the above mentioned limitations, a perfect match between traditional and HTS results can never be achieved and expected. In perspective, with the advancement of HTS technologies, and availability of novel metrics, the gap between the traditional and novel approaches is bound to increase, especially for the microbial organisms. Nevertheless, the contribution of traditional approaches based on polyphasic taxonomic identification of organisms will remain a fundamental requirement to ensure continuous coverage of taxonomic reference databases.

8. Adoption of new strategies at EU level

The development of the novel HTS methods in the EAW consortium allows fostering the adoption of metagenomic approaches in the whole Alpine Space area. The implementation is based on a high number of detailed field and laboratory protocols that cover all the single steps required for the analysis of eDNA, namely DNA extraction, library preparation, and bioinformatic analyses. These protocols have general value, and can be applied throughout the European Economic Area (EEA) (EC and CH).

From a technical point of view, the application of novel approaches does not have geographical limitations. Following the experience that characterized the adoption of the present traditional methods 30 years ago, this however requires at least two steps. The present biomonitoring methods, which are based on the identification of indicator species by morphological (polyphasic) methods, were developed within research institutions, and therefore transferred and tested for biomonitoring to government and environmental agencies. Before formalization in legislation (WFD), the methods experienced a lengthy process of testing and refinement.

The adoption of novel HTS methods in the EEA will presumably follow the same steps: testing and refinement. In this regard, the role of environmental agencies in applying and testing the new protocols is a key element in this process, and this is what was done in the EAW project, which was based on a continuous and reciprocal knowledge transfer and feedback between academia and government agencies. This process, in the Alpine Area, is also involving the EUSALP agenda, with feedback aimed at including these novel methods of biodiversity assessment in the corresponding action groups (specifically AG7 as to “blue infrastructures”).

In AG6 there is the potential to reactivate the subgroup "Integrated and sustainable water management" (sub-topic 3). Another opportunity is to bring the metabarcoding implementation on EU level, might be to propose An AC's thematic group on either integrated water basin management or participate with delegates to the “Alpine Biodiversity Board” of the AC.

The widening of the geographical area interested in the application of new methods outside the Alps already begun, with the involvement of environmental agencies in central Italy (Regione Lazio). The application to a

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set of lakes and rivers provided excellent results, which are (considering the relevant amount of data obtained from HTS) still under analysis and evaluation.

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