Standard operating procedures for lake littoral and profundal macroinvertebrate sampling- from site selection to data management

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Table of content

Monitoring site evaluation

A map based evaluation of each site is required prior to going into the field. The final sample site(s) selected must be representative of the data quality objective, waterbody, watershed being evaluated. Final selection of representative sampling sites will occur in the field. Pre-monitoring site evaluation involves reviewing point source discharge information, general topographic and site access information. Further the type of the lentic system should be specified.
Pond: Natural or man-made lentic system with an open water surface area less than 1 hectare.
Lake. Natural or man-made lentic system with an open water surface area greater than 1 hectare and more or less stable shorelines.
Reservoir. Man-made lentic/lotic system with an open water surface area greater than 1 hectare. Reservoirs generally have hypolimnetic outflow, a high drainage area to surface area ratio, and significant water level fluctuation i.e. less stable shorelines.

Obtaining drainage basin area

Drainage basin area information can be obtained from existing reports, topographic maps, or geographic information system data bases. This information and especially information on land use may be very useful in the interpretation of results.

Surface area

The surface area of waterbodies can be obtained from existing reports, topographic maps, or geographic information system data bases. For reservoirs, note whether the surface area is the minimum or maximum pool area. Report both values if available.

Shoreline development

In the pre-evaluation phase assess the major pressures on the system i.e. the presence and proximity of campgrounds, boat launches, summer or permanent residences, discharge outfalls, and swimming beaches, industry, mining or similar.

Sampling location

The general location of lentic sample sites is determined from the information gathered in the pre-monitoring evaluation. Pre-monitoring site evaluations outline the general sampling locations for each lentic system being assessed. Final decisions sample locations are made at sites, depending of their suitability. Physical measurements, and site descriptions as well as chemical samples should be collected at each sample location in addition to the macroinvertebrate samples. Studies on minimally impacted boreal lakes (Jyväsjärvi et al. 2009) indicate that depth, water color and hypolimnetic temperature are the most important environmental determinants of chironomid community variation. For shallow lakes(< 4m mean depth) biological sampling of the deepest part of the lake is not necessarily representative of actual ecological state. In shallow lakes, primarily littoral macroinvertebrate sampling is advised. Littoral sampling should be conducted on primarily rocky, representative shores of the lake. Studies in boreal lakes indicate (Suurkuukka & al 2012, in press) indicate that representative samples of common species are often caught with a few replicates but that rare species are best represented when sampling effort is spread over at

least 3 shores within the same lake. Thus sampling effort is dependant on the ultimate goal of the sampling. For biodiversity surveys a multihabitat, multisample approach on several occasions is advisable. For routine assessment studies that use common indices, a lighter sampling design is advised. In the following sampling for status assessment purposes is described.

Safety considerations

Always let colleagues know at which locations and on which days you plan to operate. Ensure that you have adequate maps with you. Preferably work in pairs especially when sampling remote or otherwise dangerous locations. In boats always use or carry life vests. Use the belt on your waders, it is a simple but effective way to improve flotation when you fall in. Always carry a mobile phone with you and have firstaid supplies ready in the car or boat. Make sure all sampling personnel is appropriately vaccinated against possible health hazards. Put mobile phones into watertight bags (e.g. ziplock) to ensure operability even if you have fallen in. When working in cold conditions have a second set of clothes with you waiting in the car.

Littoral sampling procedure

At pre-selected sites, first establish overall suitability for sampling; i.e. is this shore representative of the laketype, does it have a rocky shore? Having determined the direction of the wind set your first sampling spot downwind. By starting downwind and moving upwind you will avoid that disturbed sediments will spread unto unsampled sampling points of the same shore. Sampling points should be random but located within 25-40cm depth. Distances between sampling points should be at least 3-5m. Mark the downwind end of your sampling point using a heavy metal object (e.g. big screw) with an attached floater to help relocating the sampling point for post sampling physical measurements. Position your hand net (0.5mm mesh) very close but not onto the substratum of the sampling point. Standing in front of the net opening disturb the substratum by trampling forcefully on the bottom and move backwards 1m in about 20s parallel to the shore and in upwind direction. While trampling ,move the handnet in a "snakelike" fashion closely over the substratum to catch dislodged macroinvertebrates. Do not scrape the net along the bottom to avoid tearing of the net. Having moved 1m, raise the opening over the water surface and remove any sharp or large stones and twigs from the net. Rinse the objects in the net clear of any animal material. Rinse the net of small particles by repeatedly dipping it into the water. Be sure not to not submerge the net opening to avoid loss of sample material. Transport the sample back to shore for preservation. Empty the content of the net into a bucket and inspect the net for any additional individuals. Sieve the content in the bucket using a 500µm sieve. Mark each tightly closing sample container (250ml-1000ml volume, alcohol-prove grade plastics or glass) with an individual code (e.g. A32, P33 ect.)using an alcohol prove marking pen, and write all necessary information on a small piece of white, good quality copy paper (year, date, name of the lake, name of the shore, sample number, sample container number) with a pencil and drop it into the sample container. Be sure to write the corresponding replicate sample container code onto the field sheet as well. Do not use any other pen than a pencil and make test that the copying paper is not dissolved by alcohol before using them in the field. Put the contents retained by the sieve into the sample containers. Sample content should make up a maximum of 2/3 of the total volume of the container. If the samples contain more material use an additional container (be sure to mark this both on the field sheet as well as on the paper slip within each sample container. (i.e. put in a slip reading 1/2 or 2/2 respectively). Fill up the sample container with 90-95% ethanol. Wipe the bottle threads and the cap to remove any sand or dirt to ensure that sample containers will close tightly. Close the container and pack containers for transport to the laboratory. Before taking the next sample, rinse your net well.
At three suitable shore locations around the lake take a minimum of 2 samples per location totaling in 6 samples/lake. Include lesser locations if you cannot find three suitable locations but always take 6 samples from each lake. Do not pool samples but process and analyze them separately. When you are done with

preserving the last sample return to the sampling spots marked by the floaters and make the evaluations of physical parameters for each kicked sample according to the guidance provided in the field sheet section.

Material list

Waders
Handnet according to SFS-EN 28265 (ISO 7828:1985) standard, mesh size 500µm
Bucket (5-10 l in volume)
Funnel
Sieving cloth 500µm
Sample containers (250-1000ml in volume)
Ethanol prove marker
Field sheets
Paper
Maps
Pencils
Tweezers
Floaters and weights
Ethanol >90%
GPS
Measuring pole (e.g. 1,5m)

Optional:

Tape measure
First aid kit
20 m rope

Profundal lake sampling procedure

Profundal lake sampling should be carried out in deep lakes i.e. lakes with a maximum depth of > 10m and a mean depth of >4m according to methods described in the standard SFS 5076. Lake profundal data should be collected from mid October to mid November from the lakes deepest profundal zone (i.e. from depths that are at least 90% of maximum depth).Use bathymetric maps and GPS to navigate to the right area and verify your location with a depth meter. If bathymetric maps are lacking do a sweep of the lake with a depth probe and locate, record GPS coordinates and map the extent of the deep profundal area. For sampling, the boat should be anchored at a suitable spot on the upwind end of the deep profundal area. The first sample should be taken at the anchoring site. Measure the depth at the site and arm the Ekman grab. Use a meter marked line and lower the Ekman grab to depth -1,5 -2 m and stop the grab. Do not let lower the grab fall uncontrolled as it may tilt and hit the bottom at a wrong angle. Wait until the grab has stopped swaying (about 30seconds) and then let it fall freely to the bottom. Straighten the line without lifting and drop the bullet weight and wait for the grab to close. If the line has sufficient tension you will feel the closing of the grab. Hoist the grab at a steady pace preferably over a pulley to minimize line abrasion. At 1m from surface lean overboard and gently lift the Ekman grab into the boat. Inspect the whether the jaws of the grab have closed properly (i.e. no twigs, stones between the jaws). Open the upper lid of the grab and inspect the surface- it should look "intact". If the jaws have not closed properly or if in doubt on the representativeness of the sample discard the current sample and take another sample. Place the representative sample in a plastic container (e.g. bucket) and if possible measure the temperature and oxygen content of the water directly over the sediment. After these measurements open

the jaws and release the sample in the plastic container. Pour the sample into a bucket sieve with a µm mesh. Rinse the sample by dipping the bucket sieve into the water but make sure that the bucket sieve is never fully submersed. Use a washing bottle to aid the process if needed. Remove any larger non-organic material if necessary. Use tweezers or flush the remaining content into a plastic container using alcohol and place a paperslip with all sample site information (place, date, sample number, depth) within the sample. Fill the sample container with 80% alcohol following guidance provided in ISO-EN 5667-3. Wipe the bottle threads and the cap to remove any dirt to ensure that sample containers will close tightly. Close the container and pack containers for transport to the laboratory. Before taking the next sample, rinse your grab well. Take a total of six replicate profundal samples/ lake. Do not pool samples but process and analyze them separately.

When you use an anchor with a long line it is easy to take the next replicate samples despite strong winds. Having taken the first sample release about 3m of line on the anchor rope and refasten the anchorline to the boat let the wind transport the boat until there is tension on the anchor rope. Take your next sample by repeating the above procedure. By repeatedly releasing rope you do not have to use the motor or row between sampling.

Samples may also be sieved at shore and this may be advisable if very windy conditions prevail. If you intend to preserve your samples at the shore, reserve enough plastic containers with you in the boat. Make sure you mark the containers according to their sampling order. At shore make sure that the water used for rinsing does not include and animals (if necessary filter it) as these may interfere with the results of the profundal samples.

Material list

Boat (inflatable or other)
Ekman grab
Life vest
Anchor with long line
Depth meter
Buckets
500µm bucket sieve
Tweezers
Washing bottle
Paper
Maps
Gps
Pencil
Field sheet
Ethanol 80%

Optional:

Oxygen probe
Thermometer

Laboratory procedures

The following procedures apply to both internal and external laboratory procedures. If the samples are sent to external experts for identification, copies of the field sheets are placed in a plastic bag with the samples in the shipping container. Macroinvertebrate samples do not require cooling once the preservative has been added but should not be exposed to UV- or daylight. If handled by external consultants, the receiving laboratory checks that samples have sufficient levels of preservatives, and verifies the samples in the shipping container, noting any damage or missing samples.

Sorting

Sorting Efficiency: Sample sorting is done on white trays. Use good lighting (spot lighting using high powered halogen lamps is advised) and sharp tweezers. The tray should be divided into 6-9 equally sized fields. Mark these fields on the tray using a permanent, alcohol proof marker. Take about a spoonful of sample material onto the tray (e.g. 25x 35cm in dimension). Dilute the sample material with water and spread it evenly over the tray. Check each marked field individually for any organisms and transfer them to a separate sample vial (volume of 20-50ml) filled with 80% ethanol. Go through all the sample material and put a paperslip containing all sample information into the vial. Seal the vial firmly and store for identification. If you use plastic vials make sure that the plastic is alcohol prove as some plastics become brittle when in contact with highly concentrated ethanol. Sorting of a single sample should not take a trained sorter longer than 1 workday. If samples contain vast amounts of material they may be subsampled. In this case divide the whole sample material into smaller same sized fractions and sort one. Be sure to mark how the sample was subsampled on the paperslip within the vial (e.g. subsampled, 1/5 of original).
Sample sorting efficiency for new technicians should be checked and documented so that a minimum of 90 per cent of organisms are removed during the sample sorting process. For samples processed by an untrained technician, sorted sample fractions of the initial samples are retained and checked by an experienced sorter or taxonomical expert. If more than 10 per cent of the organisms in a sample are missed, the untrained sorter has to continue training until the QA objectives are met.

Material list

White tray (approx. 20 x 30 cm)
Tweezers
Ethanol 80%
Water
Washing bottles
Good quality office paper
Pencil
Sample vials (20-50ml)

Identification

To allow faster identification the taxonomic expert should sort the animals in samples according to rough morphotypes (e.g. sort all Baetidae into one petridish) before keying doing so will speed up the workflow. Specimens should be keyed to a standardized minimum taxonomic resolution level; the majority of organisms should be identified to genus or species. If such a national taxonomic resolution does not exist it

should be established and documented in cooperation with expert taxonomists to incorporate knowledge on distributional patterns and to ensure the availability of relevant taxonomic literature. Adults, larvae and pupae taxa should not be treated/listed as separate distinct taxa in the database , because it will inflate the number of taxa in the macroinvertebrate community and skew the evaluation of the final data set. Adult insects will not be keyed except for taxa which are aquatic as adults e.g. riffle beetles (Coleoptera: Elmidae), and other Coleoptera and certain Hemiptera.
Beginner or mid-level taxonomists should collect reference collections of identified species and have them checked by national experts. Any laboratory doing routine keying should have links to recognized national or international experts to identify rare, unusual or previously undocumented organisms. Once keyed, the physical sample material should be well preserved and stored for a minimum of at least 5-10 years to allow for checking of identifications. Preferably identified samples should be appended to permanent national natural museum collections.

Material list

Petri dishes of different sizes
Tweezers
Insect needles
Ethanol 80%
Paper
Pencil
Identification keys
Stereo microscope 10-60X or better
Compound microscope 10- 100x (immersion oil)
Immersion oil
Cover slips
Object trays

Optional:
Slide drying trays
Fixant (e.g. Eukitt, Canada balm)

Data management

Results on benthic macroinvertebrate together with all measurements should be recorded in a national database. Applying a standardized method and level of identification will allow valid comparison of macroinvertebrate data sets between different samplers, sampling locations and years. Special attention should be given to internal and possibly external ease of access to data (through designated web portals) and safe data storage (backups on multiple storage devices).

Literature

Jyväsjärvi, J, Tolonen, K & H Hämäläinen: 2009. Natural variation of profundal macroinvertebrate communities in boreal lakes is related to lake morphometry:implications for bioassessment: Canadan Journal of Fisheries and Aquatic Sciences 66:589-601.
Suurkuukka, H, Meissner, K & T. Muotka: 2012. Species turnover in lake littorals: spatial and temporal variation of benthic macroinvertebrate diversity and community composition. Diversity and Distributions (in press).
SFS 5076: 1989, 7 p. Sampling of the bottom fauna on soft bottoms with an Ekman grab

ISO 5667- 3: 1994: Water quality. Sampling. Part 3: Guidance on the preservation and handling of samples, 31p.
SFS-EN 28265: 1994. Water quality. Design and use of quantitative samplers for benthic macro-invertebrates on stony substrata in shallow freshwaters (ISO 7828:1985, ISO 8265:1988) 14 p.

Filling in the field sheet

The field sheet can be used for any benthic sampling. The first page of the field sheet is organized into different sections (Appendix 1). Most information can and should be filled into the form for the first two sections ("Site name" and "Sampling") before going into the field. Prefilled forms can then be printed (using a laser printer) and taken to the field where the rest of the information is filled in. The flipside contains short information on how to measure slope, estimate vegetation and substrate cover and current classes. Note that for littoral sampling separate sheets for each lake shore are needed.

Site name- section

Fill in the site name, type (i.e. stream, pond, lake or seashore) and name of the waterbody sampled. In the absence of accurate coordinates(from previous visits), provide approximate GPS coordinates to aid locating the right sampling sites in the field. State in which municipality and watershed the site is located. Provide a general habitat description (e.g. slow flowing section, rapids or vegetated lake shore).

Sampling- section

Fill in the date and sampling time. Provide the name of the agency in charge of sampling and indicate whether sampling is related to routine monitoring or a research project. Specify how many samples are to be taken in the field and which device to use. This is especially crucial if the person designing the sampling is not the surveyor. Indicate the mesh size and area covered by the device during sampling.

Site observations-section

State the name of the surveyor (person taking the samples) if not known in advance. At the site, if the coordinates filled in the "Site name" section were only approximate, provide measured GPS coordinates (for lake shores provide separate coordinates for each shore from the centre of the sampled area. Add any additional information (i.e. poor visibility, changed locations, number of photograph taken at site, etc.) that may be of use when evaluating results.

Substrate

This section is intended to give a general overview of the site. Describe the general substrata composition at the site by classifying particles into abundance classes ranging from 0-3 (table 1).

Class	Relative abundance
0	none
1	little (<10 %)
2	moderate (10–50 %)
3	abundant (> 50 %)

Table 1. Abundance classes and corresponding relative abundance level used for substrate, plantcover and riparian zone estimation.

Aquatic plant cover

Describe the general aquatic plant cover at a site by assigning abundance classes for different plant types.

Environmental variables

At each stream site estimate or measure the width of the section. For streams smaller than 3m width use 10cm accuracy in measurements, for bigger rivers use 0.5m accuracy. Measure or estimate overall current velocity using currentspeed classes provided in table 2.

Current speed class	Current [m s^{-1}]
Fast	>0.5
Intermediate	0.2–0.5
Slow	<0.2

Table 2: Current speed classes and their corresponding velocities.

For profundal lake sites measure or estimate maximum depth. Provide Secchi depth if available. Note wind direction and approximate wind speed. Estimate wave height and provide water level if known.

Riparian zone / shore

Describe the general characteristics of the riparian zone or lake shore using the abundance classes in table 1. State the width and inland length of your observational zone. The area should be at least 25m wide and at least 10m wide. Estimate the degree of shading the vegetation in the riparian zone exerts onto the sampled area.

Sample information

Fill in the location name. Measure the depth for each replicate sample individually. Fill in the container code the sample was put in. For littoral and stream samples, provide substrate composition and plant cover for each sample.

Field sheet flipside:

Measuring slope %

Measure slope from 5 transects perpendicular to the shore line and spaced a minimum of 5 m apart.
First note the initial or starting depth. Next wade to the depth of 50cm (depth 1) and measure distance to shore using e.g. a measuring tape. Next wade to the depth of 100 cm (depth 2) and measure distance to shore at that point. Repeat this process at each lakeshore site. In the laboratory calculate the slope for each transect separately, then calculate shore specific mean values.

Appendix 1: Field sheet

SITE NAME:			
Site type:		Waterbody name:	
GPS coordinates	accurate/approximate	N:	E:
Municipality:		Watershed :	
Habitat type:		Substratum:	
General description:			
SAMPLING		Date and time:	Agency:
Monitoring/ project name:		Number of samples to be taken at site:	
Sampling device:		Area covered by device/sample [cm^2]	
Dimensions of device (LxWxH):		Sampling time [s]:	Mesh size [mm]:
FIELD OBSERVATIONS			
Surveyor name:			
GPS coordinates	N:	E:	GPS was used:
Additional information:			

Substrate (0-3*)		Plant cover (0-3*)		Environmental variables		Riparian zone/ shore(0-3)	
Bedrock [> 4 m]						length[m]: width[m]:	
Large boulder [256 mm-4 m]		Emergent plants		Width[m]			
Boulder [64-256 mm]		Floating leaf plants		Current velocity*[m/s]		Shading [%]	
Cobble [16-64 mm]		Submergent plants				Evergreen trees	
Pebble [2-16 mm]		Isoetids				Deciduous	
Sand [0,06-2 mm]		Free floating plants		Maximum depth[m]		Mixed forest	
Silt				Secchi depth[m]		Clearcut	
Clay		Mosses		Wind direction		Field /pasture	
Mud		Macroalgae		Wind speed[m/s]		Swamp	
Peat		Algae		Wave height[m]		Shrubs/bushes	
Fine detritus				Water level[cm]		Road /settlement	
Coarse detritus		No vegetation				Forest drainage/other drainage	
Tree branches and stems							
Artificial						Else, what?	

SAMPLE INFORMATION :

Location	Depth [cm]	Container ID	Substrate composition and plant cover at sampling spot.

How to fill in the fieldsheet -short instruction

Measuring slope % and mean slope in lakes

Space slope transects about 5 m apart. First determine possible initial depth. Measure the distance from shore at two depths e.g. 0.5m and at 1m depth. Using the following equation establish mean slope using the same metric for depth and distance (i.e. either cm or m).

slope(%) = depth / distance from shore x 100

	Lake shore				
	Initial depth/ depth a & b (cm)			Distance to shore (cm) at depth a / b	
Transect 1					
Transect 2					
Transect 3					
Transect 4					
Transect 5					

Plant cover and substratum distribution can be evaluated (over the entire sampling site) as follows:

Class	Relative abundance
0	none
1	little (<10 %)
2	moderate (10–50 %)
3	abundant (> 50 %)

Current speed at stream sites can be measured with a flow meter [m s^{-1}] or evaluated as follows:

Current speed class	Current [m s^{-1}]
Fast	>0.5
Intermediate	0.2–0.5
Slow	<0.2