Feb 01, 2019

Lake plankton sample collection from the field for downstream molecular analysis

Isabelle Domaizon[1], Rainer Kurmayer[2], Camilla Capelli[3], Cécile Chardon[4], Peter Hufnagl[5], Marine Vautier[4], Nico Salmaso[6]

[1]INRA, CARRTEL, Thonon les bains, France;

[2]University of Innsbruck, Research Dep. for Limnology, Innsbruck, Austria;

[3]University of Applied Sciences Southern Switzerland, SUPSI, Cannobio, Switzerland;

[4]INRA, CARRTEL, Thonon les bains, France;

[5]Austrian Agency for Health and Food Safety, Vienna, Austria;

[6]Edmund Mach Foundation, San Michele all'Adige, Trento, Italy

dx.doi.org/10.17504/protocols.io.xn6fmhe

EcoALpsWater

Isabelle Domaizon
INRAE - UMR CARRTEL - Pole R&D Ecla

The objective of this protocol is to provide a reliable and replicable method for the sampling of lake micro-plankton to be used for downstream DNA analysis. This protocol is one of those proposed by the EcoAlpsWater consortium to promote the implementation of High Throughput Sequencing (HT S) of environmental DNA (eDNA) in the biomonitoring and ecological assessment of water bodies.
The application proposed here, in the context of EcoAlpsWater, aims at comparing DNA inventories to traditional phytoplanktonic inventories and at characterizing more broadly the micro-planktonic diversity through eDNA analysis (including bacteria).
The sampling strategy is similar to that used for classical phytoplankton survey focusing on the euphotic zone, however the procedure for filtration and preservation is adapted for DNA samples.
This protocol is part of the deliverables provided by the WP1 of the Eco-AlpsWater project. All members of the EcoAlpsWater consortium (http://www.alpine-space.eu/projects/eco-alpswater/en/home) contributed to the optimization of this protocol.

DOI

dx.doi.org/10.17504/protocols.io.xn6fmhe

Isabelle Domaizon, Rainer Kurmayer, Camilla Capelli, Cécile Chardon, Peter Hufnagl, Marine Vautier, Nico Salmaso 2019. Lake plankton sample collection from the field for downstream molecular analysis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.xn6fmhe

Plankton, lake, environental DNA, metabarcoding, biomonotoring, freshwater, biodiversity, filtration, NGS, High Throughput Sequencing

protocol ,

Feb 01, 2019

Feb 01, 2019

19902

The sampling material list is presented in the step by step protocol.

SCIENTIFIC CONTEXT & GENERAL DESCRIPTION OF SAMPLING

1 Phytoplankton (including cyanobacteria) is routinely used to define the ecological quality status of lakes. The traditional method to produce metrics/indices from phytoplankton is based on the direct observation of the organisms (morphological identification of micro-algae), which has been proven to be time and resource consuming. Recent development of DNA metabarcoding could potentially alleviate some of these limitations, by using DNA sequences instead of morphology to identify planktonic organisms.
Additionally, metagenomics allows, from a single eDNA extracts, to identify not only phytoplanktonic taxa but also all planktonic micro-organisms that are present in the water sample (heterotrophic bacteria & protists) providing therefore an in depth characterization of the microbial diversity.
The objective of this protocol is to provide a reliable and replicable method for the sampling of lake micro-plankton to be used for downstream DNA analysis. The application proposed here, in the context of EcoAlpsWater, aims at comparing DNA inventories to traditional phytoplanktonic inventories and at characterizing more broadly the micro-planktonic diversity through eDNA analysis. The sampling strategy is similar to that used for classical phytoplankton survey focusing on the euphotic zone, however the procedure for filtration and preservation is adapted for DNA samples.
NOTE: The materials list and the stepwise protocol are described for the preparation of one sample; however for each sampling station, recommendation is to duplicate the filter dedicated to DNA analysis in order to have a backup available.

SAMPLING MATERIALS CHECK LIST & PRECAUTIONS DURING SAMPLING

2

The material that is classically used for phytoplankton sampling in lake is required (please see standards for the CEN if necessary).
In addition, specific materials dedicated to the preparation of DNA samples are :
-Gloves (at least 1 pair, 2 preferably)
-Sterivex cartridge (Sterivex™ GP pressure filter units, 0.22µm; Millipore catalog# SVGPL10RC) : 1 cartridge per sample.
-Caps for Sterivex (Luer-Lock male & female : outlet & inlet) : 2 per sample
-Sterile Syringe with Luer lock (*Syringes BD Plastipack Luer Lock Vol 50-60mL (ref : BD plastipack H850LL)*: 1 syringe per sample
- Sterile bag (or tube) to store the Sterivex after filtration
- Marker pen+ label OR pre-printed label for sample identification,
- Field sheet + pen ,
- A cooling box with ice / ice packs for sample transport
- Optionally a 1 L DNA free bottle that will contain a sub-sample of water dedicated to DNA (neck bottle that is larger than the diameter of the syringe) ; alternatively the water to be filtered for DNA can be taken directly from the main carboy receiving lake water samples.
- Optional : scissors (to remove the Sterivex and syringe from their blister pack).

Specific precautions to be taken are :
- Store all materials inside DNA clean bags or containers to reduce any contamination
- Wear gloves during the whole procedure
- Prior to sampling, the bottles dedicated to DNA will need to be washed (H2O2 10% overnight incubation), rinsed 5X with distilled water and dry. On the field, the bottle can be rinsed with the sampled water.
-In the field, before successive sampling, specific care should be taken to avoid contamination of sampling equipment: we recommend using a single bottle (that has been cleaned according to the recommendations) for each sampling station.
- During the handling try to reduce exposure to sunlight if possible.
- Cleaning of the water sampler, a basic option is to rinse with lake water (just before collecting the analysed water sample).
- Blanks: At least one blank every 10 samples. Filter DNA free water following the same protocol as for environmental samples. The blanks will then be analyzed like other samples.

WHEN & WHERE TO SAMPLE

3

In the context of the Interreg Alpine Space Eco-ALpsWater project (2018-2021):
-Plankton sampling should occur once each month for 2 years long for pilot sites (6 lakes) and for at least 1 sampling per year during the summer period over 2 years for the other sites (extended implementation).
-Sampling location is as usually done for standard monitoring of phytoplankton: sampling location is generally the deepest location or may be the midpoint of the lake (the historical sampling point on which long term records are based).
-The water sample collected is representative of the euphotic zone.

Citation: Isabelle Domaizon, Rainer Kurmayer, Camilla Capelli, CÃÂ©cile Chardon, Peter Hufnagl, Marine Vautier, Nico Salmaso Lake plankton sample collection from the field for downstream molecular analysis https://dx.doi.org/10.17504/protocols.io.xn6fmhe

STEPWISE PROTOCOL

4

- LAKE WATER SAMPLING

- **Estimation of the sampling depth / euphotic zone** :

Two options are possible:
- use a direct measurement of euphotic depth using submersible sensors
- use of conversion coefficients to estimate the euphotic zone from Secchi disk measurements : Euphotic zone is estimated by multiplying the Secchi depth readings by 2.5 (if not 2.5, the choice has to be justified for each lake ; e.g. metalimnic cyanobacteria below the 2.5x Secchi depth limit).

- **Collect water sample(s) to represent the euphotic layer** :

The sampling strategy follows the integrative method applied for phytoplankton ; different sampling systems can be used to obtain the integrated sample (Integrating Water Sampler e.g. bottle IWS; a pool of discrete samples taken with a Niskin bottle; see standard procedures CEN).
In any case, verify that the final volume of water that should allow covering the different analyses; i.e. phytoplankton microscopic counts + DNA (100mL to 1L according to the lake trophic state) + Chl-a (if Chl-a is to be measured).
The collected water has to be stored in DNA free carboys/bottles (see section 2) to avoid contamination.
Use a cooling box if the samples are to be transported.
Proceed rapidly to the water filtration for DNA.
The immediate onsite filtration allows to reduce the potential damage to cells during transportation, while the filtration in the lab generally offers better conditions in terms of sterility and ease of handling.
If filtration is not done onsite, transport the samples using a cooling box and store the sample at 4°C for maximum 12 hours until filtration.

- WATER FILTRATION AND PRESERVATION FOR DNA

- **Set-up on site :**

The set up is easy and does not necessarily require countertop space, though this could be convenient; filtration could be done on boat, on land or at the lab.
Get the filtration material ready to use (gloves, Sterivex cartridge and caps, syringe, scissors, absorbent paper, indelible marker or pre-printed label, field sheet + pen).

- **Filtration procedure (sterivex) & preservation :**

Note that, due to the potential presence of large size phytoplanktonic taxa, no pre-filtration is required here to exclude multi-cellular genetic material or metazoan.
From the integrated water sample,

1. Remove the syringe and Sterivex from their blister pack (handle carefully avoiding to touch either end of the filter, to prevent contamination)
2. Homogenize gently the water sample & Fill the syringe (100mL) with water sample (you can either take directly water from the integrated sample, or you may have taken a

Citation: Isabelle Domaizon, Rainer Kurmayer, Camilla Capelli, CÃ©cile Chardon, Peter Hufnagl, Marine Vautier, Nico Salmaso Lake plankton sample collection from the field for downstream molecular analysis https://dx.doi.org/10.17504/protocols.io.xn6fmhe

subsample in a sterile bottle : in any case verify that the neck of the bottle is larger than the syringe's diameter)

3. Connect the syringe and Sterivex together
4. Filter (slowly) the water sample through the sterivex unit
5. Typical volumes filtered are 100mL to 1L : the volume of water filtered will depend on the microbial load and turbidity of the water sample. Repeat the filtration (with the same syringe and sterivex) to filter as much volume as possible until the filter is clogged and note the final volume of filtered water. The filtration process should not take longer than 15 min.
6. Remove as much of the remaining liquid as possible (using the syringe containing air)
7. Cap both ends with the inlet and outlet Caps (Or use Parafilm to wrap the ends of the Sterivex)
8. Place the Sterivex in a freezable bag, store it in ice, and freeze it as soon as possible at -20°C (if possible freeze immediately). The bag can be replaced by a tube (50mL).
9. Label the bag (or tube); use pens that can withstand temperatures of -20°, and that can resist water/humidity. Finally, complete the field sheet.

A demonstration video is available at https://www.youtube.com/watch?v=du5dfjNQr1E

- LABEL STANDARD AND SMAPLING FIELD DATASHEET

Sample labeling: "Plankton", "Lake Name", "Station", "Layer", "Date", "volume (mL)"

Sampling field datasheet should contain the classical information collected for traditional plytoplankton survey.
Additionally information specific to DNA sampling are required (as, the volume of water filtered, the time and duration of filtration process, the location of filtration, the color of filter sterivex).

Note: The type of filters and syringes used in this protocol should be as advised in the material list (see references in section II), if this is not the case, please add the information about filters and syringes used as additional info on the field datasheet.

Citation: Isabelle Domaizon, Rainer Kurmayer, Camilla Capelli, CÃÂ©cile Chardon, Peter Hufnagl, Marine Vautier, Nico Salmaso Lake plankton sample collection from the field for downstream molecular analysis https://dx.doi.org/10.17504/protocols.io.xn6fmhe

Lake Name		

		Specific comments
Lake Station		
GPS Coordinates	Longitude (X) : Latitude (Y):	
Sampling date & time		
Secchi depth		
Euphotic zone (depth in m)	☐ Sensor measurement : ☐ Secchi depth conversion factor :	

DNA filtration		Specific comments
Vol. of water filtered (mL)		
Time of filtration		
Duration of filtration (min)		
Location of filtration	☐ On-site ☐ laboratory	
Color of filter		

Field datasheet - Plankton collection

Citation: Isabelle Domaizon, Rainer Kurmayer, Camilla Capelli, CÃÂ©cile Chardon, Peter Hufnagl, Marine Vautier, Nico Salmaso Lake plankton sample collection from the field for downstream molecular analysis https://dx.doi.org/10.17504/protocols.io.xn6fmhe