# Description of the macroinvertebrate sampling methods to be applied in STAR

## The PERLA sampling method

### 1. General description

The PERLA prediction system is a biological method of ecological status assessment of running waters in the Czech Republic. The method is based on the comparison of an observed site with a reference site. It takes the natural variability of the environment and within biological communities into consideration and corresponds with present trends in the EU.
The PERLA prediction system is based on the prediction of macroinvertebrate community composition at a specific site using several environmental variables and on the subsequent comparison of the predicted (target) community with the macroinvertebrate community actually found on the site assessed. The application of PERLA requires the compilation of a reference data set for the given geographical region.
The PERLA prediction system is based on the RIVPACS approach

### 2. Methodology for the selection of reference sites

*Criteria for the definition of reference sites*
A reference site is a site where only natural stresses are present and man-made stresses are considered to be insignificant. The community present at a reference site is a natural community when it is influenced only by natural stress (e.g. flood) and man-made stress is not significant (EN ISO 8689-1:2000).

*Criteria for the definition of reference sites in the Czech Republic*

Basic statements
- A reference site must hold natural conditions.
- The reference conditions must reflect minimal anthropogenic disturbance.
- The degree of urbanisation, agriculture and silviculture in a catchment should be as low as possible.

River channel and habitat
- The reference site floodplain should preferably not be cultivated. If possible, it should be covered with natural climax vegetation and unmanaged forest.
- Coarse woody debris must not be removed.
- Stream bottoms and stream banks must not be fixed (old river bank fixation by a belt of trees was accepted).
- Natural riparian vegetation and floodplain conditions must still exist, making lateral connectivity between the stream and its floodplain possible.

Hydrological conditions and regulation

- No alterations of the natural hydrograph and discharge regime.
- No hydrological alterations such as water diversion, abstraction or pulse releases.
- No or only minor upstream impoundments, reservoirs, weirs and reservoirs retaining sediments may be present (a dam or a weir 20 km upstream was accepted for some stretches of mid-sized or large streams).

Physical and chemical conditions

- close to natural background levels describing the baseload of a specific catchment area
- no point sources of pollution or nutrients
- no sign of acidification
- no liming activities
- no impairments due to physical conditions, especially the thermal conditions must be close to natural
- no sign of salinity
- physical and chemical conditions were checked by physico-chemical and chemical analyses of water and sediment

Biological conditions

- There must not be any significant impairment of the allochtonous biota by introduced Crustacea or Mollusca.
- The value of the Czech saprobic index must not be higher than 2.2 (beta-mesosaprobity).

It was not possible to find real reference sites for all stream types present in the CR, in full compliance with the criteria listed above.
In such cases, the optimum sites present within the corresponding stream type were taken for the reference sites.
Large lowland streams were the most problematic. For the largest rivers (lowland stretches of Morava, Elbe and Moldau River) we were not able to identify any suitable reference sites.

**3. Sampling Methodology**

**A. Sampling site selection**

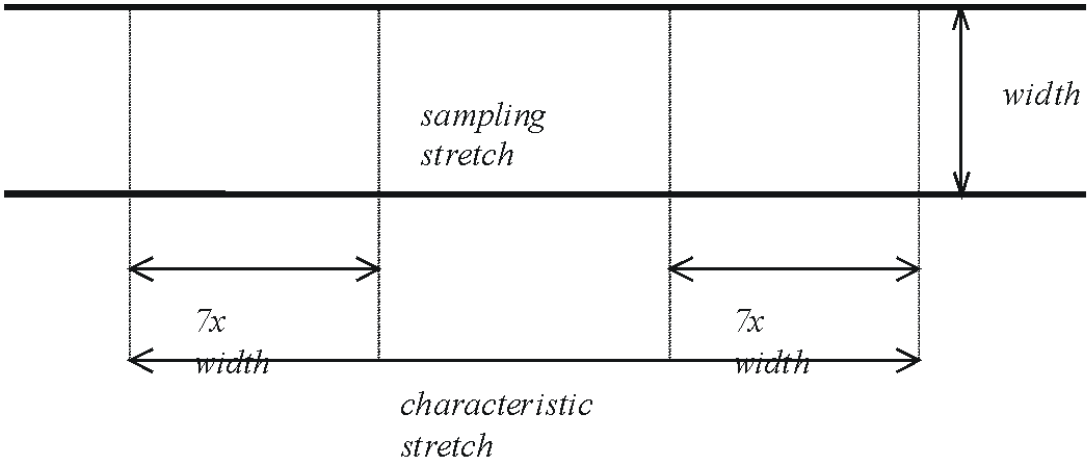
Sampling site – the site where a sample of macrozoobenthos is taken.
Sampling site identification by:

the name of the stream, its profile
the number of the hydrological order (stream order)
the river kilometre (distance from source)
altitude above sea level
latitude
longitude

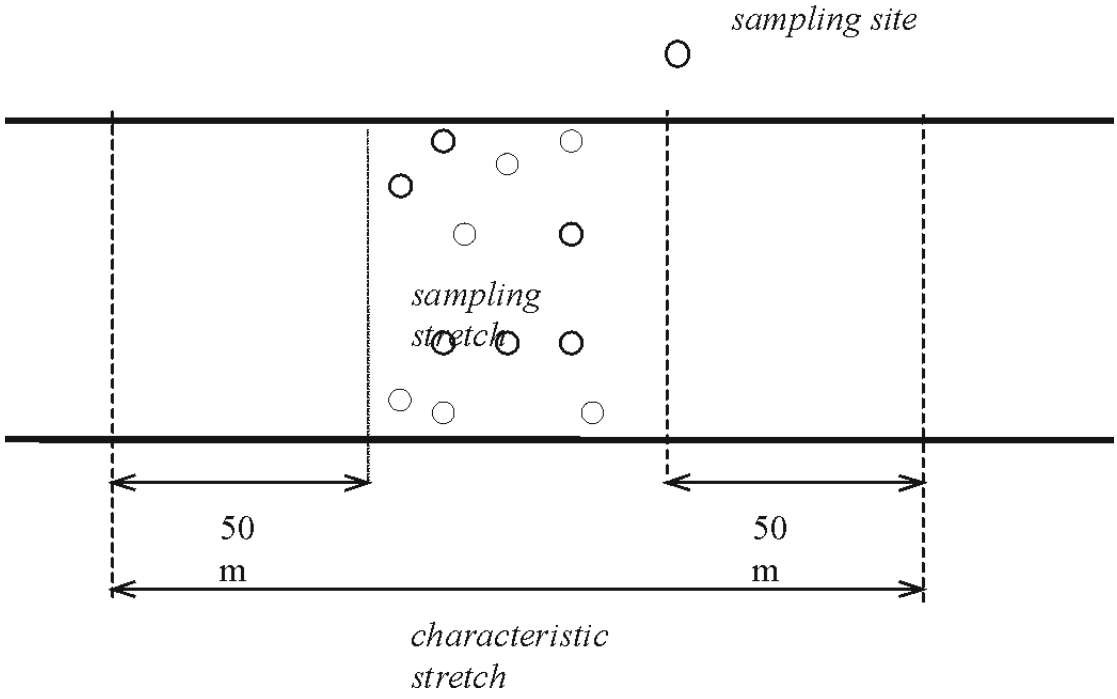
The characteristic stretch of the stream, the sampling strech of the stream , and the sampling sites are assigned on each site.

Fig. 1: Assignment of the characteristic stretch of the stream, the sampling stretch of the stream, and the sampling sites on a site with

a) a width of the river channel under 5 m




b) a width of the river channel of 5 m or above




*Characteristic stretch of the stream:* Its length is equal to the 7-fold stream width or to 50 m (depending on which distance is shorter) upstream and downstream of the *sampling stretch of the stream*. Based on the characteristic stretch the values of some environmental variables are assessed (slope, character of substrate, water plant vegetation, degree of shading, riparian vegetation).

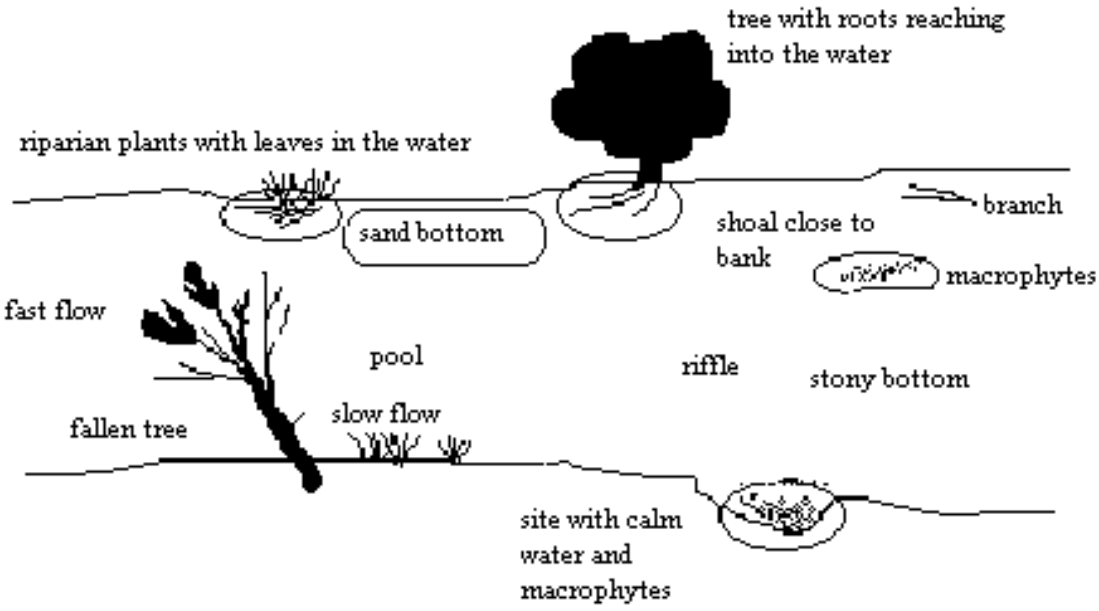
*Sampling stretch of the stream:* usually, it is not possible to sample the entire characteristic stretch, therefor, a shorter one – the sampling stretch – is defined in its centre. This has to include all habitats present within the characteristic stretch. In smaller streams (river channel width under 5 m), the entire sampling stretch and almost the entire characteristic stretch are sampled. In bigger streams (river channel width of 5 m or above), individual sampling sites are selected within the sampling stretch of the stream.

*Sampling sites:* sites where macrozoobenthos samples are taken. Selection of sampling sites: all habitats present within the sampling stretch are recorded. (see Fig. 1, 2):

- sites of various stream velocity
- various distances from the banks
- grass tufts on the banks, with parts floating in the water
- branches or trunks lying in the water
- roots
- sites with various substrates (stones, sand, fine sediments, etc.)
- tufts of water plants floating in the stream
- calmer water with water plants near to the banks etc.

For each habitat, its percentage area of the total bottom area of the *sampling stretch of the stream* is estimated. The corresponding sampling time is allocated to each individual habitat (multihabitat sampling). Total net sampling time is 3 minutes.

Fig. 2: Examples of habitats



**B. Recording of basic data on stream characteristics**

In connection to the sampling of biota, the following environmental variables are recorded and/or measured.

| | |
|---|---|
| Stream width | The flooded river channel is measured in such a way, that potential stream variability in this parameter is expressed (at least 3 times). |
| Depth | Water depth on site is measured on 5 sites of equal distance from eachother (1/8, 1/4, 1/2, 3/4, 7/8) along the cross section . Depth is measured at one or several cross sections and the computed hydraulic depth is taken for the mean depth. |
| Mean velocity | Measured with a flowmeter on the same sites as the depth. From the measured values the mean (hydraulic) velocity is computed. |
| Substrate | The percentage cover of the bottom surface area is estimated for each substrate size fraction within the characteristic stretch of the stream. The estimate is based on a view from above and includes visible particles. If larger particles are covered by a thin layer of sediment but their contours remain visible, this layer is neglected and only referred to in the minutes. However, if the particle contours are not clearly visible, only the fine substrate is recorded. The scale according to Furse et al. (1986) is used and the value of □ (phi) is computed using the following table and formula:<br><br>□ = (boulders + stones) * (-7,75) + (coarse gravel + gravel) * (-3,25) + sand * 2 + fine sediment * 8 |

Tab. 1: Particle size of the individual substrate fractions

| name of particle size fraction | particle size (mm) |
|---|---|
| fine sediment | <0,1 |
| sand | 0,1 – 2 |
| gravel | 2 – 16 |
| coarse gravel | 16 – 64 |
| stones | 64 – 256 |
| boulders | >256 |

| | |
|---|---|
| Gradient | the gradient of the characteristic river stretch is measured by a levelling instrument and measuring belt |
| Bank character | natural or type of modification |
| Bottom character | natural or type of modification |

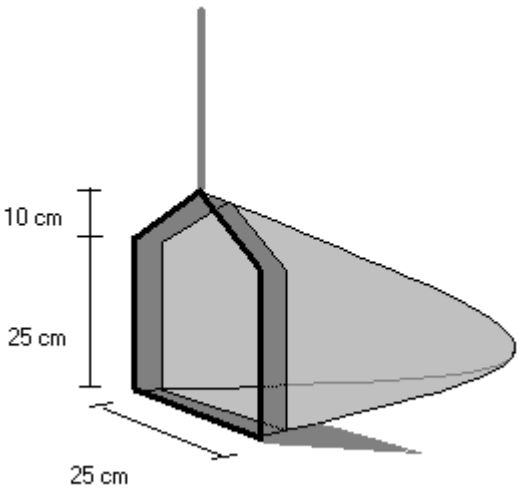
Complementary information

cross section and (longitudinal) river profile – schematic drawing of the stream and its close surroundings
pool-rapid ration – assessed as the ratio of calm stretches and stream stretches of higher flow velocity.
flow type ("laminary", turbulent)
water content of the stream
shading of the studied stretch – percentage of shaded water area at high noon (in zenith)
macrophytes
periphyton
riparian vegetation
landuse categories (CORINE – wider surroundings)

From water management maps (1: 50000): altitude (a.s.l.)
stream length
distance from source

**C. Biological sampling**

Fig. 3: Hand net




The standard method Three Minute Semiquantitative Kick Sampling using a hand net of 0,5 mm mesh size is employed (Fig. 3).
Sampling is conducted moving in up-stream direction, thus avoiding disturbance of the area not sampled yet. The substrate infront of the net is disturbed by the foot or hand of the person doing the sampling. Complementary to that, objects from the bottom are left up and investigated so that species firmly attached to them are not overlooked or underestimated. Submerged water plants, leaves of terrestrial plants, tree roots, branches and trunks fallen into the stream, etc. are also washed of infront of the net. A detailed specification of hand sampling in flowing shallow water in of foot sampling in deeper water is given in the standard ČSN EN 27828 (ISO 7828).

**4. Sample processing**

In the field, coarse anorganic sediments are removed from the sample by decanting, twigs, leaves are taken away and the sample is well washed to remove fine sediments.
On site, the sample should be presorted on white photo-pan into glas test-tubes according to taxonomic groups to reduce potential mechanical damage of brittle insect larvae. Surplus

water is removed from the rest of the sample, which is placed into a PVC container with a volume of 1 – 2 litres. The sorting of the remaining organisms is done in the laboratory. It is essential that all sampling equipment (particularly the net) are extremely thoroughly washed after each sampling.

The samples are preserved by adding a 40% formaldehyde solution to a resulting 4% concentration. Some organisms are better preserved in ethanol (molluscs, crustaceans). The use of any different preservative should be indicated directly on the sample (ČSN EN ISO 5667-3).

The samples are labled by putting lables inscribed by plain pencil (graphite) into the container or test-tubes. From the outside, the container is marked by a water-resistant felt pen. The label or description on the outside of the container should always include the code identifying the sample, and further information on the sampling date, the stream and the site. A sampling protocol is filled in for each sample.

Identification of macroinvertebrates

Biological samples are identified on the lowest taxonomic level possible, i.e. usually on the species level.

The results of identification are recorded in the identification protocol.

The identification protocol has to include:

- code identification of the sample,
- name of stream,
- name of cross section,
- sampling date,
- date of identification,
- name of the person who conducted the identification,
- list of recorded taxa with indication of development stages and their abundances,
- the percentage of sample processed.

Record-keeping

the following documentation is filed:

- sampling protocols
- original identification protocols
- processed makrozoobenthos samples including permanent microscopical preparations

Macrozoobenthos taxa lists for the individual samples and data on environmental variables are stored using the database software PERLA (in Czech).

The PERLA database is based on the programming language C++ (Paradox 7.0).

The software is available as free-ware on the address http://www.vuv.cz/Perla/main.php – in the section Software PERLA.