Study Design and Joint Quality Handbook of Methods for the EU-Biodiversa/Belmont Forum Project ARCTIC-BIODIVER

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Final Draft: June 2020

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Background

ARCTIC-BIODIVER (2019–2021) is a multi-disciplinary, multi-scale assessment that evaluates freshwater biodiversity and food web dynamics along circumpolar latitudinal temperature gradients in Europe, North America and Greenland to characterize their current state and scenarios for change in response to predicted warming scenarios. A primary focus is to develop strong links between climate change predictions, biodiversity scenarios, and the consequences for ecosystem services in Arctic freshwaters, thereby addressing the socio-economic impacts of a warming climate. ARCTIC-BIODIVER also develops more relevant assessment tools for biodiversity in Arctic freshwaters, and contributes to circumpolar harmonization of sampling methods, data storage, and large-scale analysis to promote future circumpolar assessments of biodiversity change.

This document provides a detailed description of the study design and of the common methods for field work that project partners have agreed upon. The aim of this document is to give partners detailed guidance on the methods that should be used in order to harmonize sample collection and optimize data compatibility among field sites.

ARCTIC-BIODIVER Study Design

Experimental Design (Space for Time with Latitudinal Gradients)
Questions:
Research question: How does food web structure and function change over latitudinal and/or nutrient gradients? This will be assessed for lakes and streams along latitudinal gradients in Alaska, western and eastern Canada, Greenland and Scandinavia (Sweden through Norway including Svalbard). Likely these latitudinal gradients will also include nutrient gradients.

Biotic variables that will be measured include: cellulose decomposition, primary producer biomass, microbial composition and function (i.e., DNA methods), taxonomic and traits composition of major focal ecosystem components (i.e., benthic macroinvertebrates, benthic algae and cyanobacteria, zooplankton and phytoplankton). In addition, food web structure and connectivity will be examined using fatty acid and stable isotope (C and N) analysis of basal resources (i.e. benthic and pelagic algae and cyanobacteria) and primary consumers (i.e., benthic invertebrates; and zooplankton) to indicate food availability and quality along the gradient.

Habitats to be sampled:

Stream sampling will occur in wadeable (2nd to 4th order streams, approximately) habitats, roughly 1-10 m width. If streams are sampled near lakes, the streams should preferably be inlets. If sampling of a stream takes place in a lake outlet, it must occur at least 1 km downstream of the lake to avoid lentic influence (a.k.a. lake effects). In addition, glacial streams should be avoided if possible as we want to limit the possibility of confounding latitudinal change with the effects of glaciers. We will aim to sample at least 10 streams across each latitudinal gradient.

Lake sampling will be done in the littoral zone (ca. 0.5–1.0 m depth) of lakes with a mean lake depth > 3 m. If a lake is very small (i.e., 1 m mean depth or anything that freezes completely in winter), then it should not be sampled. It is important to determine whether or not there are fish in the lake. If this is not known, collect eDNA samples for fish detection as needed. There's no need to collect eDNA samples for fish if you know that lake has or does not have fish. We will aim to sample at least 10 lakes across each latitudinal gradient.

Define Latitudinal Gradients for:

- Alaska
- W Canada
- E Canada
- Greenland W and E
- Scandinavia (Sweden, Norway, Svalbard)

Pilot Study in 2019:

- Conduct sampling sometime between June to September 2019
- Subset of lakes and rivers
- Test sampling methods with the aim to decide upon the suite of variables to be sampled in 2020

Full Study in 2020/2021:

- Conduct sampling sometime between June to September 2020 (or June to September 2021 if sampling in 2020 is restricted due to COVID-19)
- Full set of lakes and rivers across each latitudinal gradient (at least 10 lakes and 10 rivers per gradient)

Study Area Locations

Based on defined latitudes from each country:

- Alaska
    - At least 10 streams and 10 lakes along road system (63 - 71N) from the Alaska Range to the Arctic Coastal Plain. Sample sites were chosen after the Quebec meeting in February 2020.

- Canada west
    - Streams and lakes near Norman Wells (southernmost site), Sachs Harbour, NWT (northernmost site) encompassing a gradient from 65°N to 72°N.
- Canada east
    - Streams and lakes in northern Quebec, and possibly southern Baffin Island, northern Baffin Island, and Ellesmere Island. The number of sites will depend on how many can be visited and sampled in the time allotted to the sampling team for this specific project.
- Greenland
    - Streams (1-2) and lakes (2) at Qeqertarsuaq (Disko Island) near Arctic Station - 69°N
    - Streams (2) and lakes (2) at Zackenberg Research Station - 71°N
    - Streams and lakes at Villum Research Station (Station Nord) - 81°N
    - Streams (2) and lakes (2) in South Greenland (Narsarsuaq/Narsaq) - 61°N
- Norway
    - Ponds/small lakes in Svalbard around Longyearbyen (4) and Ny-Alesund (22) collected in 2019 - > 78°N
    - Ponds (4) and lakes (1) at Finse glacier collected in 2019 - 60ºN
    - Streams (7) and lakes (12) in Northern Norway (Finnmark) - 68-70 ºN
    - Streams (5) and lakes (5) at Finse glacier - 60ºN
    - Ponds/small lakes (up to 20) in Svalbard around Longyearbyen and Ny-Alesund - > 78ºN (expected 2021)
- Sweden
    - Some 10 lakes from Swedish National Monitoring and 10 nearby streams across a gradient from 62° to 69° N. Lakes and streams have been selected during spring 2020.

Site codes

Site Labeling:
[Country – Habitat – Replicate ID – Project Leader Initials]

Country abbreviations:
- USA = US
- Canada West = CW
- Canada East = CE
- Greenland = GR
- Norway = NO
- Sweden = SE

Habitat abbreviations:
- S = stream
- L = Lake

Habitat replicate ID:

- Label habitat replicates as 01-10 (if > 10 replicates, continue numbering such as 12, 13., 14…)

Sample type:

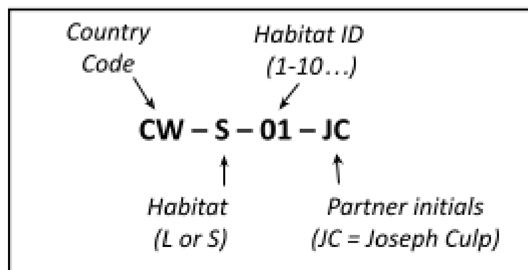
- Add the name of the sample type below the site descriptor (e.g., benthic invertebrates, algal taxonomy, algal fatty acid, etc.)

Project Leader Initials:

- Include two initials of the project leader at the end of each label. ****Make sure that labels are printed with ink that will not dissolve in alcohol!****

Here is an example label:

Canada West, Stream Habitat, Replicate 1, Joseph Culp
CW – S – 01 – JC



Methods for Sampling Lakes

Sample site selection

- Lakes with a mean depth > 3m that do not freeze completely in the winter should be chosen.
- Record maximum depth if possible (or determine from sampling records or GIS if possible).
- Determine whether or not fish are present, if that information is not known (record information if known).
- Select location in the littoral zone (ca. 0.5–1.0 m depth).
- Ideally select rocky habitat in lake littoral. Soft sediment is acceptable if rocky habitat cannot be found/accessed.
- Secondary sampling can be completed if desired (e.g., additional habitats/locations in the lake, for example, sampling soft sediment if rocky habitat has been sampled as primary location), but these samples must be kept separate for processing.

Order of sample collection

1. Sample site photos
2. Water chemistry
3. Fish eDNA sample (if required)
4. Microbial samples (eDNA)
5. Phytoplankton
    a. Chlorophyll a
    b. Taxonomy
    c. Stable isotopes
    d. Fatty acids
6. Zooplankton
    a. Taxonomy
    b. Stable isotopes
    c. Fatty acids
7. Benthic algae
    a. Taxonomy
    b. DNA
    c. Chlorophyll a
    d. Stable isotopes
    e. Fatty acids
8. Meiofauna (Optional, if time permits)
9. Benthic macroinvertebrates
    a. Taxonomy/stable isotopes
    b. Fatty acids

10. Habitat measurements
    a. Habitat
    b. Stable isotopes
11. Decomposition strips

Full list of materials for 1 lake site (to be added once edits complete)

1. Sample photos

- Take photos of the sample area, across the lake, and shoreline on either side of sampled area. Take photos of the field sheet with site name, the sample area, across the lake, shoreline on either side of sample area, and aquatic and exposed substrate. In addition, take a few "action photos" showing aquatic ecologists at work.

2. Water chemistry

Equipment:
- Sample bottles
- Nitrile gloves
- Telescoping rod + container
- Multimeter
- Fixer (for later analysis of metals, optional)
- Filter (Dissolved P)
- Pump
- 60-mL syringe with filter holder
- 0.45-μm cellulose acetate membrane filter

Method:
- Use a telescoping sampling rod with water chemistry container attached to the end to collect a sample from shoreline. Samples should be collected from ~30-50 cm below the surface. Turn the bottle upside down before dipping below the surface and turn upright when below surface to fill the bottle and collect the sample. Rinse the bottle three times before collecting the final sample (unless not required by lab), pouring rinse water away from where the final sample will be collected. Repeat for all water chemistry bottles (unless lab specifies no rinsing).
- Filter samples for dissolved phosphorus using either 1) a 0.45 μm cellulose acetate filter and hand pump, or 2) a 60-mL syringe attached to a filter holder containing a 0.45-μm cellulose acetate membrane filter. For the syringe method 2), refill the syringe as needed to obtain sufficient water to fill the sample bottle, detaching the syringe from the filter holder while refilling, and replacing the filter with a new one if the filter clogs.

- Add fixer for metals if collected according to laboratory instructions
- Optional: If required by the analytical lab or national monitoring protocol, take duplicate sample for 10% of sites (i.e., collect duplicate samples at 1 lake if sampling 10 lakes or fewer).
- Optional: To collect a sample for specific ultraviolet absorbance (SUVA) of colored dissolved organic matter (CDOM), filter sample using the same method as for dissolved phosphorus. Filter sample through a 0.45-μm cellulose acetate membrane filter and use the first 5 ml to rinse a pre-combusted (4h, 400°C) 250-ml brown glass bottle. Repeat the rinsing 3 times. Fill the bottle to the top with no air. Samples should be kept in the cold and the dark if possible until analysis in the lab (using a spectrophotometer).
- Optional: Collect field blanks using deionized water taken with you into the field.
- If you have a multi-meter, measure listed parameters (see table below). Extend the probe on a pole if possible, otherwise sample from shore. Make sure the multi-meter is not lying on the bottom of the lake.

Variables measured	
Water chemistry samples	
Cations, anions	Al, Ca, Fe, K, Mg, Mn, Na, Br, Cl, F, PO_4, SO_4 (note that NO_3-NO_2, NH_4, and HCO_3/CO_3 will all be captured by nutrient analyses and allow for ionic balance calculation).
Other lab variables	Alkalinity, pH, specific conductivity
Nutrients	TP, TN, TOC, DP or TDP (see note below), nitrite/nitrate, ammonium, DOC, DIC, HCO_3/CO_3 NOTE: If handling time restrictions can be met then measure DP. When holding times are long due to shipping from remote locations (e.g., western Canada samples), then field filter for TDP.

Metals (optional)	
CDOM (optional)	Specific ultraviolet absorbance (SUVA)
Multi-meter	
Physicals	Conductivity, temperature, pH, dissolved oxygen, total dissolved solids (TDS) (optional), turbidity (optional)

3. Microbial samples (eDNA)

Equipment:

- Telescoping rod (https://bit.ly/38aUzFr)
- Bucket
- Spray bottle (or similar) with 70% Ethanol
- Brush
- Marker pen
- Electrical tape
- 0.22 um cartridge filter (https://bit.ly/2xJKO4B, provided by UiO) (1 per sampling site)
- 50/60 mL syringe (provided by UiO) (1 per sampling site)
- 5 mL syringe (provided by UiO)
- RNAlater (https://bit.ly/2TYE1Nm, provided by UiO)
- Nitrile gloves

Method:

1. First, put on nitrile gloves and keep them clean – only touch sampling equipment. Prior to sampling at a new site, ensure the sampling rod, sampling vessel and buckets have been disinfected with 70% ethanol by: spraying the equipment with ethanol, scrubbing with the brush and waiting for 5 minutes before putting the sampling equipment in contact with a new water body.

2. Rinse thrice the sampling vessel with lake/river water before beginning sample collection. Rinsing water is to be dumped on ground rather than in the lake.
3. Extend the sampling rod to full length so as to collect water at a distance of 3-4 meters from the shore (if possible). Avoid excessive mixing when lowering the sampling vessel in the water. Draw with the sampling rod and vessel and collect in the bucket. Important: do not sample water affected by your activity (i.e. resuspended sediments, discarded samples).

4. Rinse thrice the 50/60 mL syringe by drawing water from the lake and ejecting it on the ground.

5. eDNA collection on 0.22 um cartridge filters. This is done using the 50/60 mL syringe. To filter, fill a syringe with water from the bucket, remove air bubbles, then fasten a filter cartridge to the syringe and expel the water volume through the filter*. Important: keep count of the volumes! It is essential to record the cumulative volume filter through the cartridge.

6. Filter water until it becomes hard to expel water. Then fill syringes with air and push through the filter to remove residual water. Continue until no water is visible in the filter cartridge. Usually this takes three repetitions and it is easier if you keep the syringe upright (vertical).

7. Once the cartridge is dry, add 2 mL of RNAlater solution into the cartridge using the 5 mL syringe.

8. Label filter cartridges with ID and sample volume. Close the two openings of the cartridge using the electrical tape. Keep the cartridges at 4°C in the field (or as cold as possible). If you do not have access to freezers, as they are preserved with RNAlater, you can try to keep samples cold using ice in coolers while on the road/field.

9. In the laboratory, storage at -20°C until shipping.

10. For shipping, pack samples in a EPS box (expanded polystyrene) using gel packs or rigid cold accumulators, to keep the cartridges as cold as possible (not necessarily frozen, which can avoid shipping problems and extra costs). Samples will be sent to UiO, where eDNA** extraction will be performed.

(*) The water expelled through the filter can be collected for chemical analysis (nutrients, cations, anions).

(**) Fish presence can be determined by using eDNA. Thus, DNA extracted at UiO will be used for metabarcoding analysis with the right fish primers.

4. Phytoplankton samples

Equipment for taxonomy

- 20-µm-mesh plankton net (with cod-end) attached to a 3-5 m length of rope
- Falcon tube (one each for taxonomy, stable isotopes, and fatty acids)
- Squeeze bottle
- Acidic Lugol's solution
- 95% ethanol (enough for one falcon tube + spraying net to clean it)
- Telescoping rod (optional)

Equipment for chlorophyll

- Container or bucket for collecting 1L water
- GFC filters (47 mm in diameter
- Filtering setup (funnel, filter holder and vacuum bottle) See photo
- Pump (operated manually by hand)
- Aluminum foil
- Ziploc bags
- 20 and 200 um mesh on a plastic ring (for filtering out zooplankton and finally to collect the <200 and >20 material)

a. Chlorophyll a (quantitative sample)

Method

1. Note that the collection method differs in lakes with rocky versus fine-grained substrates. When the lake bottom is composed of hard, rocky substrate, walk out as far as possible to collect a minimum of 1 L of water in a sample container. In contrast, use the telescoping sample rod when the lake bottom is composed of fine-grained substrate.
2. Use a filtering device with a GF/C filter to filter 1 L if possible (always record how much is filtered). You should see residue on the filter.
3. Fold filter, wrap in aluminum foil to protect from sunlight, put in a ziploc bag, squeeze out air and freeze it.
4. Pack with dry ice or as many ice packs as possible for sample shipping.

Photo of filtration setup. Vacuum is made by an electric pump in the lab but can be exchanged with a hand operated pump in the field. See illustration to the right.

b. Taxonomy (qualitative sampling)

Method

1. Wash 20-µm-mesh plankton net well with water or spray with ethanol if it was previously used at another site (to avoid cross-contamination of lakes).
2. From the shore, throw the 20-um plankton net as far as the rope length out into the lake and tow it in. Throw with the wind and not in the shallowest part. Repeat about 10 times in different areas of the lake until there is some visible material in the cod-end.
3. Put the material from the cod-end into a falcon tube. Add lugol's to the tube until it's tea- or whiskey-coloured to fix the sample. Store in the dark at 4°C if possible or room temperature otherwise.

c. Stable isotopes

Method

1. From the shore, throw the 20-µm plankton net out into the lake as far as the rope length and tow it in. Throw with the wind and not in the shallowest part of the area. Make sure not to touch or get too near the bottom as this will "contaminate" your sample with benthic material. Repeat approximately 10 times in different areas of the lake (walk 5-10 m if possible and repeat) until there is visible material in the cod-end.
2. In order to exclude larger zooplankton from the sample, filter the material from the cod-end through a piece of 200-µm mesh net (mounted on a plastic ring = sieve) and collect the filtered water with phytoplankton in a sample jar. [The 200-µm mesh net with filtered zooplankton can be used for zooplankton stable isotopes (see zooplankton section, below)].
3. Pour phytoplankton material through a 20-µm-mesh net that is mounted on a plastic ring to concentrate the phytoplankton material and remove all water.

a. (Option 1): Cut the piece of mesh net out of the ring using a scalpel, roll it up carefully with the plankton on the inside, and place it in a falcon tube and freeze (-20°C or preferably -80°C, i.e. dry ice).
b. (Option 2): Alternatively, you can aspire the concentrated phytoplankton biomass with a plastic pipette and place it in an Eppendorf or Falcon tube. One ml of very dense phytoplankton biomass (once sedimented) is enough for stable isotope analyses. Leave 10% of the tube empty and freeze it (leaving a portion of the tube empty will prevent expanding ice from opening the tube once frozen).

4. Pack frozen samples with dry ice or as many ice packs as possible for shipping (ensure container is full of samples and ice packs with little open space). Keep frozen during transport (e.g. cool freight).

d. Fatty acids

Method

1. From the shore, throw the 20-µm plankton net out into the lake as far as the rope length and tow it in. Throw with the wind and not in the shallowest part of the area. Make sure not to touch or get too near the bottom as this will “contaminate” your sample with benthic material. Repeat approximately 10 times in different areas of the lake (walk 5-10 m if possible and repeat) until there is visible material in the cod-end.
2. In order to exclude larger zooplankton from the sample, filter the material from the cod-end through a piece of 200-µm mesh net (mounted on a plastic ring = sieve) and collect the filtered water with phytoplankton in a sample jar. The 200-µm mesh net with filtered zooplankton can be used for zooplankton stable isotopes (see zooplankton section, below).
3. Pour phytoplankton material through a 20-µm-mesh net that is mounted on a plastic ring to concentrate the phytoplankton material and remove all water.
   a. (Option 1): Cut the piece of mesh net out of the ring using a scalpel, roll it up carefully with the plankton on the inside, and place it in a falcon tube and freeze (-20°C or preferably -80°C, i.e. dry ice).

   b. (Option 2): Alternatively, you can aspire the concentrated phytoplankton biomass with a plastic pipette and place it in an Eppendorf or Falcon tube. One ml of very dense phytoplankton biomass (once sedimented) is enough for stable isotope analyses. Let 10% of the tube empty and freeze it (leaving a portion of the tube empty will prevent expanding ice from opening the tube once frozen).
4. Pack samples with dry ice or as many ice packs as possible for shipping (ensure container is full of samples and ice packs with little open space). Keep frozen during transport (e.g. cool freight).

6. Zooplankton samples

Equipment

- 200-µm-mesh plankton net with cod-end
- Falcon tube (one each for taxonomy, stable isotopes, and fatty acids)
- Lugol's solution
- 95% ethanol (enough for falcon tube and for spraying net between sites)
- Spray bottle
- Squeeze bottle
- 200-µm-mesh piece of net glued to plexiglass ring (one per site)
- Funnel

a. Taxonomy (qualitative)

Method

1. Wash 200-µm-mesh plankton net well with water or spray with ethanol if it was previously used at another site (to avoid cross-contamination of lakes).
2. From the shore, throw a 200-µm net as far as the rope length allow into the lake and tow it in. Throw with the wind and not in the shallowest part. Throw in different areas of the lake. Repeat about 10 times throwing into different areas of the lake. If the net is larger, you may need fewer tosses of the net. Do not touch the bottom. If it happens, discharge sample and start over again.
3. Concentrate the material using the net as a sieve. Empty the sample from the cod-end/cup into a falcon tube. Add lugol's to the tube until it's tea- or whiskey-coloured to fix the sample. Store in the dark (and at room temperature).

b. Stable isotopes

Method

1. If there was not enough zooplankton in the 200-µm mesh net when you collected the phytoplankton sample, collect additional material. From the shore, throw a zooplankton net with 200-µm net-mesh as far as the rope length out into the lake and tow it in. Throw with the wind and not in the shallowest part. Make sure not to touch or get too near the bottom as this will "contaminate" your sample with benthic material. Repeat about 10 times throwing into different areas of the lake (walk 5-10 m if possible and repeat). If the net is larger, you may need fewer tosses of the net.

2. Pour material through a piece of 200-µm-mesh net mounted to a plastic ring to remove water and concentrate sample on the net. You need about 0.5 ml (= 1 mg of dry weight) of zooplankton biomass for stable isotope analyses. If a net of about 10 cm diameter is fully covered by zooplankton individuals distributed evenly, this is enough biomass for stable isotope analyses. Detach ring from net by cutting along the edge with a scalpel/knife, roll up net carefully (without squeezing the animals inside the net), place it in a falcon tube, and freeze (-20°C or preferably -80°C, i.e. dry ice).
3. Pack frozen samples with dry ice or as many ice packs as possible for shipping (ensure container is full of samples and ice packs with little open space). Keep frozen during transport (e.g. cool freight).

c. Fatty acids

Method

1. If not enough zooplankton in the 200-µm mesh net when you collected the phytoplankton sample, collect new material. From the shore, throw a zooplankton net with 200 µm net-mesh as far as the rope length out into the lake and tow it in. Throw with the wind and not in the shallowest part. Make sure not to touch or get too near the bottom as this will "contaminate" your sample with benthic material. Throw in different areas of the lake (walk 5-10 m if possible and repeat). Repeat about 10 times throwing into different areas of the lake. If the net is larger, you may need fewer tows of the net.
2. Pour the material in the net onto a 200-µm net mounted on a plexiglas ring and concentrate the samples on the net. You need a few mg of zooplankton dry weight for fatty acid analyses. If a net of about 10 cm diameter is fully covered by zooplankton individuals distributed evenly, this is enough biomass for stable isotope analyses.
3. Detach ring from net by cutting along the edge with a scalpel/knife, roll up net carefully (without squeezing the animals inside the net), and place it in a Falcon tube. Freeze net in tube at -20 °C to -80 °C.
4. Pack frozen samples with dry ice or as many ice packs as possible for shipping C(ensure the container is full of samples and ice packs with little open space). Keep frozen during transport (e.g. cool freight).

7. Benthic algae

Equipment

- Tray (size that can contain at least five cobbles)
- Toothbrush - one per site (semi-quantitative samples)
- 250 mL flasks
- Squirt bottle
- Sharpie or wax pencil
- Camera
- Falcon test tube (marked at 50 mL) or syringe (50 mL)
- Alkaline Lugol's solution (alkaline solution will preserve diatom shells; see below for recipe)

- 95% ethanol (enough for chlorophyll a sample, stable isotope sample and DNA sample)
- Sample containers (one each for taxonomy, chlorophyll a, stable isotopes, and fatty acids)

Prepare alkaline Lugol’s as follows: Dissolve 2 g potassium iodide and 1 g iodine crystals in 300 ml distilled or demineralized water. The resultant liquid should be dark brown coloured. It should be stored in an air-tight and light-proof container to minimize sublimation.
Add 1 to 5 drops of Lugol’s iodine to 100 ml sample to give a dark brown colour. More alkaline Lugol’s may be necessary if samples are rich in organic matter and are stored for a longer time.

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a. Taxonomy

Method

1. Use a shoreline reach about 10 m long, go as deep into the water as possible for your arms to reach the stones. Don’t collect directly at the shore - risk for parts that have been dry.
2. Collect > five stones (fist-sized or bigger) and brush the tops of them with a toothbrush into a tray (video here). Make sure not to get the dirt of the bottom side into the tray. The toothbrush should be new or clean for each site.
3. Rinse the material off the toothbrush into a tray (composite all five samples).
4. Add more stones in case the water in the tray is not yet brown/green from algae, ideally such as here, or here (in Swedish only) or here. “Whisky”-color is ok.

5. Remove any large debris particles and pour the sample into a 250 mL flask. Put a mark on the flask to indicate the volume of the sample. This sample will be split into sub-samples for taxonomy, chlorophyll a, DNA, stable isotopes, and fatty acids.

6. Put the rocks in the tray and take a picture from above (make sure the size of the tray is recorded so the area scraped can be estimated later on). You may also add the flask with label to the tray, as this facilitates later identification of the picture.
7. Shake flask and pour out 50 mL homogeneous sub-sample into a Falcon test tube (or use large syringe to take a 50 mL aliquot)
8. Add alkaline Lugol's solution to the test tube until it's tea- or whiskey-coloured to fix the sample.
9. Use the remainder of the sample in the 250 mL flask for samples of DNA, chlorophyll, SI and FA (see below).

b. DNA

Method

1. Swirl the flask containing the toothbrush sample collected for taxonomy to homogenize the sample, and take out a 10-50 mL sub-sample for DNA (record volume) and add 95% ethanol to preserve the sample (> 80% volume of the final sample).
2. Ethanol samples will need special containers to ship by air. Please send DNA samples to: Eva Herlitz, SLU, Department of Aquatic Sciences and Assessment, Gerda Nilssons väg 5, SE 756 51 Uppsala, Sweden. Mark the package with some note saying, ”ArcticBiodiver DNA samples - Maria Kahlert”. In case you are sending as a “letter”, you need to use a different address: Eva Herlitz, SLU, Department of Aquatic Sciences and Assessment, Box 7050, SE 750 07 Uppsala, Sweden.

c. Chlorophyll a

Method

1. Swirl the flask containing the toothbrush sample collected for taxonomy to homogenize the sample, and take out a 10-50 mL sub-sample for chlorophyll (record volume) and add 95% ethanol to preserve the sample (> 80% volume of the final sample). Collect a larger subsample (50 mL rather than 10 mL) if algal biomass seems low. Record the volume of the subsample!

2. Ethanol samples will need special containers to ship by air. Ship by ground if possible.

d. Stable isotopes

Method

1. Swirl the flask containing the toothbrush sample collected for taxonomy to homogenize the sample, and take out a 10-50 mL sub-sample from the flask and freeze (-20°C or preferably -80°C, i.e. dry ice) .

Pack frozen samples with dry ice or as many ice packs as possible for shipping (ensure container is full of samples and ice packs with little open space). Keep frozen during transport (e.g. cool fright).

e. Collection for fatty acids

Method

1. Freeze the remainder of the original (fresh) sample of benthic algae as soon as possible (-80C freezer if possible). This can preferably be done in the flask where it was collected.
2. Pack frozen samples with dry ice or as many ice packs as possible for shipping (ensure container is full of samples and ice packs with little open space).

8. Biofilm meiofauna samples

Equipment

- Cylindrical brush sampler (from Willem)
- Filter for cylindrical brush sampler
- Container for sample
- Flask
- 4% formaldehyde

Method

1. Use the cylindrical brush sampler (area 3.14 cm^2) to collect five quantitative samples of epilithic algal communities from five cobbles, according to Peters et al. (2005, Arch. Hydrobiol. 163: 133–). See photo below or video here.
    a. Put filter on tool. Place on flat rock, turn the top of the tool to brush the rock. The brushing is done for 30 seconds on the upper (water-exposed) side of the cobble.
    b. Then close the valve while still pressing the sampler to the cobble and pull up the piston to collect the algal suspension, then turn valve to release sample.
2. Collect five replicates, cleaning the brush between samples. Empty each sample into separate flasks and fix with 4% formaldehyde. Store at room temperature.

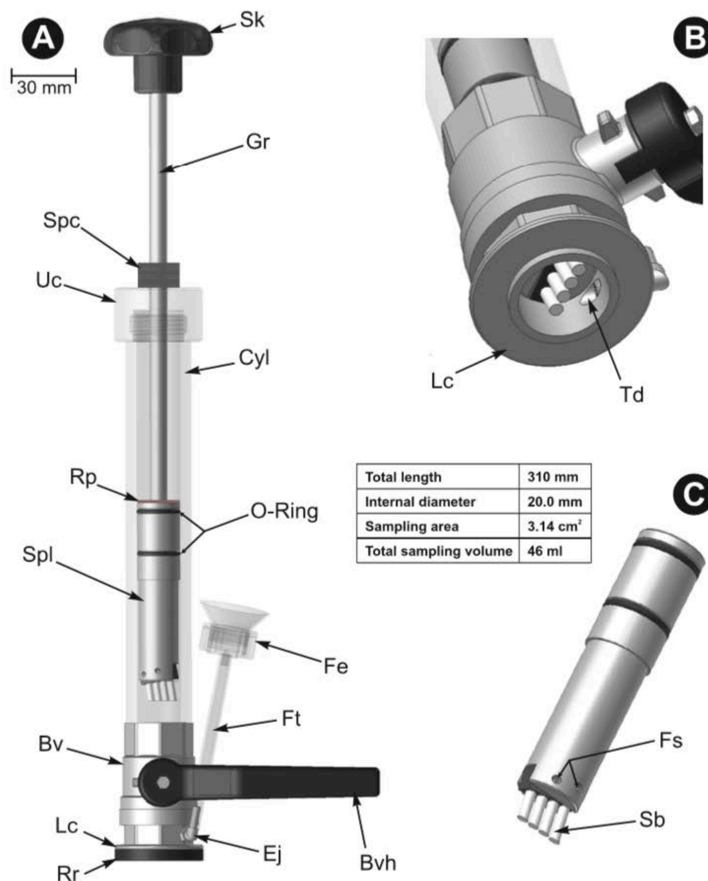


Fig. 1. The modified Brush Sampler device: **A**, location drawing; **B**, front view of sampling area and brush without rubber ring sealing; and **C**, detail of the plunger with brush retainer. (Bv) Ball valve, (Bvh) Ball valve handle, (Cyl) Plexiglas® cylinder, (Fe) Filtering element, (Fs) Fixation screws, (Ft) Flexible tube, (Gr) Guide rail, (Lc) Lower collar (stainless steel), (Ej) Plastic elbow joint, (Rp) Rubber pad, (Rr) Rubber ring, (Sb) Strip brush, (Sk) Star-shaped knob, (Spc) Spacer, (Spl) Stainless steel plunger, (Td) Tangential drilling, (Uc) Upper collar.

9. Benthic macroinvertebrate samples

Equipment

- 400-500-µm-mesh kick net, preferably with cod-end if available.
- Squirt bottle
- 95% ethanol (enough to fill 1-5 sample jars - 5 for Sweden and 1 or more for other countries, depending on size of sample)
- Sample containers
- Smaller vials (e.g. cryo-vials) for fatty acid samples (sorted in the field)
- 500-um-mesh netting for storing fatty acid samples (if not sorting in the field)
- Falcon tube for fatty acid samples (if not sorting in the field) or cryo-vials (if predominant taxa are sorted in the field).

a. **Taxonomy/Stable isotopes**

Method

1. *Hard bottom*: Kick and sweep the net back and forth along five 1-metre-long transects radiating out from shore. Each transect should be sampled for 30s.
    a. Sweden: keep the sample from each transect separate.
    b. All other countries: all transects combined into a single sample.
2. *Soft bottom (if no hard bottom can be found)*: Jab the lake bottom from the shoreline. Take five 30-second samples, radiating out in a line transect from shore as possible.
    a. Sweden: keep the sample from each transect separate.
    b. All other countries: all transects combined into a single sample.
3. Rinse water down the side of the net to ensure all invertebrates are rinsed into the cod-end. The contents of the net should be rinsed into the sampling cup by splashing/spraying water onto the outside of the net, washing organisms down into the sample cup from the sides of the net, while avoiding pouring water inside the net (i.e., don't pour water into the net to wash organisms down, because you might add more organisms).
4. If there is a large amount of sand and gravel in the net, the bucket swirl method can be employed. See method here: https://www.waterboards.ca.gov/water_issues/programs/swamp/docs/cwt/guidance/3522.pdf. Note that this method describes using a separate sieve to pour the sample over. We pour the sample back through the sampling net to avoid bringing extra equipment.
5. Remove sample from the cod-end place into a sample jar (do this separately for each sub-sample for Sweden, and use 5 separately-labelled sample jars). Add 95% ethanol to sample jar to preserve sample (70% final concentration as a minimum). Ensure a label is placed in the jar (written in pencil or ink that will not dissolve in ethanol)
6. Ethanol samples will need special containers to ship by air. Ship by ground if possible.
7. After samples are returned from the taxonomy lab, use the same samples for stable isotopes (Need to specify that lab should keep different taxa in separate containers).

b. **Fatty acids**

Method

1. *Hard bottom*: Kick and sweep the net back and forth radiating out from shore to collect macroinvertebrates of predominant taxa. Note that you are not bound to any time limit, but that this is sampling that aims at collecting sufficient biomass (5–10 ind.) for predominant taxa.
2. *Soft bottom*: Jab the lake bottom from the shoreline. Take samples, radiating out from shore as possible. Collect sufficient biomass (5-10 ind.) of predominant taxa. .
3. Sort predominant taxa in the field. Predominant taxa will likely be larval stages of chironomids, gammarids, tipulids, stoneflies, mayflies, as well as worms, but other may occur. Sort 5–10 ind. of the same taxa (that appear similar) into cryo-vials for freezing and preserve another 2–3 ind. in 70% ethanol for later taxonomic analysis. If not sorting the sample in the field, pour the sample over a piece of 500-μm netting (preferably

mounted on a plexiglas ring), spread the invertebrates out evenly on the net, cut out and roll the net (inverts on the inside) and store rolled netting in falcon tube.

4. Freeze sample as soon as possible at -20 C to -80 C. Pack frozen samples with dry ice or as many ice packs as possible for shipping (ensure container is full of samples and ice packs with little open space). Keep frozen during transport (e.g. cool freight).

10. Habitat measurements

Habitat measurements should be made in the riparian and the littoral zone where sampling occurs. Plot sizes for estimating percent cover of different habitat types should be 15 m x 10 m for the littoral zone and 15 m x 15 m for the riparian zone. Smaller areas are acceptable if it is not possible to sample these large areas. In all cases, an estimate of the area sampled should be recorded.

a. Habitat

Equipment

- GPS
- Field sheets
- Pencils
- Metre stick
- Ruler
- Tape measure

Method

1. Record coordinates (latitude and longitude) of sample location, including datum (coordinate system of GPS unit)
2. Record elevation in m
3. Record information about presence/cover of macrophytes (not mosses) on field sheet (0%, 1-25%, 26-50%, 51-75%, 76-100%)
4. Record % cover of woody debris
5. Record description of riparian vegetation on field sheet
    a. Estimate percent canopy coverage over the habitat is best estimated using a densiometer (0%, 1-25%, 26-50%, 51-75%, 76-100%). A densiometer can be purchased from forestry suppliers (~$100 USD) and consists of a convex or concave mirror that is gridded such that you assess the number of grids covered by the canopy. See the website as an example: https://www.forestry-suppliers.com/Documents/1450_msds.pdf. An alternative method could be to estimate the percentage of the littoral zone shaded by the canopy along a 15 m shoreline length (measured 2 m out from the shore).
    b. Record dominant lakeside vegetation (presence of ferns/grasses, shrubs, deciduous trees, coniferous trees)
6. Record depth at sampling location. Measure depth at five locations where benthic macroinvertebrate and benthic algal samples were collected and average.

7. Characterize the dominant substrate type by observing the substrate and comparing with visual substrate assessment chart below.

Code	Size Class	Size Range (mm)	Description
RS	Bedrock (Smooth)	>4000	Smooth surface rock bigger than a car
RR	Bedrock (Rough)	>4000	Rough surface rock bigger than a car
HP	Hardpan	>4000	Firm, consolidated fine substrate
LB	Boulders (large)	>1000 to 4000	Yard/meter stick to car size
SB	Boulders (small)	>250 to 1000	Basketball to yard/meter stick size
CB	Cobbles	>64 to 250	Tennis ball to basketball size
GC	Gravel (Coarse)	>16 to 64	Marble to tennis ball size
GF	Gravel (Fine)	> 2 to 16	Ladybug to marble size
SA	Sand	>0.06 to 2	Smaller than ladybug size; gritty between fingers
FN	Fines	≤0.06	Silt Clay Muck (not gritty between fingers)
WD	Wood	Regardless of Size	Wood & other organic particles
RC	Concrete	Regardless of size	Record size class in comment field
OT	Other	Regardless of Size	Metal, tires, car bodies etc. (describe in comments)

Note: Table is from U.S. EPA National Rivers and Streams Assessment 2013/14 Field Operations Manual, pg. 73.

Variables measured	
Coordinates	Latitude, longitude
Depth	Average and/or maximum of lake; depth at sampling location
Elevation	
Macrophytes	Presence and coverage
Inlets/outlets	Presence/absence
Substrate	Description where sampled (hard/soft)
Riparian vegetation	Presence, coverage, and type
Fish	Presence/absence
Woody debris	% coverage
Other observations	Write down any observations that you think may be of interest

b. Stable isotopes

Collect organic material from within the littoral zone of the lake and directly from the riparian vegetation to represent allochthonous subsidies to lake food webs.

- Collect the dominant riparian vegetation from the shoreline and place in a ziploc. Stable isotope analysis only need a few mg of dry weight. Try to be representative by collecting the dominant plant species.
- Collect organic material from the macroinvertebrate kick net or from directly within the stream and place in a ziploc Only a few mg of dry weight is necessary.
- Keep samples fridge-cold during transport and store samples at -20°C a soon as possible.

12. Decomposition strip samples

Equipment

- Zip-ties (5+ per site)
- Cotton strips (provided by Brianna)
- Chain or clothesline wire (with clothesline wire attachments)
- Temperature logger
- 1-2 Stakes or pieces of rebar per site
- 1 tent peg per site (if not using 2 stakes)
- 95% ethanol (return trip - enough to rinse strips)
- Labelled bags (return trip - one per site)
- Aluminum foil (return trip)

Method

Prior to deployment (can be prepared in lab)

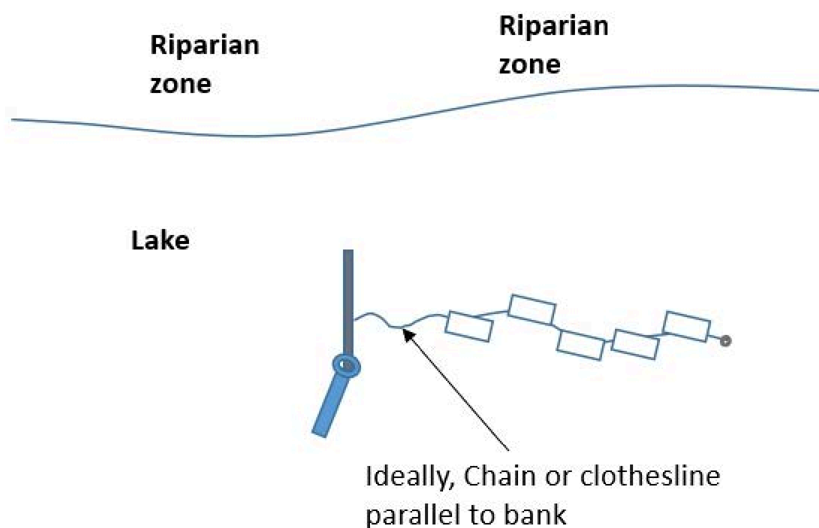
1. *Separate the threads of the strip at one end (0.5 cm from the edge) with a sharp object (e.g., nail, forceps, zip-tie tip) to create a small opening*

2. *Put a zip-tie through each strip. Restrict handling of cotton strips to 1-cm wide area at each end of the strip to avoid contaminating the middle portion strip where tensile-strength measurement occurs.*

In the field

1. Secure a stake/rebar at a safely wadeable depth in lakes to make sure strips are not exposed to drying. When securing the chain with cotton strips into the lake bottom, make sure to select an area that will not become exposed to air if water levels drop and that is not an area of natural deposition.
2. Attach five strips to a chain (if hard-bottomed) or clothesline (if soft- or hard-bottomed) making sure they are evenly spaced. Secure the chain/clothesline with attached strips to the stake/rebar. Align strips parallel to shore and secure the other end of the chain/clothesline with a tent peg or stake.
3. Deploy temperature logger with strips (e.g., HOBO TidBit, Pendant or similar).

Placement of cotton strips in lake.



Cotton strips attached to chain; Chain and loggers attached to rock with clothesline

1. Strips need to be deployed and retrieved ~6 weeks later. Anyone can retrieve the samples, so someone outside the project can do so. Plan accordingly, so that cotton strips are not lost under ice.
2. To collect strips, remove chain/clothesline from lake. Collect temperature logger if deployed and record date collected. On bank, cut each ziptie to remove each strip from the clothesline/chain. Either in field or back at lab on the day of sampling, rinse with 95% ethanol, dry strips, and wrap in aluminum foil. Place in labelled bag (site code).

Methods for Sampling Rivers

Selection of sample site

- Select wadeable (2nd to 4th order, approximately) streams, roughly 1-10 m width. Sample reaches are approximately six times the wetted stream width.
- If streams are sampled near lakes, the streams should preferably be inlets. If sampling takes place in a lake outlet, it must occur at least 1 km downstream of the lake to avoid lentic influence.
- Avoid glacial streams.
- Sample in rocky habitats, with a chosen stream reach including riffle and run habitats. Samples will be composite across riffle and run. Sampling soft-bottomed habitats is acceptable if no hard substrates are available.

Order of collection

1. Sample site photos
2. Water chemistry
3. Microbial samples (eDNA)
4. Benthic algae
   a. Taxonomy
   b. DNA
   c. Chlorophyll a
   d. Stable isotopes
   e. Fatty acids
5. Meiofauna (Optional, if time permits)
6. Benthic macroinvertebrates
   a. Taxonomy/stable isotopes
   b. Fatty acids
7. Habitat measurements
   a. Habitat
   b. Stable isotopes
8. Decomposition strips

Full list of materials for 1 river site (to be added once edits complete)

1. Sample photos

- Take photos of the field sheet with site name, the sample reach, upstream of sample reach, downstream of sample reach, shoreline near sample reach, and aquatic and exposed substrate.

2. Water chemistry samples

Equipment:

- Sample bottles with labels
- Marker pen
- Nitrile gloves if recommended
- Telescoping rod
- Multimeter
- Fixer (optional for samples for analysis of metals concentrations)
- Filter (DP)
- Pump
- 60-mL syringe
- 0.45-µm cellulose acetate membrane filter

Method:

1. Using the water chemistry bottles provided by the lab, collect an upstream sample from within the flow in mid-channel and below the surface. Use telescoping rod if necessary. Rinse the sample bottle three times (omit if not required by lab), pouring out water away from where the sample will be collected.
2. Filter samples for total dissolved phosphorus using either 1) a 0.45 µm cellulose acetate filter and hand pump, or 2) a 60-mL syringe attached to a filter holder containing a 0.45-µm cellulose acetate membrane filter. For the syringe method 2), refill the syringe as needed to obtain sufficient water to fill the sample bottle, detaching the syringe from the filter holder while refilling, and replacing the filter with a new one if the filter clogs.

3. Add fixer for metals if collected
4. Take duplicate sample for 10% of sites (i.e., collect duplicate samples at 1 river site if sampling 10 river sites or fewer)
5. Collect field blanks using deionized water taken with you into field (optional)
6. If you have a multi-meter, measure parameters from within the flow of the channel.

Variables measured	
Water chemistry samples	
Cations, anions	Al, Ca, Fe, K, Mg, Mn, Na, Br, Cl, F, PO_4, SO_4

	(note that NO_3-NO_2, NH_4, and HCO_3/CO_3 will all be captured by nutrient analyses and allow for ionic balance calculation).
Other lab variables	Alkalinity, pH, specific conductivity
Nutrients	TP, TN, TOC, DP or TDP (see note below), nitrite/nitrate, ammonium, DOC, DIC, HCO_3/CO_3 NOTE: If handling time restrictions can be met then measure DP. When holding times are long due to shipping from remote locations (e.g., western Canada samples), then field filter for TDP.
Metals (optional)	
Multi-meter	
Physicals	Conductivity, temperature, pH, dissolved oxygen, total dissolved solids (TDS) (optional), turbidity (optional)

3. Microbial samples (eDNA)

Equipment:

- Telescoping rod (https://bit.ly/38aUzFr)
- Bucket
- Spray bottle (or similar) with 70% Ethanol
- Brush
- Marker pen
- Electrical tape
- 0.22 um cartridge filter (https://bit.ly/2xJKO4B, provided by UiO) (1 per sampling site)
- 50/60 mL syringe (provided by UiO) (1 per sampling site)
- 5 mL syringe (provided by UiO)
- RNAlater (https://bit.ly/2TYE1Nm, provided by UiO)
- Nitrile gloves

Method:

1. First, put on nitrile gloves and keep them clean – only touch sampling equipment. Prior to sampling at a new site, ensure the sampling rod, sampling vessel and buckets have been disinfected with 70% ethanol by: spraying the equipment with ethanol, scrubbing

with the brush and waiting for 5 minutes before putting the sampling equipment in contact with a new water body.

2. Rinse thrice the sampling vessel with river water before beginning sample collection. Rinsing water is to be dumped on ground rather than in the river.

3. Extend the sampling rod to full length so as to collect water at a distance of 3-4 meters from the shore (if possible). Avoid excessive mixing when lowering the sampling vessel in the water. Draw with the sampling rod and vessel and collect in the bucket. Important: do not sample water affected by your activity (i.e. resuspended sediments, discarded samples).

4. Rinse thrice the 50/60 mL syringe by drawing water from the river and ejecting it on the ground.

5. eDNA collection on 0.22 um cartridge filters. This is done using the 50/60 mL syringe. To filter, fill a syringe with water from the bucket, remove air bubbles, then fasten a filter cartridge to the syringe and expel the water volume through the filter*. Important: keep count of the volumes! It is essential to record the cumulative volume filter through the cartridge.

6. Filter water until it becomes hard to expel water. Then fill syringes with air and push through the filter to remove residual water. Continue until no water is visible in the filter cartridge. Usually this takes three repetitions and it is easier if you keep the syringe upright (vertical).

7. Once the cartridge is dry, add 2 mL of RNAlater solution into the cartridge using the 5 mL syringe.

8. Label filter cartridges with ID and sample volume. Close the two openings of the cartridge using the electrical tape. Keep the cartridges at 4°C in the field (or as cold as possible). If you do not have access to freezers, as they are preserved with RNAlater, you can try to keep samples cold using ice in coolers while on the road/field.

9. In the laboratory, storage at -20°C until shipping.

10. For shipping, pack samples in a EPS box (expanded polystyrene) using gel packs or rigid cold accumulators, to keep the cartridges as cold as possible (not necessarily

frozen, which can avoid shipping problems and extra costs). Samples will be sent to UiO, where eDNA** extraction will be performed.

(*) The water expelled through the filter can be collected for chemical analysis (nutrients, cations, anions).

(**) Fish presence can be determined by using eDNA. Thus, DNA extracted at UiO will be used for metabarcoding analysis with the right fish primers.

4. Benthic algae

Equipment

- Tray (size that can contain at least five cobbles)
- Toothbrush - one per site (semi-quantitative samples)
- 250 mL flasks
- Squirt bottle
- Sharpie or wax pencil
- Camera
- Falcon test tube (marked at 50 mL) or syringe (50 mL)
- Alkaline Lugol's solution (alkaline solution will preserve diatom shells; see below for recipe)
- 95% ethanol (enough for chlorophyll a sample, stable isotope sample and DNA sample)
- Sample containers (one each for taxonomy, chlorophyll a, stable isotopes, and fatty acids)

Prepare alkaline Lugol's as follows: Dissolve 2 g potassium iodide and 1 g iodine crystals in 300 ml distilled or demineralized water. The resultant liquid should be dark brown coloured. It should be stored in an air-tight and light-proof container to minimize sublimation.
Add 1 to 5 drops of Lugol's iodine to 100 ml sample to give a dark brown colour. More alkaline Lugol's may be necessary if samples are rich in organic matter and are stored for a longer time.

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a. Taxonomy

Method

1. Use a shoreline reach about 10 m long, go as deep into the water as possible for your arms to reach the stones. Don't collect directly at the shore - risk for parts that have been dry.
2. Collect > five stones (fist-sized or bigger) and brush the tops of them with a toothbrush into a tray (video here). Make sure not to get the dirt of the bottom side into the tray. The toothbrush should be new or clean for each site.
3. Rinse the material off the toothbrush into a tray (composite all five samples).
4. Add more stones in case the water in the tray is not yet brown/green from algae, ideally such as here, or here (in Swedish only) or here. "Whisky"-color is ok.

5. Remove any large debris particles and pour the sample into a 250 mL flask. Put a mark on the flask to indicate the volume of the sample. This sample will be split into sub-samples for taxonomy, chlorophyll a, DNA, stable isotopes, and fatty acids.

6. Put the rocks in the tray and take a picture from above (make sure the size of the tray is recorded so the area scraped can be estimated later on). You may also add the flask with label to the tray, as this facilitates later identification of the picture.
7. Shake flask and pour out 50 mL homogeneous sub-sample into a Falcon test tube (or use large syringe to take a 50 mL aliquot)
8. Add alkaline Lugol's solution to test tube until it's tea- or whiskey-coloured to fix the sample.
9. Use the remainder of the sample in the 250 mL flask for samples of DNA, chlorophyll, SI and FA (see below).

b. DNA

Method

1. Swirl the flast containing the toothbrush sample collected for taxonomy to homogenize the sample, and take out a 10-50 mL sub-sample for DNA (record volume) and add 95% ethanol to preserve the sample (> 80% volume of the final sample).
2. Ethanol samples will need special containers to ship by air. Please send DNA samples to: Eva Herlitz, SLU, Institutionen f vatten och miljö, Gerda Nilssons väg 5, SE 756 51 Uppsala, Sweden. Mark the package with some note saying, "ArcticBiodiver DNA samples - Maria Kahlert". In case you are sending as a "letter", you need to use a different address: Eva Herlitz, SLU, Institutionen f vatten och miljö, Box 7050, SE 750 07 Uppsala, Sweden.

c. Chlorophyll a

Method

1. Swirl the flask containing the toothbrush sample collected for taxonomy to homogenize the sample, and take out a 10-50 mL sub-sample for chlorophyll (record volume) and add 95% ethanol to preserve the sample (> 80% volume of the final sample). Collect a larger subsample (50 mL rather than 10 mL) if algal biomass seems low. Record the volume of the subsample!
2. Ethanol samples will need special containers to ship by air. Ship by ground if possible.

d. Stable isotopes

Method

1. Swirl the flask containing the toothbrush sample collected for taxonomy to homogenize the sample, and take out a 10-50 mL sub-sample from the flask and freeze (-20°C or preferably -80°C; i.e., dry ice).
2. Pack frozen samples with dry ice or as many ice packs as possible for shipping (ensure container is full of samples and ice packs with little open space). Keep samples frozen during transport (e.g. cool freight).

e. Collection for fatty acids

Method

1. Freeze the remainder of the original (fresh) sample of benthic algae as soon as possible (-80C freezer if possible). This can preferably be done in the flask where it was collected.
2. Pack frozen samples with dry ice or as many ice packs as possible for shipping (ensure container is full of samples and ice packs with little open space).

5. Biofilm meiofauna samples

Equipment

- Cylindrical brush sampler (from Willem)
- Filter for cylindrical brush sampler
- Container for sample
- Flask
- 4% formaldehyde

Method

1. Use the cylindrical brush sampler (area 3.14 cm^2) to collect five quantitative samples of epilithic algal communities from five cobbles, according to Peters et al. (2005, Arch. Hydrobiol. 163: 133–). See photo below or video here.
    a. Put filter on tool. Place on flat rock, turn the top of the tool to brush the rock. The brushing is done for 30 seconds on the upper (water-exposed) side of the cobble.
    b. Then close the valve while still pressing the sampler to the cobble and pull up the piston to collect the algal suspension, then turn valve to release sample.
2. Collect five replicates, cleaning the brush between samples. Empty each sample into separate flasks and fix with 4% formaldehyde. Store at room temperature.

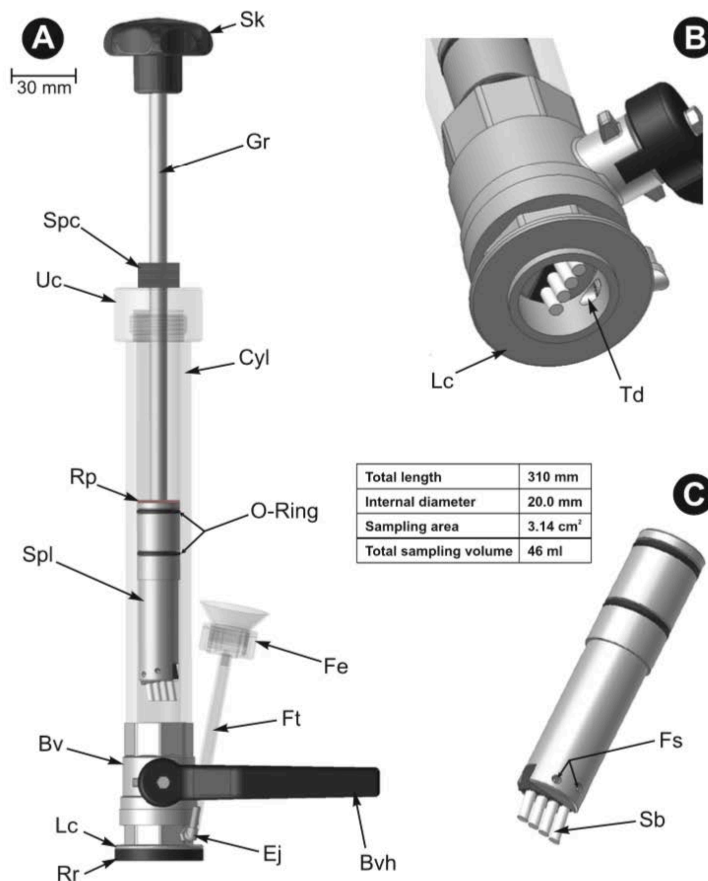


Fig. 1. The modified Brush Sampler device: **A**, location drawing; **B**, front view of sampling area and brush without rubber ring sealing; and **C**, detail of the plunger with brush retainer. (Bv) Ball valve, (Bvh) Ball valve handle, (Cyl) Plexiglas® cylinder, (Fe) Filtering element, (Fs) Fixation screws, (Ft) Flexible tube, (Gr) Guide rail, (Lc) Lower collar (stainless steel), (Ej) Plastic elbow joint, (Rp) Rubber pad, (Rr) Rubber ring, (Sb) Strip brush, (Sk) Star-shaped knob, (Spc) Spacer, (Spl) Stainless steel plunger, (Td) Tangential drilling, (Uc) Upper collar.

6. Benthic macroinvertebrate samples

Equipment

- 400-500-um-mesh kick net with cod-end
- Squirt bottle
- 95% ethanol (enough to fill 1-5 sample jars - 5 for Sweden and 1 or more for other countries, depending on size of sample)
- Sample containers
- Smaller vials for fatty acid samples (if sorting in the field)
- 500-um-mesh netting for storing fatty acid samples (if not sorting in the field)
- Falcon tube for fatty acid sample (if not sorting in the field)
- Soft forceps (for collecting individual benthic invertebrates)

a. Taxonomy/Stable isotopes

Method

1. *Hard bottom*:
    a. Method 1: Collect five kick samples (1 minute along a 1-m stretch each) from riffle/run habitat, collecting sample in kick net.
        i. *Sweden*: Keep 5 samples separate and store in separate jars.
    b. Method 2: Perform 3-minute traveling kick through stream reach, zig-zagging across and through channel in riffle/run habitat, collecting a single sample in the kick net
2. *Soft bottom*: Use more of a jab than a kick while collecting samples but follow the same procedure.
3. Rinse water down the side of the net to ensure all invertebrates are rinsed into the cod-end. The contents of the net should be rinsed into the sampling cup by splashing/spraying water from the sides of the net, while avoiding pouring water inside the net
4. If there is a large amount of sand and gravel in the net, the bucket swirl method can be employed
5. Remove sample from the cod-end place into a sample jar (do this separately for each sub-sample for Sweden, and use 5 separately-labelled sample jars). Add 95% ethanol to sample jar to preserve sample (70% final concentration as a minimum). Ensure a label is placed in the jar (written in pencil or ink that will not dissolve in ethanol)
6. Ethanol samples will need special containers to ship by air. Ship by ground if possible. After samples are returned from the taxonomy lab, use the same samples for stable isotopes (Need to specify that lab should keep different taxa in separate containers).

b. Fatty acids

Method

- *Hard bottom*: Kick and sweep the net back and forth to collect macroinvertebrates of predominant taxa. Note that you are not bound to any time limit, but that this is sampling that aims at collecting sufficient biomass (5–10 ind.) for predominant taxa.
- *Soft bottom*: Jab the lake bottom from the shoreline. Take samples, radiating out from shore as possible. Collect sufficient biomass (5-10 ind.) of predominant taxa. .
- Sort predominant taxa in the field. Predominant taxa will likely be larval stages of chironomids, gammarids, tipulids, stone flies, mayflies, as well as worms, but other may occur. Sort 5–10 ind. of the same taxa (that appear similar) into cryo-vials for freezing and preserve another 2–3 ind. in 70% ethanol for later taxonomic analysis. If not sorting the sample in the field, pour the sample over a piece of 500-μm netting (preferably mounted on a plexiglas ring), spread the invertebrates out evenly on the net, cut out and roll the net (inverts on the inside) and store rolled netting in falcon tube.
- *Hard bottom*:
    - Method 1: Collect predominant fauna by using the kick net from riffle/run habitats, collecting sample in kick net. Composite all samples.

    - Method 2: Perform 3-minute traveling kick through stream reach, zig-zagging across and through channel in riffle/run habitat, collecting a single sample in the kick net.
- *Soft bottom*: Use more of a jab than a kick while collecting samples but follow the same procedure and collect predominant fauna from these samples.
- If not sorting the sample in the field, pour the sample over a piece of 500-um netting, spread the invertebrates out evenly on the net, and roll the net. Store rolled netting in falcon tube.
- Freeze samples as soon as possible at -20 C to -80 C. Pack frozen samples with dry ice or as many ice packs as possible for shipping (ensure container is full of samples and ice packs with little open space). Keep samples frozen during transport (e.g. cool freight).

7. Habitat measurements

a. Habitat

Equipment

- GPS
- Field sheets
- Pencils
- Metre stick
- Ruler
- Tape measure

Method

1. Record coordinates (latitude and longitude) of sample location, including datum (coordinate system of GPS unit)
2. Record elevation in m
3. Record location in relation to lakes (inlet/outlet)
4. Record information about presence/cover of macrophytes (not mosses) on field sheet (0%, 1-25%, 26-50%, 51-75%, 76-100%)
5. Record information about presence/cover of mosses on field sheet (0%, 1-25%, 26-50%, 51-75%, 76-100%)
6. Record information about presence/cover of periphyton on field sheet (scale from 0 to 5 describing cover and thickness: 1 - not slippery; <0.5 mm thick to 5 - extensive, thick algal mat; >20 mm thick. Details to be added to field sheets)
7. Record % cover of woody debris
8. Record description of riparian vegetation on field sheet
    a. Estimate percent canopy coverage estimated using a densiometer (0%, 1-25%, 26-50%, 51-75%, 76-100%)
    b. Record dominant streamside vegetation (presence of ferns/grasses, shrubs, deciduous trees, coniferous trees)
9. Measure reach length (length of reach should be six times the wetted stream width)
10. Record depth and wetted width

    a. Divide the sample reach into 5 evenly-spaced transects
    b. Along each transect, measure wetted width
    c. Measure depth at 5 evenly-spaced locations along each transect

11. Measure velocity at multiple locations along each transect (optional)
12. Measure discharge (optional)
13. Measure substrate particle size:
    a. Option 1 - Use a ruler to measure median axis of 100 particles (to the nearest 0.1 cm) randomly throughout the reach. Select particles at random by taking 2 steps through the reach and measuring the particle in contact with the toe of your boot (including sand). Do not bias measurements by picking the largest particle to measure. Median axis (or b-axis) is the intermediate axis of the rock (neither the longest axis nor shortest axis). Record bedrock as RS for smooth bedrock or RR for rough bedrock, and anything in sand or fines category (< 0.2 cm) as < 0.2 cm. If time-limited, measure a smaller number of rocks (minimum 20).
    b. Option 2 - Visually estimate size class of median axis of 100 particles along transects or throughout reach (using random selection method described above). Use US chart for visual estimation of particle size, but combine sand and fines for compatibility with measurements. If time-limited do a smaller number of rocks (minimum 20).

Code	Size Class	Size Range (mm)	Description
RS	Bedrock (Smooth)	>4000	Smooth surface rock bigger than a car
RR	Bedrock (Rough)	>4000	Rough surface rock bigger than a car
HP	Hardpan	>4000	Firm, consolidated fine substrate
LB	Boulders (large)	>1000 to 4000	Yard/meter stick to car size
SB	Boulders (small)	>250 to 1000	Basketball to yard/meter stick size
CB	Cobbles	>64 to 250	Tennis ball to basketball size
GC	Gravel (Coarse)	>16 to 64	Marble to tennis ball size
GF	Gravel (Fine)	> 2 to 16	Ladybug to marble size
SA	Sand	>0.06 to 2	Smaller than ladybug size; gritty between fingers
FN	Fines	≤0.06	Silt Clay Muck (not gritty between fingers)
WD	Wood	Regardless of Size	Wood & other organic particles
RC	Concrete	Regardless of size	Record size class in comment field
OT	Other	Regardless of Size	Metal, tires, car bodies etc. (describe in comments)

Note: Table is from U.S. EPA National Rivers and Streams Assessment 2013/14 Field Operations Manual, pg. 73.

Variables measured	

Coordinates	Latitude, longitude
Mean Depth	dMean and maximum; measure 5 times across channel for five transects. Take average of maximum depth at each transect as maximum depth of sample reach. Take average of all depth measurements for mean depth of sample reach.
Elevation	
Wetted width	Mean of five measurements spaced along reach
Macrophytes	Presence and coverage
Current velocity	Measured at multiple locations along transects (optional)
Discharge	(optional)
Periphyton	Presence and coverage
Location relative to lake	Inlet/outlet
Riparian vegetation	Presence/absence; canopy coverage
Woody debris	% coverage

b. Stable Isotopes

Collect organic material from within the littoral zone of the lake and directly from the riparian vegetation to represent allochthonous subsidies to lake food webs.

1. Collect the dominant riparian vegetation from the shoreline and place in a ziploc. Stable isotope analysis only need a few mg of dry weight. Try to be representative by collecting the dominant plant species.
2. Collect organic material from the macroinvertebrate kick net or from directly within the stream and place in a ziploc Only a few mg of dry weight is necessary.
3. Store samples at -20°C if possible, or fridge cold if not possible. Pack frozen samples with dry ice or as many ice packs as possible for shipping (ensure container is full of samples and ice packs with little open space) to keep samples frozen during transport.

8. Decomposition strip samples

Equipment

- Zip-ties (5+ per site)
- Cotton strips (provided by Brianna)

- Chain or clothesline wire (with clothesline wire attachments)
- Temperature logger (optional, but highly recommended)
- 1-2 Stakes or pieces of rebar per site
- 1 tent peg per site (if not using 2 stakes)
- 95% ethanol (return trip - enough to rinse strips)
- Labelled bags (return trip - one per site)
- Aluminum foil (return trip)

Method

Prior to deployment (can be prepared in lab)

1. *Separate the threads of the strip at one end (0.5 cm from the edge) with a sharp object (e.g., nail, forceps, zip-tie tip) to create a small opening*

2. *Put a zip-tie through each strip. Restrict handling of cotton strips to 1-cm wide area at each end of the strip to avoid contaminating the middle portion strip where tensile-strength measurement occurs.*

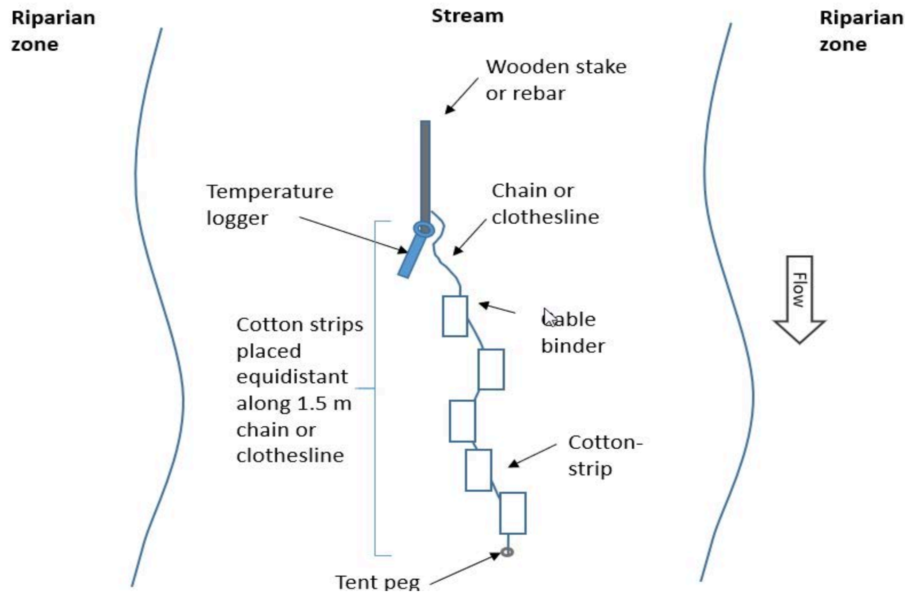
In the field

1. Secure a stake/rebar in the main channel of the river bottom or at a safely wadeable depth in lakes to make sure strips are not exposed to drying. When securing the chain with cotton strips in the river, make sure to select an area that will not become exposed to air if water levels drop and that is not an area of natural deposition.
2. Attach five strips to a chain (if hard-bottomed) or clothesline (if soft- or hard-bottomed) making sure they are evenly spaced. Secure the chain/clothesline with attached strips to

the stake/rebar. Align strips downstream and secure the other end of the chain/clothesline with a tent peg or stake.

3. Deploy temperature logger with strips (e.g., HOBO TidBit, Pendant or similar).

Placement of cotton strip in stream/river.



Cotton strips attached to chain; Chain and loggers attached to rock with clothesline

Installation in stream; submerged in stream

1. Strips need to be deployed and retrieved ~6 weeks later. Anyone can retrieve the samples, so someone outside the project can do so. Plan accordingly, so that cotton strips are not lost under ice.
2. To collect strips, remove chain/clothesline from stream. Collect temperature logger if deployed and record date collected. On bank, cut each ziptie to remove each strip from the clothesline/chain. Either in field or back at lab on the day of sampling, rinse with 95% ethanol, dry strips, and wrap in aluminum foil. Place in labelled bag (site code).