May 29, 2024 Version 2

River biofilms sampling for both downstream DNA analysis and microscopic counts V.2

DOI
dx.doi.org/10.17504/protocols.io.e6nvw9mjdgmk/v2

Frederic Rimet[1], Marine Vautier[1], Rainer Kurmayer[2], Nico Salmaso[3], Camilla Capelli[4], Agnès Bouchez[1], Peter Hufnagl[5], Isabelle Domaizon[1]

[1]INRAE, CARRTEL, Pole R&D ECLA, 74200 Thonon les bains, France;
[2]University of Innsbruck, Research Dep. for Limnology, Innsbruck, Austria;
[3]Edmund Mach Foundation, San Michele all'Adige, Trento, Italy;
[4]University of Applied Sciences Southern Switzerland, SUPSI, Cannobio, Switzerland;
[5]Austrian Agency for Health and Food Safety Ltd.(AGES), Austria

EcoALpsWater

Cecile Chardon
UMR CARRTEL

DOI: https://dx.doi.org/10.17504/protocols.io.e6nvw9mjdgmk/v2

Protocol Citation: Frederic Rimet, Marine Vautier, Rainer Kurmayer, Nico Salmaso, Camilla Capelli, Agnès Bouchez, Peter Hufnagl, Isabelle Domaizon 2024. River biofilms sampling for both downstream DNA analysis and microscopic counts. **protocols.io https://dx.doi.org/10.17504/protocols.io.e6nvw9mjdgmk/v2**Version created by **Cecile Chardon**

Manuscript citation:
GUIDANCE ON THE MONITORING OF THE BIOLOGICAL QUALITY ELEMENTS PART A3 – PHYTOBENTHOS (Jan 2015) Federal Ministry of Agriculture, Forestry, Environment and Water Management

Protocol status: Working
We use this protocol and it's working

Created: May 29, 2024

Last Modified: May 29, 2024

Protocol Integer ID: 100825

Keywords: river biofilms, sampling, DNA analysis, microscopic counts, river biofilm, benthic diatoms from river, sampling of biofilm, ecoalpswater consortium, context of ecoalpswater, members of the ecoalpswater consortium, downstream dna analyses of microbial assemblage, ecological assessment of running water, downstream dna analysis, ecoalpswater, analysis of benthic diatom, microscopic enumeration of the phytobenthic community, associated microbes in biofilm, organic matter pollution in river, algal microscopic count, benthic diatom, biofilm, comparing dna inventory, algae, eu water framework directive, preparation of benthic diatom, direct observation of the organism, water quality, part of the eu water framework directive, groups of algae, macrophyte ecology method assessment, microbial eukaryote, phytobenthos assessment, microbial assemblage, based ecological assessment, traditional biological monitoring, dna inventories to traditional inventory, alpswater project, microbial, organism, diatoms community, microbe,

Abstract

The objective of this protocol is to provide a reliable and replicable method for the sampling of river micro-phytobenthos and associated microbes in biofilms, to be used in both downstream DNA analysis and algal microscopic counts.
The filed protocol is optimized for routine sampling and is in agreement with CEN guidance (NF EN 13946) and CEN technical report (CEN/TR 17245) for the analysis of benthic diatoms from rivers and lakes.
The application proposed here in the context of EcoAlpsWater aims in comparing DNA inventories to traditional inventories (microscopy).
This protocol is part of the deliverables provided by the WP1 of the Eco-AlpsWater project. All members of the EcoAlpsWater consortium (involving 12 partners : http://www.alpine-space.eu/projects/eco-alpswater/en/home) have contributed to the optimization of this protocol.

Phytobenthos-based ecological assessment of running waters is part of the EU Water Framework Directive 2000/60/EC (European Commission) allowing to evaluate the level of nutrients and organic matter pollution in rivers. According to the countries, phytobenthos assessment includes all groups of algae or is based only on diatoms communities. Other periphytic organisms such as fungi, bacteria or microbial eukaryotes as cilates or planktonic (metaphytic) algae are not considered in this assessment method. Further the group of charophytes is covered by the macrophyte ecology method assessment.
The recent development of DNA metabarcoding has the potential to complement the traditional biological monitoring based on the direct observation of the organisms, both by reducing sample-processing cost and time and by offering the opportunity to consider a larger taxonomic diversity (e.g. bacteria, heterotrophic micro-eukaryotes ...).
The protocol described here is dedicated to the sampling of biofilms in rivers both for microscopic enumeration of the phytobenthic community and for downstream DNA analyses of microbial assemblages.
This field protocol is based on routine methods used for biofilms sampling and is in agreement with:
- CEN, 2014. Water quality - NF EN 13946 - Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes. Afnor, 1-23.
- CEN 2018. Water quality - CEN/TR 17245 - Technical report for the routine sampling of benthic diatoms from rivers and lakes adapted for metabarcoding analyses. CEN, 1-8.

Guidelines

- When & where to sample
  - Choice of the sampling season and period
  - Choice of the sampling stations
- Biofilms sampling procedure & preservation
  - If stones are available
  - If stones are not available
  - Blank samples
- Label standard and sampling filed datasheet

Materials

- Reagents
  - for materials cleaning
    * 10% H_2O_2 solution
    * DNA free water (Millipore Water (Milli-Q) 18.2 MΩcm (at 25 °C)

  - for sampling:
    * DNA free water (Millipore Water (Milli-Q) 18.2 MΩcm (at 25 °C), plan 300mL for 3 stations
    * for the preservation of DNA samples : absolute ethanol (quality : for analysis), plan approx. 150mL for 3 stations
    * for the preservation of samples dedicated to microscopic counts: absolute ethanol, plan approx. 150mL for 3 stations **OR** another solution as formaldehyde (according to the countries the traditional protocol for fixation of samples can differ).
    * for blank sample: DNA free water (Millipore Water (Milli-Q) 18.2 MΩcm (at 25 °C), plan 50mL per blank sample and absolute ethanol (quality: for analysis), plan approx. 50mL per blank sample

- Materials
  - DNA free tray, 1 per sampling station and 1 per blank sample

- Consumables
  - new nylon brush (e.g. toothbrush), 1 per sampling station (to avoid contaminations)
  - 50mL Falcon tube (sterile):
    * at least 3 tubes per sampling station:
1 tube for DNA analysis, 1 tube for microscopic counts and 1 tube for additional sample kept without fixation (if some samples have to be inspected live under the microscope, or for cyanotoxins analysis)
    * 1 per blank sample: it is recommended to make a sample blank every 10 samples approximately
  - gloves

Troubleshooting

Safety warnings

- Absolute ethanol
  CAS number : 64-17-5
  Signal word : Harmul and Flammable
  Hazard phrases : 225, 319
  Precaution phrases : 210, 305+351+338

- Formaldehyde solution
  CAS number : 50-00-0
  Signal word : Flammable, Corrosive substance, Toxic, Health hazard
  Hazard phrases : 226, 301+311+331, 314, 317, 335, 341, 350, 370
  Precaution phrases : 201, 210, 280, 301+310+330, 303+361+353, 305+351+338+310

- 10% H_2O_2 solution, Hydrogen peroxide solution at 10%
  CAS number : 7722-84-1
  Signal word : Harmful and Corrosive substance
  Hazard phrases : 302, 318, 412
  Precaution phrases : 273, 280, 301+312+330, 305+351+338+310

Before start

- Read and follow the step 1 - When & where to sample
- Watch the sampling desmonstration video: **https://www.youtube.com/watch?v=Y_3YaOc6WWo**
- The following cleaning precautions must be applied, to avoid contaminations:
  - Nylon brushes (e.g. toothbrush) must be new
  - At the lab, the tray is cleaned with 10% H_2O_2, then rinsed with DNA free water and dried
  - Wear gloves throughout the sampling process and change them between different sampling stations

When & where to sample

1

- **Choice of the sampling season an period**

- Season:

Sampling in the framework of national river monitoring networks is usually carried out during low flow season, optimally during the natural low-water period of the respective water body under clear water conditions (i.e. summer in Europe).

- Flood events must be taken into consideration:

If low intensity hydrological events appeared (low intensity floods, floods of a few days duration, aerial exposure of a few days), it is recommended to wait a few days before taking samples. After intense hydrological events (lasting floods, floods causing a reworking of the supports), it is recommended to wait 3-4 weeks before taking the samples.

In the particular case of a sampling station subjected to strong artificial variations of the flow (after dams for instance), the artificial hydrological regime is assimilated to a stabilized flow.Choice of the sampling stations (example of application in Eco-AlpsWater monitoring)

- **Choice of the sampling stations**

Benthic algae biomonitoring in rivers (e.g. to establish diatom indices as IBD, IPS, TDI) is applicable to stations located on natural or artificialized watercourses with the exception of naturally salted stretches (e.g. estuaries). It is also necessary that the substrates used for sampling have been submerged during enough time (several weeks) and are accessible.

If the sampling station has been previously defined (for instance by an environmental agency, or a water agency), the samples will be taken at the defined place, unless the sampling conditions (representativeness, accessibility ...) lead the person in charge of the sampling to choose another place.

In the framework of EcoAlpsWaters, we recommend to sample on pre-existing stations defined by the environmental agency in charge of the regular monitoring of the river.

If the sampling station is not defined, then a stretch of minimum 20m/40m or 4-5 times the width of the running water body has to be sampled (Federal Ministry of Agriculture, Forestry, Environment and Water Management 2015).

Biofilms sampling procedure & preservation

2 It is important that the substrates have been submerged during enough time in order to have a biological community representing the chemical conditions of the water. Therefore, avoid sampling too close to the surface.

Make only one sample per station, regardless of the number of substrates used. A sample contains only one type of substrate (i.e. natural hard substrate, or non-natural hard substrate).

Perform the sampling as a priority:
- on natural substrates and as stable as possible (e.g. stones), also in slow-flowing riverbeds stones should be preferred
- otherwise, on non-natural hard substrates (e.g. tiles) or macrophytes

Do not collect on loose or unstable sediments (mud, sand, ...) or on wood.

The surface from stones to be sampled is 100 cm² or more. If there are not enough substrates, mention it in the field sheet.

The following cleaning precautions must be applied, to avoid contaminations:
- Nylon brushes (e.g. toothbrush) must be new
- At the lab, the tray is cleaned with 10% H_2O_2, then rinsed with DNA free water and dried
- Wear gloves throughout the sampling process and change them between different sampling stations

2.1 **If stone are available** (figure 1)**:**

A demonstration video is available at https://www.youtube.com/watch?v=Y_3YaOc6WWo

- Take at least 5 stones (it can be more, depending on stones sizes and biofilms amounts), for a total brushed surface of at least 100 cm^2. Stones are taken at 20-50 cm depth from the minimal water level (annual data) in an area of 100 m². The area is at least 2 m wide (a 2 m wide strip corresponds to a 50 m long stretch can be sampled).
- Let the stones drain for a few minutes
- Fill the bottom of the tray with 50 ml of absolute ethanol

- Brush the stones in the tray
- The obtained biological material (mixture of biofilms and water) is then sub-sampled

* For DNA samples (figure 1):

- Take the biofilm/water mixture from the tray and fill the 50mL tube up to 10 ml
- Complete the 50mL tube with absolute ethanol up to 50 ml (add ~40 mL of absolute ethanol)
- Shake to homogenize, label
- Store at ~ +4°C, in the dark for a maximum of 1 month, or can also be frozen at -20 or -80°C for a maximum of 3 months.

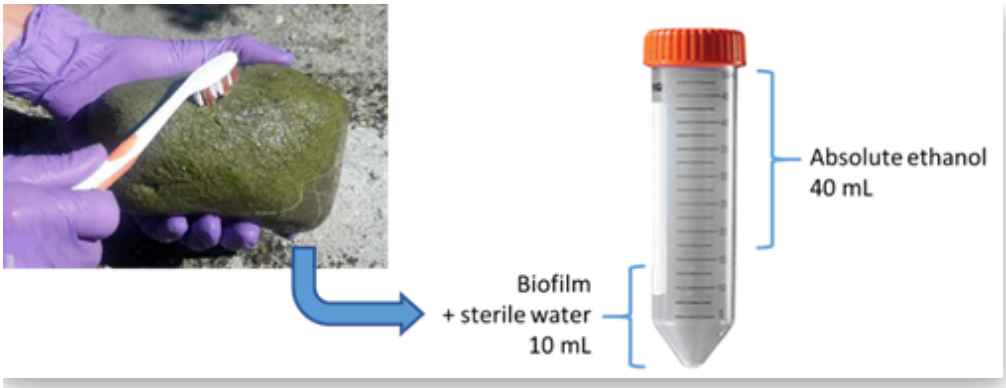

Figure 1: Schematic sampling procedure in the field when stones are available

* For microscopy samples:

- Take a second sub sample (10 mL) of the biofilm/water mixture from the same tray
- Add the appropriate preservative solution for the microscopy analysis according to the case, preservative solution can be Ethanol (70%) or Formaldehyde (2%)
- Store at ~ +4°C, in the dark.

- If the sample has to be inspected live under the microscope (in particular for soft algae), a third sub-sample can be kept without any fixation. Store at ~ +4°C, in the dark.

2.2 **If stones are not available:**

- Sample on artificial and hard substrates (figure 2) (e.g. riprap, artificial concrete banks)
  - Use a hoe equipped with a net
  - Scrap a minimal surface of 100 cm^3 at 20-40 cm depth

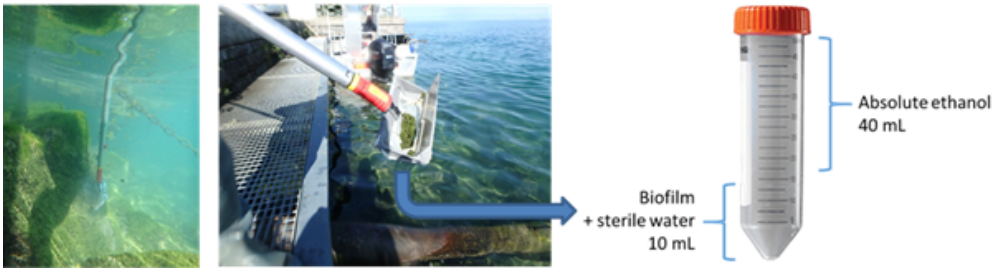

Figure 2: Schematic sampling procedure in the field when no stone is available

- If artificial and hard substratesare not available, sample on macrophytes
  - At 20-40 cm depth: squeeze submerged filaments (e.g. Elodea, Potamogeton) or scrap the macrophyte stem (e.g. Typha). At least 20 cm of stem must be scrapped.

Important note: if samples are taken on macrophytes, this must be indicated on the sample, and only diatom analyses will be performed on these samples.

2.3 **Blank sample**

It is recommended to make a blank sample during the sampling of the sample for DNA analysis:

- If number of stations < 10, make 1 blank sample
- If number of stations > 10, make 1 blank sample every 10 samples approximately

- Fill the bottom of a clean tray with 50 ml of free DNA water.
- Fill a 50 mL sterile Falcon tube up to 10 ml with the water from the tray.
- Complete the tube with absolute ethanol up to 50 ml (add ~40 mL of absolute ethanol).
- Shake to homogenize and label.
- Store the blanks like the other samples (at ~ +4°C in the dark for a maximum of 1 month, or frozen at -20 or -80°C for a maximum of 3 months).

Label standard & sampling field datasheet

3

- Sample labeling: “Biofilm”, “River Name”, “Station”, “Date”
- Accompanying documents:
  - field sheets : we propose a field sheet (Table 1) for mesological accompanying data
  - photographic documentation : if possible, add a photographic documentation of the sampling area

Biofilm sampling - river	ECO-ALPSWATER INTERREG	
Station code		
Name of the river		
Name of the sampling site		
Sampling date		
Time		
Sampling person name		
Location of biofilm sampling vs limnimetric scale		**GPS coordinates**
Height on limnimetric scale (cm)		X : Y :
Sampling at the planned site	yes / no	
Comment about the sampled site:	Acces to site (explain) Precise location (River kilometre, nearest place, municipality...) :	Position VS site of GPS measure
Average width of the water (m)	*(estimated / measured)*	
Average sunshine of the sampled site	*<10% / 10 à 50% / 50 à 90% / >90%*	
Previous hydrological situation	*Water raise .....days before / no significant rain since ..... days / other :*	
Apparent hydrological situation	*flood / full or nearly full bed / medium water / low water / water holes, puddles / no water*	
Mean Flow Velocity (estimate)		
Flow-off amount (estimate)		
Obsevations about the riverbed:	recalibration reprofiling rectification	channelisation dredging cattle in minor bed
Clogging	clogging of the bottom by mud: Yes / No clogging of bottom by sand: Yes / No or other:	
Presence	Weir causing a reach upstream: Yes / No Ford: Yes / No	
Discharge (Current discharge, discharge forecast (to the extent identifiable) :		
Plant prolifération:	**yes / No**	
Other observations on the site and its environment		
Comments on sampling: sampling conditions: *good / complicated* (depth, turbidity)		
Présence of algae on substrates	*Yes / No if yes, on the sampled substrates: Yes / No*	
Kind of algae	*filamentous, turf, in plates, encrusting*	
Overall degree of covering of phytobenthos [%]		
Occurrence of other groups of aquatic plant organisms (lichens, mosses, macrophytes)		
Choriotope/habitat distribution [%] estimate		
Presence of sediments	*Yes / No if yes, on the sampled substrates: Yes / No*	
Number of substrates sampled:		
Kind of substrates sampled	*Natural hard substrates: blocks of> 25cm, stones (6-25cm), pebbles (1-6cm)* *Non-natural hard substrates (tile, brick, concrete, metal), plants:*	
Sampling material:	*brush / knife / hoe / hand (plant expression)*	
Current facies	*lotic / lentic*	
Morphodynamic facies sampled	*Lotic channel, Lentic channel, Border, Dead Water, Current, ... waterfall*	
Distance to bank		
Depth		
Notes:		

Table 1: Field datasheets