List of parameters to be measured

- **Meteorological data**

  Gather these data from the nearest meteorological station (KMI) from May –October :

  - Air temperature (°C)
  - direction and velocity of wind (m/s)
  - Rainfall (mm)
  - Irradiance (W/m^2)

- **Physical variables** (surface in shallow lakes < 3 m; vertical profile from different depths in deeper lakes)
  - Temperature(°C)
  - pH
  - Oxygen (mg/l, % saturation)
  - Conductivity (µS/cm at 25°C)
  - Depth (at the sampling site ; mean depth only once) Do we have to make a bathymetric map?NO Just mesure depth at the sampling site, and sample the lake at different places to have an estimate of mean depth
  - Euphotic depth (Secchi depth or irradiance profile with light sensors)
  - Depth of the mixed layer (in lakes > 3 m) : determined from T / O_2 vertical profiles

- **Chemical variables** :

Measurements on filtered water, unfiltered water or filter of:

  - NO_3^- on GF/C or GF/F filtered water (for ex: we determine N-NO_3 by an adapted cadmium reduction method to reach a minimum detectable concentration of about 10 µg N-NO_3/L)
  - NO_2^- on GF/C or GF/F filtered water (for ex: we determine N-NO_2 by the sulfanilamide method to reach a minimum detectable concentration of about 10 µg N-NO_2/L
  - NH_4^+ on GF/C or GF/F filtered water (for ex: we determine N-NH4 by the salicylate-dichlorocyanurate method to reach a minimum detectable concentration of about 20 µg N-NH4/L)
  - PO_4 (SRP),on GF/C or GF/F filtered water (for ex: we determine P by the molybdate method to reach a minimum detectable concentration of about 10 µg P/L);
  - P total, see P after persulfate digestion (**unfiltered water**).
  - PON (particulate organic N) (elemental analysis, Carlo Erba NA 1500, nitrogen/carbon analyser), **filtration of a known volume on GF/F previously incinerated at 500 °C**
  - POC Particulate organic C) (elemental analysis, Carlo Erba NA 1500, nitrogen/carbon analyser); **carried out on the same filter as for PON**
  - Dissolved Si, on GF/C or GF/F filtered water

- **Plankton examination and biomass** :
  - Phytoplankton composition and biomass
    - Chla content and pigments composition (HPLC),
    - microscope examinations
  - Zooplankton composition and biomass (microscope examinations)

In the field

- Measure physical variables from the water column :

(a depth profile in deep stratified lakes or only at the surface in shallow, well mixed lakes)

  - Temperature
  - pH
  - Oxygen (mg/l, % saturation)
  - Conductivity
  - depth

- Transparency : use a Secchi disc (do not measure in the shadow of the boat)

- Gather water samples with a Van Dorn bottle on regular depths in the stratified lakes or with a tube samples in the shallow lakes from at least three different localities in each lake. From the integrated sample (gathered in a big container of 100L) take subsamples for phytoplankton microscopy and laboratory analysis and transport these to the lab in an ice-cooled box.

- Take a zooplankton sample with a Schindler Patalas sampler at regular depths in the deep lakes at each locality; alternatively, filter water from the big container with the integrated sample over a 64 µm mesh size net in case of shallow lakes, write down the volume filtered and fix with sugared formaldehyde to an end concentration of ~ 10%. (add 10 ml formaldehyde (35 %) in 100 ml sample).

- Take extra water samples in case of a clear phytoplankton bloom :
  - Gather water from the surface in a graduated bucket of 10L
  - Filter this water over a net of 20-25 µm meshsize until the net cloggs, take care to remove organic debris with tweezers.
  - Rinse the concentrate from the net with a small amount of filtered water (in a rinsing bottle) and gather in a glass beaker
  - In case of a scum layer on the water surface, sample directly with a beaker, no concentration over a net is necessary
  - Bring the concentrate in a 100 ml PE vial with a broad opening (to fasten lyophilisation) and store in the deepfreeze (-20°C).

- In case of evident blooms, collect fresh sample of water (simply 15 ml of “green” water in a sterile Falcon tube or equivalent) and as soon as possible deliver (prior) to ULG in a brown envelope for isolation by culture methods. (not on Friday or just before a day-off)

- Fill in the sampling form

In the laboratory (the same day) :

For each collected sample :

➢ Whole water :

- Fill a **500ml bottle (we take 250 ml and fix it with 125 µl alkaline lugol, 6.25 ml borax buffered formaldehyde and 250 µl sodium thiosulphate)** with concentrated Lugol (final color « tea ») for counting / for identification of phytoplankton, settle for at least 24h and concentrate; add formaline according final volume for a 2% final concentration.

- Fill a 50 ml PE bottle (we use Falcon-tubes) for total phosphorus , add **0,2 ml H_2SO_4 5N**, to be kept at 4°C

- Into 4 labelled 1.5ml microcentrifuge tubes, pipette 1ml of mixed environmental water sample. (see Codd laboratory detailed protocols for further operations).

  a) Store 2 tubes at -20°C. (TM by ELISA)
  b) Store 1 tubes at +4°C. (Samples for single colony and filament ELISA, we sometimes concentrate the samples first to make isolation easier)
  c) Centrifuge 1 tube and remove supernatant and place into new labelled 1.5ml microcentrifuge tube, store sample at -20°C. (SM by ELISA)

➢ Filtrations :

- **1 filter GF/C whatman previously incinerated** (500°C during 4h) **for particulate C and N**

  Filter a sufficient amount of water (until the filter gets clogged and/or gets a clear colour), place the filter in a special filter cap, note volume filtered and dry weight and store in the deep freezer(-20°C)

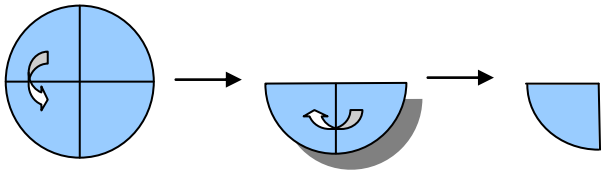
- **1 filter GF/C whatman for chlorophyll a and pigments analysis by HPLC**

  Filter an adequate volume of a well-mixed environmental water (do not exceed 200mbar of vacuum pressure). Roll the filter on itself and place it in a glass or PE tube. Pigments have to be extracted using 8 mL of 90% aqueous acetone in an adequate vial (glass vial, as those used for scintillation); extraction is carried out with 15 minutes sonication in a sonication bath with ice (~ 0-2°C) (for ex.: bath style sonicator, Branson. The vials are then stored overnight in a refrigerator (4°C); then a second 15 min sonication is performed;); alternatively, a one-step sonication can be performed with a point sonicator; centrifuge the extract and keep at least 2 ml in an amber vial for HPLC analysis. If you have to store the filter before extraction, keep them in a deep freezer or liquid nitrogen but pigment extraction should de done within 2 weeks of collection. It's definitely better to carry out the extraction on the fresh filters and to store the 2 mL extract after centrifugation. The pigments analysis will be done at Namur laboratory. Store the extracts in the deep freezer at -80°C.

- **2 filters 0.2 µm for molecular analysis** (PCR for microcystin genes)

  A volume from 100 to 500 ml (or part if the filter is severely clogged, in that case record the volume filtered!) is filtered onto a 0.2 µm Supor Filter, PES, Pall Life Science, 47 mm, 0,2 µm, VWR, (Catalogue #: 60 300).

  Do this twice – so we will have one filter in reserve. Pay attention not to contaminate the filter by putting it on the floor or taking it with fingers: manipulate it carefully with a tweezers all time. After filtration, fold it into four parts, put in lysis buffer, label it properly and immediately frozen at –20°C (if possible). The samples have to be sent, still frozen, to Ulg for DNA extraction. (see ULG sampling procedure for details).



- **1 filter GF/C whatman for particulate microcystin content** (PM)

  Filter 1 liter of a mixed environmental water or an appropriate reduced volume on a pre-weighted GF/C filter (precision 0.1 mg), and record volume filtered. Retain the filtrate in a labelled 1L glass. (see below); Place the filter disc in a labelled plastic Petri dish and store at -20°C. (see Codd laboratory detailed protocols for further operations).

Filtered water :

- 1 or more PE bottle for storage of an adequate volume of water for analysis of dissolved nutrients (volume depending of your laboratory test needs, store at -20°C if needed) We store these in a Falcon tube of 50 ml.

- 1 L glass bottle for soluble microcystin content (SM), store the filtrate (from the filtration for particulate microcystin) at +4°C until SPE. (see Codd laboratory detailed protocols for further operations).