Deliverable D.T3.1.1 Pilot actions plans: finetuning of approach to the 6 Alpine Space countries (6 key lakes and 5 rivers). Intermediate reports. 6,00

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from the proposal:
Innovative monitoring approaches will be fine tuned for each lake and river, taking into account peculiarities and challenges of single waterbodies and watersheds. Tuning during preparatory phase (WPT2) and stakeholder involvement (D.T3.1.2).

Pilot actions plans, finetuned for each waterbodies (lakes and rivers) in the 6 countries. Reports should be prepared by each country (internal cooperation between institutes). WP leader will coordinate the activity. This is 1 deliverable with 6 sub-reports.
The protocols described in WPT1 will be adapted to each waterbody, including information such as exact position (geographical coordinates) of stations, depths (euphotic depth), sampling equipment etc. Moreover, for each waterbody, specific details on the workflow of samples – from the field to the sequencing lab – should be provided. Moreover, besides eDNA, these reports should include the list of metadata (supporting field and lab variables, i.e. physical, chemical and biological variables). Metadata will be presented and discussed at the Mondsee meeting.

Includes adaptation of sampling protocol developed in WP1 (version 21 Dec 2018):

a) lake plankton sampling

+) Including information on sampling such as sampling date, sampling depth, type of integration, transport of samples and to be recorded parameters, possibly sampling pathogenic bacteria
+) Including information on processing, i.e filtration in the laboratory, live sample inspection in the microscope, microscopical analysis, additional parameters according to WFD/WPO
+) including information on processed sample storage (-20°C or in lysis buffer)

b) lake biofilm sampling

+) including information on sampling gradient (e.g. by eutrophication), substrate type, sampling depth below minikmum Waterlevel (as observed during the last year), actual sampling depth, transport of samples and to be recorded parameters,
+) location of processing of samples (lab vs. field), (live) microscopical inspection, microscopical analysis (type of fixation),
+) including information on processed sample storage (fixation and -20°C/fridge)

c) river biofilm sampling

+) Including information on sampling such as sampling date, sampling depth, substrate type, sampling depth below minimum Water level (as observed during the last year), actual sampling depth, transport of samples and to be recorded parameters,
+) location of processing of samples (lab vs. field), (live) microscopical inspection, microscopical analysis (type of fixation),
+) including information on processed sample storage (fixation and -20°C/fridge)

d) fish river sampling

+) Including information on sampling such as sampling date, sampling stretch, and to be recorded parameters (e.g. water level, discharge), substrate type, sampling strategy (e.g. integrated point

sampling or peristaltic pump integrated sampling), i.e. using a pump-driven integrated sampling for a sampling stretch or a water sampler with discrete samples along the stretch
+) processing of samples in the field or in the lab, e.g. filtration by a peristalitic pump or vacuum filtration,
+) storage (and transport) of samples

e) fish lake sampling

+) Including information on sampling site number, and location, sampling date, sampling stretch, and to be recorded parameters (e.g. sampling depth), for example substrate or habitat type, sampling strategy (e.g. integrated point sampling or peristaltic pump integrated sampling), i.e. using a pump-driven integrated sampling for a sampling stretch or a water sampler with discrete samples along the stretch
+) processing of samples in the field or in the lab, e.g. filtration by a peristalitic pump or vacuum filtration,
+) storage (and transport) of samples

A) Lake plankton sampling (countries in alphabetical order)

Note: In general for lake plankton sampling the collection of a spare sample for eDNA extraction is recommended. This sample aliquot should be stored at -20°C and preserved for eventual DNA extraction and metabarcoding analysis in the course of the EcoAlpsWater framework.

Austria

Key pilot site:

Lake Mondsee (site details are given in WP2 report)

Sampling:

will be performed monthly from January 2019 – December 2020 by PP2 (LFUI) according to the WP1 lake plankton sampling protocol, at the regular WFD site „Mondsee gesamt“ (at max. depth). The last regular GZÜV sampling was 2016-2018 (and is still ongoing). The relevant GZÜV data have been requested from the State government of Upper Austria (21 Dec 2018). The sampling depth is 0-20 m and the water column will be sampled (depth-integrated) using an automated integrated sampler (Hydrobios, 2 Liter volume). Metalimnetic nuisance algae *Planktothrix rubescens* will be included. We will sample also pathogenic bacteria (July, Aug, Sept) and the samples will be sent to PP8 (AGES).

Metadata:

Additional parameters include Chlorophyll a, Phytoplankton species + biovolume as estimated from Lugol fixed samples, TP, cyanotoxins, Secchi depth, physical parameters through the water column (oxygen, temperature, pH, Chl. A and Phycoerythrin fluorescence) monitored by a YSI multiprobe.

Sample Processing:

Samples will be transported to the laboratory and the total transport time until filtration in the laboratory is estimated with 2 h. Standard vacuum filtration with vacuum pressure control (< 0.4 bar) is available. Microscopical inspection of live plankton net sample (semiquantitative) will be performed on a routine basis. Microscopical Phytoplankton counting of Lugol samples will be subcontracted. DNA samples will be stored at -20°C.

France

Key pilot site:

Lake Bourget (site details are given in WP2 report)

Sampling:

Will be performed monthly from January 2019 – December 2020 by PP6 (INRA) according to the WP1 lake plankton sampling protocol, at the regular WFD site (point ‘B’ (E 5° 51’ 35,7’’, N 45° 44’ 49,7’’) located in the center of lake, at the maximum depth) . The regular WFD sampling is done 4 times per year

; however additional sampling is organized each month since 2004 by the Observatory on Alpine Lakes (INRA CARRTEL) and is still ongoing. The relevant data are available from Observatory on Alpine Lakes (https://www6.inra.fr/soere-ola/). The sampling depth is 0-18 m and the water column will be sampled depth-integrated using an automated integrated sampler (Hydrobios, 5 Liter volume). Metalimnetic nuisance algae *Planktothrix rubescens* is measured and samples for cyanotoxins can be sampled when it is relevant.(i.e. according to the presence of cyanobacterial pigments in the metalimnion) . Nointerest in pathogenic bacteria sampling.

Metadata:

Additional parameters include Chlorophyll a, Phytoplanktonspecies + biovolume as estimated from Lugol fixed samples, , Secchi depth, physical and chemical parameters through the water column (oxygen, temperature, pH, Chl. A, conductivity, NH_4^+, NO_3^-, Ntot, TOC, PO_4^{3-}, Ptot, SiO_2, SO_4^{2-}, Cl^-, $Ca2^+$, Mg^{2+}, K^+, Na^+ and Phycoerythrin fluorescence) monitored by a multiprobe and laboratory analyses.

Sample processing:

In the absence of buffer, the water samples will be transported to the laboratory (approximately 6 hours between the sampling time and the filtration time), to be filtered and then frozen directly. In parallel, for one year, another sample will be preserved in lysis buffer to allow the comparison between the two methods of preservation (February 2019 to December 2019). Microscopical inspection of live plankton net sample (semiquantitative) will be performed on a routine basis. Microscopical phytoplankton counting of Lugol samples will be performed in house by PP6. Standard vacuum filtration with vacuum pressure control (<0.4 bar) is available. DNA samples will be stored at -20°C.

Germany

Key pilot site:

Lake Starnberg (site details are given in WP2 report)

Sampling:

Will be performed monthly from January 2019 – December 2020 by PP10 (LfU) according to the WP1 lake plankton sampling protocol, at the regular WFD site at max. depth. The last regular WFD sampling was in the course of BP2 in 2017. The relevant WFD data is available from PP10. The sampling depth is 0-20 m according to Bavarian WRRL guideline and the water column will be sampled (depth-integrated) using an automated integrated sampler (2.5 Liter total volume). Significant metalimnetic layer formed by *P. rubescens* is expected during summer and autumn mainly in 10-15m depths. No interest in pathogenic bacteria sampling.

Metadata:

Additional parameters include Chlorophyll a, Phytoplankton species + biovolume as estimated from Lugol fixed samples, Diatom taxa based on diatom valves preparation, TP, PO4-P, NH4-N, NO3-N, TN, Oxygen, temperature, Secchi depths, pH, conductivity, ICPOS: Ca, Fe, Na, Mn, Mg, Sulphate.

Sample Processing:

Samples will be transported to the laboratory and the total transport time until filtration in the laboratory is estimated with 2 h. Standard vacuum filtration with vacuum pressure control (< 0.4 bar) is available. In parallel, for one year, another sample will be preserved in lysis buffer to allow the comparison between the two methods of preservation (January 2019 to December 2019). Live inspection of plankton net sample (semiquantitative) will be performed on a routine basis. Microscopical phytoplankton counting of Lugol samples will be performed inhouse by PP10. DNA samples will be stored at -20°C.

Italy

Key pilot site:

Lake Garda (site details are given in WP2 report)

Sampling:

will be performed monthly from January 2019 – December 2020 by PP3 (ARPAV) according to the WP1 lake plankton sampling protocol, at the regular WFD site. The last regular WFD phytoplankton sampling was from 2014-2016. The relevant WFD data is available from PP3. Sampling is performed according to the Italian Manuals and Guidelines 111/2014: Biological methods for inland surface waters, issued by ISPRA. The sampling depth is 0-20 m and the water column will be sampled (depth-integrated) using an automated integrated sampler (5l, going to be purchased). Until now for WFD sampling a tube sampler has been used. No interest in pathogenic bacteria sampling.

Metadata:

Additional parameters include Chlorophyll a, Phytoplankton species and biovolume (from Lugol fixed samples), TP, nutrients (P+N), Oxygen, Transparency, cyanotoxins (microcystins), and relevant parameters as highlighted from the ecological status assessments.

Sample Processing:

The filtration for DNA will be done on the boat. No routine live inspection of plankton net samples. Microscopical phytoplankton counting of Lugol samples will be performed inhouse by PP3.

Slovenia

Key pilot site:

Lake Bled (site details are given in WP2 report)

Sampling:

will be performed monthly from January 2019 – December 2020 by PP5 (ARSO) according to the WP1 lake plankton sampling protocol, at the regular WFD site in the West basin (ZK), Gauss Kruger coordinates Y 430175 ; X 135820. The last regular WFD sampling was performed 2016-2018 and is

still ongoing. The relevant WFD data is available from PP5 (i.e. all basic phytoplankton parameters i.e. species list, biovolume of each species and chlorophyll-a concentration).

Sampling is in accordance with national and international standards and protocols, from the euphotic zone (= 2,5 x Secchi depth) during physically stratified conditions, or during homothermic conditions from 0-20 m using an automated depth-integrated sampler (Hydrobios, 5 Liter volume). Deep chlorophyll maximum will be sampled (by using a chlorophyll -a fluorescent probe measurement before sampling). Metalimnetic layer formed by nuisance algae *P. rubescens* is expected during summer and autumn.

Metadata:

Additional parameters include chlorophyll-a, phytoplankton species list and biovolume determined from the alive samples, (samples for counting and measurements are fixed with formaldehyde), diatom taxa based on diatom valves preparation, TP, dissolved orthophosphate (PO4), nitrate (NO3), ammonium (NH4), total nitrogen (N), oxygen, temperature, Secchi depths, pH, conductivity and relevant parameters as highlighted from previous ecol. status assessments.

Sample Processing:

e- DNA filtration will be carried out on the boat in the field. Filters (Sterivex) will be stored at -20°C and frozen transported in cooling box to the laboratory of PP4 (NIB) in 1 h. Species composition of plankton net sample and phytoplankton quantitative analyse will be performed according to the national methodology. Microscopical phytoplankton counting of formaldehyde fixed samples will be performed in-house by PP5 ARSO. Standard vacuum filtration with vacuum pressure control (< 0.4 bar) is not available.

Switzerland

Key pilot site:

Lake Lugano (site details are given in WP2 report)

Sampling:

will be performed monthly from January 2019 – December 2020 by PP12 (SUPSI) according to the WP1 lake plankton sampling protocol, at the regular WPO site “Gandria” which is the long-term limnological station for CIPAIS programme. The regular WPO sampling is ongoing and the relevant WPO data is available from PP12. The sampling depth is 0-20 m and the water column will be sampled (depth-integrated) using an integrated sampler (Schröder bottle). Metalimnetic nuisance algae *P. rubescens* will be included. Pathogenic bacteria will be sampled (July, Aug, Sept) and the samples will be sent to PP8 (AGES).

Metadata:

Additional parameters include Chlorophyll a, Phytoplankton species + biovolume as estimated from Lugol fixed samples, nutrients.

Sample Processing:

Samples will be transported to the laboratory and the total transport time until filtration in the laboratory is estimated with 2 h. Standard vacuum filtration with vacuum pressure control (< 0.4 bar) is available. Live inspection of plankton net sample will be performed on a routine basis. Microscopical phytoplankton counting of Lugol samples will be performed inhouse by PP12. DNA samples will be stored at -20°C.

B) Lake biofilm sampling (countries in alphabetical order)

Austria

Key pilot site:

Lake Mondsee (details are given in WP2 report)

Sampling:

will be performed once a year in summer 2018 and 2019 by PP2 (LFUI) according to the WP1 lake biofilm sampling protocol. No regular WFD sampling of lake biofilms is performed. The last biofilm sampling with focus on diatom assemblage was in 2004 (Poulickova et al. 2004, Eur J. Phycol. 39/2:143-153). Previous data are not available. Records on annual minimum water level are available from the hydrographical service of the state government of Upper Austria. Meteorological data (recent wind activity, precipitation = 2 weeks before sampling date) are recorded online and made available by ZAMG (Austrian central service of Meteorology). Ten sampling stations distributed over the entire lake shore have been defined to sample an eutrophication gradient as wide as possible.

Middle sized stones (64-256 mm) constitute the expected substrate. No difficulty to find stones for each of the stations. An underwater glass will be used (Aquascope) to sample in areas that are mostly free of submerse macrophytes (incl. charophytes) as well as free of emerse vegetation.

Metadata:

Additional parameters include TP, and standard physical parameters (oxygen, conductivity, temperature), as well as meteorological data (2 weeks before the sampling data) and annual water level variability, cyanotoxins, and phytobenthos taxa list (relative frequencies) from formaldehyde-fixed samples

Sample Processing:

Samples stones will be put into one-way plastic bags and transported to the laboratory in a cooling box and the total transport time until processing in the laboratory will be < 4h. Microscopical Phytobenthos counting (incl diatoms) will be subcontracted. Fixed DNA samples will be stored at 4°C in the dark.

France

Key pilot site:

Lake Bourget (details are given in WP2 report)

Sampling:

will be performed once a year in summer 2018 and 2019 by PP6 (INRA) according to the WP1 lake biofilm sampling protocol. No regular WFD sampling of lake biofilms is performed. The last biofilm sampling with focus on diatom assemblage was in 2016 (Rivera et al., 2018, Hydrobiologia. 807/1:37-51)Lake biofilm sampling will include a pollution (eutrophication) gradient induced by the Leysse River (and sampling stations will be arranged accordingly on both sides of the River income).

Previous data are not available. Records on annual minimum water level are available from Hydroreel, the French server of real-time hydrometric data (https://www.rdbrmc.com/hydroreel2/station.php?codestation=698). Meteorological data (recent wind activity, precipitation = 2 weeks before sampling date) are available fromMeteo France (the Official Service of Meteorology and Climatology in France).

Middle sized stones (64-256 mm) constitute the expected substrate. No difficulty to find stones for each of the stations.

Metadata:

Additional parameters include NH_4^+, NO_3^- , Ntot, PO_4^{3-}, Ptot, TOC, DCO, Ca^{2+}, K^+ , Mg^{2+}, Na^+, Cl^- SO_4^{2-}, total and organic suspended matter, SiO_2, Chla, CAROT, pheopigments, and standard physical parameters (oxygen, conductivity, temperature). As well as meteorological data (2 weeks before the sampling data) and annual water level variability, , and diatom taxa list (relative frequencies) from fixed samples (diatom valves preparations).

Sample processing

Sampled stones will be brushed in the field and brushed algae DNA will be preserved immediately in ethanol. Stones will be transported to the laboratory for additional microscopical analysis of fixed samples performed by PP6. Total sampling and transport time is estimated with 6-8 h. Fixed DNA samples will be stored at 4°C in the dark.

Germany

Key pilot site:

Lake Starnberg (details are given in WP2 report)

Sampling:

will be performed once a year in July 2019 and 2020 by PP10 (LfU) according to the WP1 lake biofilm sampling protocol. Thirty regular WFD sampling transects will be chosen which have been regularly sampled in the course of BP2 in 2017. The relevant WFD data is available from PP10. Records on

annual minimum water level are available from the hydrographical service, www.gkd.bayern.de (in 2017 the range in water level variation was 0.39 m). Meteorological data (recent wind activity, precipitation = 2 weeks before sampling date) are available from meteorological station near Starnberger See. To sample a gradient of eutrophication as wide as possible thirty sampling stations will be used, which are distributed over the entire lake.

Previous data from recent site protocols from transects will be used to find the locations without macrophytes growth and to improve the site protocol information. We will check underwater for submerse macrophytes growth when sampling. As a substrate cobbles and mid- sized stones will be sampled; no difficulties to find stones are expected. In summer of both years, also benthic algae between macrophytes areas will be preserved in 10 additional samples to improve habitat sampling of potential toxic cyanobacteria such as *Trichonema* (May to October) with microscopic analysis and eDNA samples.

Metadata:

Additional parameters include classes of riparian shading; substrate classes; routine physiographical protocol including: Water temperature; oxygen, pH, conductivity at site. SRP, TP and nitrate from available routine monthly sampling at main station as well as meteorological data (2 weeks before the sampling data) and annual water level variability, and benthic diatoms taxa lists (relative frequencies) from fixed samples (diatom valves preparations).

Sample Processing:

Sampled stones will be brushed in the field and brushed algae DNA will be preserved immediately. Stones will be transported to the laboratory for additional microscopical analysis fresh and fixed samples (Lugol) performed by PP10. Total sampling and transport time is estimated with 4-6 h. Fixed DNA samples will be stored at 4°C in the dark.

Italy

Key pilot site:

Lake Garda (details are given in WP2 report)

Sampling:

will be performed once a year in summer 2019 and 2020 by PP3 (ARPAV) according to the WP1 lake biofilm sampling protocol. Regular sites will be used and the last WFD Diatoms Sampling was performed during 2014-2016, according to UNI EN 13946:2014 (Water quality – Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes) and the Italian Manuals and Guidelines 111/2014 (Biological methods for inland surface waters, issued by ISPRA). The relevant WFD data is available from PP3. Records on annual minimum water level are available from Agenzia Interregionale per il fiume Po (A.I.PO) - Ufficio di Mantova. No records on recent (2 weeks before sampling date) wind activity from (nearby) meteorological stations are available. Ten sampling stations distributed over the entire lake shore have been defined to sample an eutrophication gradient as wide as possible.

For each station a selection of 5 cobbles or 5 small boulders or 10 pebbles is collected (preferably without filamentous algae, otherwise these are removed). No difficulty to find stones for each of the stations are expected. We watch underwater without aquascope to sample diatoms in areas that are mostly free of submerse macrophytes (incl. charophytes), we don't need aquascope for Lake Garda.

Metadata:

Additional parameters include chemical-physical parameters (pH, DO, cond, T°C), annual water level variability, cyanotoxins, and diatom taxa list (relative frequencies) from samples (diatom valves preparations).

Sample Processing:

Sampled stones will be brushed in the field and brushed algae DNA will be preserved immediately. Samples will be transported in the dark and in a cooling box to the laboratory, and treated for the microscopical analysis , performed by PP3. A part of samples will be fixed with ethanol. Total sampling and transport time is estimated in 2-- 6h. Fixed DNA samples will be stored at 4°C in the dark.

Slovenia

Key pilot site:

Lake Bled (details are given in WP2 report)

Sampling:

Will be performed once a year in summer (June – September) 2019 and 2020 by PP5 (ARSO) according to the WP1 lake biofilm sampling protocol. Three regular sampling stations are included in national monitoring, all of which will be used also in Eco-AlpsWater project. Additional 7 sampling stations were selected. Among those, 5 were regularly monitored until 2009. The last WFD diatom sampling was performed at 7 Sept 2016 by PP4 (NIB) and the relevant WFD data is available from PP5. Records on annual minimum water level are available from national hydrographical service. Meteorological data (recent wind activity, precipitation = 2 weeks before sampling date) are available from meteorological station at Lesce- Bled. The sampled ecological gradient will include eutrophication pressure.

We will sample middle sized stones (cobbles and very coarse gravel) from 5 to 20 cm. We don't expect difficulty to find suitable substrate. We don't use Aquascope. According to national protocol phytobenthos sampling is performed up to 60 cm depth. Moreover, lake water is clear and thus submersed macrophytes (incl. charophytes?) can easily be seen when sampling.

Metadata:

Additional parameters at each station include water temperature, pH, conductivity, oxygen content and saturation are measured on the field with transportable WTW multimeter, as well as meteorological data (2 weeks before the sampling date) and annual water level variability. TP,

dissolved orthophosphate (PO4), nitrate (NO3), ammonium (NH4), total nitrogen (N), can be used from the phytoplankton sampling. Diatom taxa based on diatom valves preparation.

Sample Processing:

Sampled stones will be brushed in the field and brushed algae DNA will be preserved immediately and transported in the refrigerator box. Transport from the field to the lab will take few hours, depending on the location of the sampling station. For example, there is one hour drive from the Lake Bled to the lab. However, sampling at 10 stations will take some time. So there will be one hour transport from the last sampled station and approximately 8-10 hours transport from the first sampled station.

Switzerland

Key pilot site:

Lake Lugano (details are given in WP2 report)

will be performed once a year in summer 2019 and 2020 by PP12 (SUPSI) according to the WP1 lake biofilm sampling protocol. No regular WFD sampling of lake biofilms is performed. Previous data are not available. Records on annual minimum water level are available from CIPAIS ProgrammeMeteorological data (recent wind activity, precipitation = 2 weeks before sampling date) are available from meteorological station at Lugano. Ten sampling stations distributed over an eutrophication gradient will be defined.

Middle sized stones (cobbles and very coarse gravel from 5 to 20 cm) will be sampled. No difficulty to find stones for each of the stations. An underwater glass will be used (Aquascope) to sample in areas that are mostly free of submerse macrophytes (incl. charophytes).

Metadata:

Additional parameters include chemical-physical parameters (pH, DO, cond, T°C), annual water level variability, as well as meteorological data (2 weeks before the sampling data), cyanotoxins, and diatom taxa list (relative frequencies) from fixed samples (diatom valves preparations).

Sample Processing:

Sampled stones will be brushed in the field and brushed algae DNA will be preserved immediately. Microscopical analysis of fixed samples will be subcontracted. Total sampling and transport time is estimated with 2h. Fixed DNA samples will be stored at 4°C in the dark.

C) **River biofilm sampling (countries in alphabetical order**)

Austria

Key pilot site:

River Steyr (details are given in WP2 report)

Sampling:

will be performed once a year in summer 2019 and 2020 by PP2 (LFUI) according to the WP1 river biofilm sampling protocol at the 3 regular WFD monitoring sites (Steyr_Hinterstoder, Steyr_Schrattentalerbruecke, Steyr_Schmiedleiten). The last regular GZÜV sampling was at 27 June 2015 and the relevant GZÜV data have been requested from the State government of Upper Austria.

Records on naturally low water level are available from the hydrographical service of the state government of Upper Austria. Meteorological data (recent precipitation = 2 weeks before sampling date) are made available by ZAMG (Austrian central service of Meteorology).

Middle sized stones (64-256 mm) constitute the expected substrate. No difficulty to find stones for each of the stations. Sample stations are free of submerse macrophytes (incl. charophytes) as well as free of emerse vegetation.

Metadata:

Additional parameters include TP, and standard physical parameters (oxygen, conductivity, temperature), as well as meteorological data (2 weeks before the sampling data) and annual water level variability, cyanotoxins, and phytobenthos taxa list (relative frequencies) from formaldehyde-fixed samples.

Sample Processing:

Sample stones will be put into one-way plastic bags and transported to the laboratory in a cooling box and the total transport time until processing in the laboratory will be 2h. Microscopical Phytobenthos counting (incl diatoms) will be subcontracted. Fixed DNA samples will be stored at 4°C in the dark.

<u>France</u>

Key pilot site:

Drôme River (details are given in WP2 report)

Sampling:

will be performed once a year in summer 2018 and 2019 by a private office (SAGE environnement) mandated by the French Water Agencyaccording to the WP1 river biofilm sampling protocol at the 4 regular WFD monitoring sites. Sites have been sampled previously, every year since 2008http://sierm.eaurmc.fr/surveillance/eaux-superficielles/liste-stations.php?donnees=signaletique&codeRegion=&codeDept=&codeCommune=&bassin=&sousBassinVersant=&coursdeau=DROME

Records on naturally low water level are available from Hydroreel, the French server of real-time hydrometric data (https://www.rdbrmc.com/hydroreel2/listestation.php?dep=26). Meteorological

data (recent precipitation = 2 weeks before sampling date) are made available by Meteo France (the Official Service of Meteorology and Climatology in France).

Middle sized stones (64-256 mm) constitute the expected substrate. No difficulty to find stones for each of the stations. Sample stations are free of submerse macrophytes (incl. charophytes) as well as free of emerse vegetation.

Metadata:

Additional parameters include Ptot, Ntot, sulphates, calcium, magnesium, potassium, sodium, bicarbonates, nitrates, nitrites, ammonium, phosphates, chlorophyl a, pheopigments, , and standard physical parameters (oxygen, conductivity, temperature, pH), as well as meteorological data (2 weeks before the sampling data) and annual water level variability, , and diatom taxa list (relative frequencies) from fixed samples (diatom valves preparations).

Sample processing

Sampled stones will be brushed in the field and brushed algae DNA will be preserved immediately in ethanol. Stones will be transported to the laboratory for additional microscopical analysis of fixed samples performed by the DREAL (French Regional Directions for the Environment, Planning and Housing) and the SAGE (Mixed Syndicate of the Drôme River) . Total sampling and transport time is estimated with 4-6 h. Fixed DNA samples will be stored at 4°C in the dark.

Germany

Key pilot site:

River Wertach (details are given in WP2 report)

Sampling:

Will be performed once a year in July 2019 and 2020 by PP10 (LfU) according to the WP1 river biofilm sampling protocol at two regular WFD monitoring stations (Wertach, Ettringer Wehr, Unterwasser; Brücke Görrisried-Wald) and at least 8 additional stations including 2 Interreg HyMoCares sites near city Augsburg. The last regular WFD sampling was at 31 Aug 2017 in the course of BP2 in 2017. The relevant WFD data is available from PP10.

Records on naturally low water level are available from the hydrographical service for current and previous years (www.gkd.bayern.de; gauge: tuerkheim-12406008). Records will be used to guide the sampling date (summer floods might change the sampling plan). Meteorological data (recent precipitation = 2 weeks before sampling date) are available from station Kirchheim (https://www.gkd.bayern.de/en/meteo/precipitation/iller_lech/kirchheim-200137)

Middle sized stones (64-256 mm) constitute the expected substrate. No difficulty to find stones for each of the stations. It is unknown, if complete stretches of additional stations are free of submerse

macrophytes (incl. charophytes) as well as free of emerse vegetation, but stones will be selected from free areas.

Metadata:

Additional parameters include classes of riparian shading; substrate classes; the routine physiographical protocol including: Water temperature; oxygen, pH, conductivity. Nutrients SRP, TP, Nitrate and other parameters are measured monthly at main station Ettringer Wehr.

Meteorological data (2 weeks before the sampling data) and annual water level variability are available. Phytobenthos (diatoms and other phytobenthos) taxa list (relative frequencies) from fixed samples (diatom valves preparations).

Sample Processing:

Sampled stones will be brushed in the field and brushed algae DNA will be preserved immediately. Stones will be transported to the laboratory for additional microscopical analysis of frehs and fixed samples (Lugol) performed by PP10. Total transport time is estimated with 3-5 h. Fixed DNA samples will be stored at 4°C in the dark.

Italy

Key pilot site:

River Adige (details are given in WP2 report)

Sampling:

will be performed once a year in late spring to early summer 2019 and 2020 by PP3 (ARPAV) according to the WP1 river biofilm sampling protocol at 3 regular WFD monitoring sites (Ponte Adige-BZ, Pescantina and Zevio-VR). The last WFD (Diatoms) sampling was performed during 2014-2016, according to UNI EN 13946:2014 Water quality – Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes and the Italian Manuals and Guidelines 111/2014: Biological methods for inland surface waters, issued by ISPRA. The relevant WFD data is available from PP3.

Records on naturally low water level are available from PP3for the previous year, and will be used to guide the sampling date (summer floods might change the sampling plan). Meteorological data (recent precipitation = 2 weeks before sampling date) are available from PP3

For each station a selection of 5 cobbles or 5 small boulders is collected (preferably without filamentous algae, otherwise these are removed). We will sample in areas that are mostly free of submerse macrophytes and emerse vegetation

Metadata:

Additional parameters include chemical-physical parameters (pH, DO, cond, T°C), annual water level variability, cyanotoxins, and diatom taxa list (relative frequencies) from samples (diatom valves preparations).

Sample Processing:

Sampled stones will be brushed in the field and brushed algae DNA will be preserved immediately. Samples will be transported in the dark and in a cooling box to the laboratory, and treated for the microscopical analysis , performed by PP3. A part of samples will be fixed with ethanol. Total sampling and transport time is estimated in 2-- 6h. Fixed DNA samples will be stored at 4°C in the dark.

Slovenia

Key pilot site:

River Soča (details are given in WP2 report)

Sampling:

Will be performed once a year in summer (June – September) 2019 and 2020 by PP5 (ARSO) according to the WP1 river biofilm sampling protocol at three regular sampling stations (sampling site Spodnja Trenta, Kamno and Solkanski jez). The last regular WFD sampling was at 18 Jun 2018 in sampling site Spodnja Trenta, 2 Aug 2012 in sampling site Kamno and 18 Jul 2017 in sampling site Solkanski jez. The relevant WFD data is available from PP5.

Records on naturally low water level are available from national hydrographical service. Meteorological data (recent precipitation = 2 weeks before sampling date) are made available from meteorological station at Tolmin. Cobbles and very coarse gravel (5-20 cm) constitute the expected substrate. We expect no difficulty to find stones. Sample stations are free of submersed macrophytes (incl. charophytes) as well as free of immersed vegetation.

Metadata:

Additional parameters at station include Water and air temperature, pH, conductivity, dissolved oxygen and oxygen saturation will be measured on field with transportable WTW multimeter. Suspended solid, COD (Mn and Cr), BOD5, TOC, total nitrogen, ammonium, nitrite, nitrate, sulphate, chloride, total phosphorus, phosphate, SiO2, calcium, magnesium, sodium, potassium, total hardness and carbonate hardness. Meteorological data (2 weeks before the sampling data) and annual water level variability. Diatom taxa list (relative frequencies) from fixed samples (diatom valves preparations).

Sample Processing:

Sampled stones will be brushed in the field and brushed algae DNA will be preserved immediately and transported in a refrigerator box. Transport from the field to the lab will take 2 h. Fixed DNA samples will be stored at 4°C in the dark.

D) fish river sampling (countries in alphabetical order)

Austria

Key pilot site:

River Steyr (details are given in WP2 report)

Sampling:

will be performed during low water period once a year in summer/autumn 2019 and 2020 by PP2 (LFUI) according to the WP1 river fish sampling protocol at one regular WFD monitoring sites (Steyr_Schrattentalerbruecke, or Steyr_Schmiedleiten). The last regular GZÜV sampling was at 27 June 2015 and the relevant GZÜV data have been requested from the State government of Upper Austria.

Records on naturally low water level are available from the hydrographical service of the state government of Upper Austria. Meteorological data (recent precipitation = 2 weeks before sampling date) are made available by ZAMG (Austrian central service of Meteorology).

All different habitats in the given stretch will be covered (i.e. ten (10) individual point samples along the regular river stretch (100m) using a water sampler (Schindler-Patalas). No (carry on) peristaltic pump based integrated sampling equipment is available.

Metadata:

Additional parameters include standard physical parameters (oxygen, conductivity, temperature), as well as meteorological data (2 weeks before the sampling data) and annual water level variability.

Sample Processing:

The filtration for DNA will be done in the field. And Sterivex filters will be transported frozen to the laboratory of PP2 in 2 h. DNA samples will be stored at -20°C.

France

Key pilot site:

Drôme River (details are given in WP2 report)

Sampling:

will be performed during low water period once a year in summer 2019 by PP6 (INRA) according to the WP1 river fish sampling protocol at 10 regular WFD monitoring sites. According to the WFD

monitoring fish survey. Sites have been sampled previously by the National Institute for Research in Science and Technology for the Environment and Agriculture (DREAL) every five years.

Records on naturally low water level are available from Hydroreel, the French server of real-time hydrometric data (https://www.rdbrmc.com/hydroreel2/listestation.php?dep=26). Meteorological data (recent precipitation = 2 weeks before sampling date) are made available by Meteo France (the Official Service of Meteorology and Climatology in France).

One sample per station will be obtained. Different habitats in the given stretch will be covered using integrated sampling using filter capsules (i.e. compiling ten (10) individual point samples along the regular river stretch (100m)).

Metadata:

Additional parameters include standard physical parameters (oxygen, conductivity, temperature, pH), as well as meteorological data (2 weeks before the sampling data) and annual water level variability.

Sample processing

The filtration for DNA will be done in the field. The filters will be preserved with lysis buffer at 4 ° C until transport to the laboratory at the end of the sampling (2 days). DNA samples will be stored at -20°C.

Germany

Key pilot site:

River Wertach (details are given in WP2 report)

Sampling:

will be performed during low water period once in October 2019 by PP7(LfL) according to the WP1 river fish sampling protocol at two sampling stretches (Görisried; Tuerkheim uh. Wehr). Species composition is different between the two regular WFD monitoring stations. The last regular WFD sampling was for Görisried (3063) in Sept/Oktober 2018; and for Tuerkheim uh. Wehr in Okt/Nov 2018 by PP10 (LFU). The relevant WFD data have been requested.

Records on naturally low water level are available from the hydrographical service for the previous year. Meteorological data (recent precipitation = 2 weeks before sampling date) are available from ….

All different habitats in the given stretches will be covered (i.e. by distributing 5 individual point samples along the regular river stretch (100m). No (carry on) peristaltic pump based integrated sampling equipment is available.

Metadata:

Additional parameters include standard physical parameters (oxygen, conductivity, temperature), as well as meteorological data (2 weeks before the sampling data) and annual water level variability.

Sample Processing:

The filtration for DNA will be done in the field. And Sterivex filters will be transported frozen to the laboratory of PP2 in 1 h. DNA samples will be stored at -20°C.

Italy

Key pilot site:

River Adige (details are given in WP2 report)

Sampling:

will be performed during low water period once a year in summer 2019 and 2020 by PP3 (ARPAV) according to the WP1 river fish sampling protocol at 10 (?) regular WFD monitoring sites (…). No data on previous WFD fish survey are available from PP3 but have been requested from ….

Records on naturally low water level are available from the hydrographical service for the previous year. Meteorological data (recent precipitation = 2 weeks before sampling date) are available from ….

One sample per station will be obtained. Different habitats in the given stretch will be covered using integrated sampling (i.e. compiling 10(?) individual point samples along the regular river stretch (100m). No (carry on) peristaltic pump based integrated sampling equipment is available.

Metadata:

Additional parameters include standard physical parameters (oxygen, conductivity, temperature), as well as meteorological data (2 weeks before the sampling data) and annual water level variability.

Sample Processing:

The filtration for DNA will be done in the field. And filters will be transported to the laboratory of PP6 within …. h. DNA samples will be stored at -20°C.

Slovenia

Key pilot site:

River Soča (details are given in WP2 report)

Sampling:

Will be performed during low water period once a year in summer (June – September) 2019 and 2020 by PP5 (ARSO) according to the WP1 river fish sampling protocol at two regular sampling stations (sampling site Spodnja Trenta and Kamno). The last regular WFD sampling was in July 2017. The relevant WFD data from traditional monitoring is available from Observer 22 (Fisheries Research Institute of Slovenia).

Records on naturally low water level are available from the hydrographical service for the previous year. Meteorological data (recent precipitation = 2 weeks before sampling date) are available from meteorological station at Tolmin.All different habitats in the given stretches will be covered (i.e. by distributing 10 individual point samples along the regular river stretch (100m). No (carry on) peristaltic pump based integrated sampling equipment is available.

Metadata:

Additional parameters include standard physical parameters (pH, oxygen, conductivity, temperature) measured on the field with transportable WTW multimeter. Suspended solid, COD (Mn and Cr), BOD5, TOC, total nitrogen, ammonium, nitrite, nitrate, sulphate, chloride, total phosphorus, phosphate, SiO2, calcium, magnesium, sodium, potassium, total hardness and carbonate hardness. Meteorological data (2 weeks before the sampling data) and annual water level variability.

Sample Processing:

The filtration for DNA will be done in the field. Filters will be transported to the laboratory of PP4 within 2 h on ice in cooling box. DNA samples will be stored at -20°C.

E) fish lake sampling (countries in alphabetical order)

Austria

Key pilot site:

Lake Mondsee (site details are given in WP2 report)

Sampling:

will be performed in summer/autumn 2019 + 2020 by PP2 (LFUI) according to the WP1 lake fish sampling protocol (High effort), at sampling sites according to the CEN guideline for exposure of fishing nets + electro fishing stretches to cover all lake habitats. The last regular GZÜV sampling was 2010 and the next regular GZÜV sampling is performed in autumn 2019 by observer 1 (BAW Scharfling). The relevant GZÜV data have been requested from the State government of Upper Austria (21 Dec 2018).

The high effort lake sampling protocol will consist of point sampling along with gill net sites and electrofishing sites, i.e. for Mondsee this will make 14 (littoral) + 64 (benthic) + 6 (pelagic) = 84 point samples (= sampling stations), taken with an automated integrated water sampler, Hydrobios, 2L volume). The sampler will be rinsed with sample water between the sampling stations. No peristaltic pump based integrated sampling equipment is available.

Metadata:

Additional parameters include standard physical parameters (oxygen, conductivity, temperature), as well as meteorological data (2 weeks before the sampling data) and annual water level variability and habitat related parameters (water depth, macrophytes, etc.).

Sample Processing:

The filtration for DNA will be done on the boat (?) in the field. And Sterivex filters will be transported frozen to the laboratory of PP2 within 2h. DNA samples will be stored at -20°C.

France

Key pilot site:

Lake Bourget (site details are given in WP2 report)

Sampling:

Lake Bourget (site details are given in WP2 report)

Sampling:

Will be performed in October 2018 and 2019 by PP6 (INRA) according to the WP1 lake fish sampling protocol (High effort)... The sampling effort is inspired from the CEN standard for fish survey (littoral electrofishing + benthic and pelagic gillnets sampling) and included pelagic (11 stations) and benthic (34 stations), littoral (28 stations pooled in 4 samples) and integrated samples (5). The last regular WFD sampling was done in autumn 2018 and is done every year. The relevant WFD data are available from Observatory of Alpine Lakes (https://www6.inra.fr/soere-ola/)…

Metadata:

Additional parameters include meteorological data (2 weeks before the sampling data) and annual water level variability and habitat related parameters (water depth, , etc.)

Sample Processing:

the filtration will be done on the shore of the lake (equipped van) within 2 hours after samplingAnd Sterivex filters filled with lysis buffer will be transported to the laboratory at the end of the sampling (2.5 days). Filters are kept at 4 ° C until transport to the laboratory DNA samples will be stored at -20°C in the laboratory.

Germany

Key pilot site:

Lake Starnberg (site details are given in WP2 report)

Sampling:

will be performed in September 2019 by PP7 (LfL) according to the WP1 lake fish sampling protocol (High effort), at sampling sites according to the CEN guideline for exposure of fishing nets + electro fishing stretches to cover all lake habitats. There was no regular WFD sampling previously but WFD Fish sampling will be performed in parallel incl gill nets, electrofishing and hyroacoustics. The lake

will be divided in 3 smaller parts, each including 64 benthic nets (192 nets in total) and one pelagic net at the deepest point.

The high effort lake sampling protocol will consist of point sampling along with gill net sites and electrofishing sites, i.e. for Starnberg this will make … = … point samples (= sampling stations), taken with an automated integrated water sampler, Hydrobios, 2L volume). The sampler will be rinsed with sample water between the sampling stations. No peristaltic pump based integrated sampling equipment is available.

Metadata:

Additional parameters include standard physical parameters (oxygen, conductivity, temperature), as well as meteorological data (2 weeks before the sampling data) and annual water level variability and habitat related parameters (water depth, macrophytes, etc.)

Sample Processing:

The filtration for DNA will be done on the boat (?) in the field. And Sterivex filters will be transported frozen to the laboratory of PP7 within 2h. DNA samples will be stored at -20°C.

Italy

Key pilot site:

Lake Garda (site details are given in WP2 report)

Sampling:

will be performed in summer/autumn 2019 + 2020 by PP3 (ARPAV) according to the WP1 lake fish sampling protocol (minimum effort). The last regular WFD based monitoring was performed during the Survey of fish fauna of the Alpine Lakes in Lombardia 2012-2015, carried out by CNR-ISE and other research institutions.

The minimum effort lake sampling protocol is based on the minimum Number of samples scaled to the lake surface area and related to electrofishing sites as foreseen by the CEN Standard. In total 10 stations = Pelagic (5 stations) and shores (5 stations) will be sampled depth-integrated using an automated integrated water sampler, Hydrobios, 2L volume). The sampler will be rinsed with sample water between the sampling stations. No peristaltic pump based integrated sampling equipment is available.

Metadata:

Additional parameters include standard physical parameters (oxygen, conductivity, temperature), as well as meteorological data (2 weeks before the sampling data) and annual water level variability and habitat related parameters (water depth, macrophytes, etc.)

Sample Processing:

The filtration for DNA will be done in laboratory.

Standard vacuum filtration with vacuum pressure control (< 0.4 bar) is available in laboratory. DNA samples will be stored at -20°C.

And Sterivex filters will be transported frozen to the laboratory of LP within 2h. DNA samples will be stored at -20°C.

Slovenia

Key pilot site:

Lake Bled (site details are given in WP2 report)

Sampling:

Will be performed once in a year (September, October) 2019 by PP5 (ARSO) according to the WP1 lake fish sampling protocol (minimum effort). The last regular WFD fish sampling (CEN norm) was done in September 2018 and September 2014 by Observer 22 (Fisheries Research Institute of Slovenia). The relevant WFD data from traditional monitoring is available.

The minimum effort protocol includes pelagic (2 stations) and shores (10 stations), to be sampled depth-integrated using an automatic integrated sampler (HydroBios 5L) or Van Dorn depth point sampler. No peristaltic pump based integrated sampling equipment is available.

Metadata:

Additional parameters include standard physical parameters (oxygen, conductivity, temperature), as well as meteorological data (2 weeks before the sampling data) and annual water level variability and habitat related parameters (water depth, macrophytes, etc.)

Sample Processing:

The filtration for DNA will be done in the field. Filters will be transported to the laboratory of PP4 within 1 h on ice in the cooling box. DNA samples will be stored at -20°C.

Switzerland

Key pilot site:

Lake Lugano (site details are given in WP2 report)

Sampling:

will be performed once a year in summer 2019 and 2020 by PP12 (SUPSI) according to the WP1 lake fish sampling protocol (minimum effort). No regular WPO based fish sampling is performed. Species lists are available from previous studies (ProjectLac Ceresio 2014) and fishing activity (CIPAIS and CISPP report).

The minimum effort protocol includes 10 stations (… pelagic and … shores, to be sampled depth-integrated using an integrated sampler (Schröder bottle). No peristaltic pump based integrated sampling equipment is available.

Metadata:

Additional parameters include standard physical parameters (oxygen, conductivity, temperature), as well as meteorological data (2 weeks before the sampling data) and annual water level variability and habitat related parameters (water depth, macrophytes, etc.)

Sample Processing:

The filtration for DNA will be done on the boat (?) in the field. And Sterivex filters will be transported frozen to the laboratory of PP12 within 2h. DNA samples will be stored at -20°C.