

**Deliverable D.T3.1.2 Local stakeh. involvement and feedback: final revision and tuning of approaches in single waterbodies. Final reports.**

**Compiled by Rainer Kurmayer (PP2,LFUI)**

From the proposal

Local meetings carried out in the 6 countries, sharing knowledge and feedback from observers and interest groups, evaluating general pilot actions plans and downscaling to the geographic and local anthropogenic pressures specificities; final reports.

**1) Information on local (national) meetings**

**Austria**

a local informal meeting was organized at 4 Dec 2018 (before the official project meeting in Mondsee 4-5 Dec) , Topic: Presentation of the project itself, its success so far, and what our observers might expect from us PP in the future and vice versa.

Participating:

Project Partners:

Rainer Kurmayer, Josef Wanzenböck, Hans Rund (LFUI), Peter Hufnagl, Stefanie Dobrovolny (AGES),

Observers:

Maria Friedl (KIS) representing observer 15 (Regional Government of Carinthia),

Elisabeth Sötz (WWF) representing World Wide Fund for Nature (WWF) Austria

Didier Pont (Boku) representing observer No35 (University of Natural Resources and Life Sciences Vienna and coordinator of the SPARE project),

**France**

A meeting was organized in the framework of EcoAlpsWaters at 19 November 2018 in Paris (Vincennes). This meeting took place in the AFB (Agence Française pour la Biodiversité) head office. The protocols for eDNA were presented and their applicability in routine was discussed. People from the Environmental Ministry, the French Biodiversity Agency, Regional Environmental Agencies, Water Agencies, the French standardization bureau (AFNOR) and private offices in charge of hydrobiological monitoring were present.

Participating: 25 people

INRA: F. Rimet, A. Bouchez, S. Rivera, C. Chardon

**Environmental Agencies & field operators in charge of biomonitoring**

Environmental Ministry: B. Jeannot, M. Le Loarer

AFB: B. Augeard, S. Couprie, O. Monnier,

Dreal: H. Abdallah, V. Peeters, F. Petel, N. Zydek

Water agencies: L. Imbert, A. Cattan

Private offices: P. Blancher, G. Gassiole, C. Geret, L. Kermarrec, E. Lefrançois, R. Marcel, N. Marie, P. Prompt, J. Vizinet

AFNOR (french standardisation bureau): B. Laurent

## **Germany**

a local informal meeting was organized at 6 February 2019 in Wielenbach as a common event by Bavarian Environment Agency (LfU, project partner 10) and Bavarian State Research Center for Agriculture (project partner 7), Topic: Presentation of the project itself, its success so far, and what we plan to investigate in our pilot lake and river. We collected what our observers might expect from us in the future and vice versa.

Participating: 26 people

Project Partners:

Jochen Schaumburg, Ute Mischke, Christine Schranz (LfU, Ref. 84), Michael Schubert, Christian Vogelmann (LfL, IFI)

Observers:

Dr. Bernhard Gum observer 9 (District of Upper Bavaria, Fishery Advice Unit),

Markus Heim observer 10 (District government of Upper Bavaria)

Dr. Franz-Xaver Heinle observer 11 (District government of Swabia)

Diana Schokrowski, also observer 11 (District government of Swabia).

17 further participants from 3 departments of 2 Universities (LMU Munich, TU Munich) and from local fish agencies (LFV), commercial fishermen (FG-Würmsee) and representatives from the Interreg project HyMoCares

Sudden illness hindered Observer 8 to come (District of Swabia, Fishery Advice Unit)

## **Italy**

On 11 October 2018, FEM has organized a meeting at S. Michele with the ARPAV of Verona (PP3) and Trento (Observer). These two public bodies are officially in charge of the WFD monitoring of Lake Garda and River Adige. The meeting provided important elements to support the strategy to be adopted from the 2nd project period (January 2019) onward for the evaluation of water quality based on traditional and innovative eDNA methods (see WPT3).

## **Slovenia**

Local meeting in Slovenia for observers, stakeholders and other interest groups was organized by PP4 and PP5 at the ARSO premises on 20th of December 2018. Meeting was to present Eco-AlpsWater project, to share knowledge and obtain feedback on the protocols, and to evaluate general pilot action plans. Since Slovenia is a small country, we recognize this as a success. People from Slovenian Water Agency and Slovenian Environment Agency responsible for biological monitoring were present. We are confident that next event will attract more people (e.g. interested groups including NGOs, infrastructure and service providers).

Participating: 11 people

Project Partners:

Tadeja Balanč (PP5), Tina Eleršek (PP4), Špela Remec-Rekar (PP5), Karmen Stanič (PP4), Nika Žalec - Interreg IVY volunteer (PP4)

Observers:

Nataša Smolar-Žvanut representing observer 16 (Slovenian Water Agency)

Other interested groups:

Tanja Menegalija from Triglav national Park

Igor Zelnik from Biotechnical Faculty, University of Ljubljana

Nataša Dolinar, Elizabeta Gabrijelčič, Edita Sodja and Tjaša Muc from Slovenian Environment Agency

### **Switzerland**

Since the beginning of the project, there have been frequent contacts with the Swiss Observer, Canton Ticino. A formal meeting for Swiss and Italian Observers, planned for the 11<sup>th</sup> of April 2019, was organized together with Italian PPs in Verona.

## 2) Potential requirements highlighted by observers

### Austria

species-specific DNA information:

We were discussing additional optional information, such as fish species occurrence. For example several oligotrophic-mesotrophic lakes in Carinthia are currently evaluated using status “good” by GZÜV (the national legislative for WFD), because certain native fish species are missing as recorded by electrofishing (e.g. minnow). Thus the recording of Fish DNA of minnows would be a potentially useful information with regard to fish BQE based evaluation.

Potential quantitative information on taxa from eDNA sampling is highlighted:

Currently Metabarcoding can give qualitative and semi-quantitative information of taxa which can be used also, e.g. species (OTUs) occurring with many 1000nds of reads probably occur in higher numbers than species (OTUs) occurring with a low read number only. Absolute quantitative information is possible only with independent PCR-based tools such as qPCR or ddPCR.

### France

The people in charge of routine sampling on French lakes validated the planned activities for 2019 on Lake Bourget and extended sites.

They agreed on applying the DNA sampling protocol for plankton but highlighted that the on-site filtration (with syringe) could be long and an alternative was chosen (bring back the samples to the lab for filtration).

Regarding fish sampling, they suggest collecting DNA samples a little bit in advance (approx. 2 weeks before traditional fisheries are performed).

For biofilms sampling, no specific requirement ; the protocols were found operational and environmental agencies as DREAL have started collecting DNA on Drome river and Arve river in 2018.

### Germany

Additional sampling for phytobenthos without diatoms is a traditional method for German rivers. It include more than the habitat “stones”, and is recommended to sample also other habitats in Eco-AlpsWater by observer 11.

Observer 10 recommended to harmonize the eDNA protocols with other regional project to have not different protocols in lowland and alpine regions. We are requested to contact other German projects, which are working on the same topic.

The regional district want to be involved closely into the sampling process.

Discussion about the concept of the project result into following points:

1. The timeframe of 3 years is very ambitious, and determination by morphology will still be necessary.
2. Sampling protocol might not fit to all water types (example humic water)
3. Biofilm on stones do not reflect the main part of total biomass in rivers and there are other missing WFD biological components
4. Bioinformatic, who has the expertise in the project and who will provide access?
5. How to deal with DNA sequence matches without taxa names or with incorrect names, which can be up to 90% of matches with international data bases? New questions about the interpretation of the matches might need as much times as the traditional methods.

## **Italy**

From the 11th October meeting and from other direct phone contacts (ARPA Lombardia) Observers highlighted the urgency to extend the analyses to a group of waterbodies hosting several toxigenic cyanobacteria; a few of these underwent also spring an/or summer blooms. These are lakes Serraiia, Ledro, Levico, Caldonazzo, Varese. It was stressed the necessity to accompany the analyses with a wide regional survey on the presence of cyanotoxins. Taking into consideration these requirements and indications, the sampling plans have been modified accordingly 1) including the suggested lakes among those that will be sampled at least for bacteria, cyanobacteria and phytoplankton eDNA analyses; and 2) including the analyses of cyanotoxins in the sampling programme, and for all the lakes studied in the 6 countries.

## **Slovenia**

We presented Eco-AlpsWater project, its main objectives and pilot sites. After presentation we played movies presenting plankton and biofilm sampling for eDNA analyses.

In discussion about phytobenthos sampling protocol it was highlighted that different habitats defined by substrate, water velocity, water depth, and shading have to be sampled regarding on the ratio of habitat coverage, if biodiversity is measured. It was exposed that 3 sampling sites for are enough for ecological status assessment with phytobenthos in the Lake Bled. The result with 3 sampling sites is comparable with the result with 7 sampling sites.

When discussing phytoplankton protocols filtration at the site with Sterivex filters was proposed as better technique for e-DNA sampling.

## **Switzerland**

The Observer revised the planned activities for 2019 on Lake Lugano and extended sites. The most of the feedback was focused on phytobenthos and fish sampling plans, because these biological indicators are not currently monitored on Lake Lugano. The choice of sampling area for phytobenthos representative of the trophic gradient was discussed, as well as previous record on fish species and breeding, nursery and feeding area.

### **3) Additional sampling strategies for consideration during implementation period**

#### **Austria**

Integrative sampling (by filtering large amounts of water onto cartridges) as opposed to point (integrated) sampling (as described in the current WP1 protocol has been discussed) with observers at the national meeting (see above). J. Wanzenböck is proposing to organize a one day workshop on this issue in February 2019 using an easy access location.

#### **France**

French partners (AFB) propose to formalize a protocol for integrative sampling for fish eDNA

French partners (INRA) propose to consolidate the protocols dedicated to plankton sampling : this could be done by comparing 2 protocols using Sterivex, with 2 options for DNA preservation (one with buffer , one with direct freezing).

#### **Germany**

Additional sampling for phytobenthos without diatoms is a traditional method for German rivers. It include more than the habitat stones and is recommended by observer 11.

Observer 10 recommended to harmonize the eDNA protocols with other regional project to have not different protocols in lowland and alpine regions.

Sampling process might be not sterile enough.

#### **Italy**

During the different samplings, collecting 1 additional eDNA sample, to store in the respective institutes as spare samples.

#### **Slovenia**

Different habitats defined by substrate, water velocity, water depth, and shading have to be sampled regarding on the ratio of habitat coverage when sampling phytobenthos.

#### **Switzerland**

The Swiss Observer highlighted the importance of extending the analysis to 4 watercourses in the catchment area of Lake Lugano, representative of different ecomorphological and qualitative states.

#### **4) interactions with ongoing networks**

##### **Austria**

Preparation of CEN-Standard for eDNA monitoring:  
current discussions on European level (cooperation with Michael Traugott, national representative through national project eDNA-AlpFisch (FFG))

COST network DNAqua (COST Action DNAqua-Net (CA15219):  
co-supervision of PhDthesis (Teofana Chonova) by R. Kurmayer and Agnes Bouchez (Cost network DNAqua), Josef Wanzenböck is applying as project observer.

ABOL Meeting, Austrian Barcode of Life, 6.-7. December 2018 in Vienna:  
Presentation of the alpine space project by Hans Rund, followed by discussions of the sampling activities with participants, i.e. Florian Leese (Cost network DNAqua), Hubert Blatterer (State of Upper Austria): For the pilot river site Steyr 4 native fish species would be expected, but only 2 fish species have been detected by traditional sampling methods (one native, one introduced). The potential value of eDNA based methods is anticipated (potential artefacts also have been highlighted, e.g. fish restaurants).

##### **France**

Preparation of CEN-Standard for eDNA monitoring: A Bouchez F Rimet INRA & AFB

COST network DNAqua (COST Action DNAqua-Net (CA15219): A Bouchez Co Chair of DNAqua Net

##### **Italy**

Interaction with COST Action DNAqua-Net (CA15219); Agnes Bouchez, member of the Project Steering Group, is vice-chair of the action.

Activities in Lake Garda are carried out in the framework of the Long-Term Research (LTER) network. The network is providing expertise (labs, personnel) and, with the collaboration with PP3 (ARPAV), logistic support in the field activities for the implementation of the project.

##### **Slovenia**

Presentation of the Eco-AlpsWater project in Expert meeting on waters in Triglav national park (15. 11. 2018, Bled, Slovenia) by Špela Remec-Rekar. Among other representatives of the Local municipality, the Ministry of the Environment and Spatial Planning and the University of Ljubljana were present. The eDNA sampling method was identified also as a good opportunity for sampling high mountains difficult accessible alpine springs and lakes.

##### **Switzerland**

The Swiss Observer will participate in a practice-oriented course “eDNA und Metabarcoding: neue Methoden für das Biomonitoring von Gewässern” at Eawag in April 2019.