Sampling protocol and audit non-diatom benthic algae

1 Goal

The identification and enumeration of benthic micro algae, other than diatoms, attached to stones, macrophytes or macroalgae, or present in sediments of European river systems. Habitats are sampled according to the methods described in the "Sampling protocol and audit benthic diatoms".

Most benthic algae in freshwater habitat are blue-green algae (Cyanophyta), green algae (Chlorophyta), diatoms (Bacillariophyta) or red algae (Rhodophyta). Most other divisions of algae can also occur in freshwater benthic habitats. These divisions seldom constitute more algal biomass in a benthic habitat than blue-green algae, green algae, diatoms or red algae (Stevenson 1996).

The Bacillariophyta (diatoms) have been studied extensively and ample information is available on the taxonomy and autecology of the taxa belonging to this group. Less information is available on the benthic algae of the other divisions. This can be due to the fact that these groups are often not readily identifiable, as more live-stages need to be studied before a positive identification can be made. Some growth forms of some algal species are morphologically indistinguishable with the light microscope (e.g. zoospores of many green algae). Autecological information on 'non diatom' taxa is, consequently, less widespread as is the case for the benthic diatoms.

STAR partners can choose to include the non-diatom benthic algae in their sampling, identification and counting effort.

2 Principle

Benthic micro algae are sampled from different substrata in European streams and rivers in order to generate representative collections of naturally occurring communities. The relative abundance of the taxa in these samples are used to assess water quality.

3 Applicability

European streams and rivers.

4 Definitions

Benthic algae are those that live on or in association with substrata (Stevenson 1996). The substrata can be natural or artificial. The substrata considered in this protocol are stones (boulders, cobbles, pebbles, concrete man-made constructions), macroalgae and macrophytes (emergent and submersed) and sediments (mineral sand and silt).

5 Recommendations on safety and environmental issues

Appropriate health and safety guidelines must be followed when using preservatives (e.g. formalin), or colouring agents. The name of the preservative should be marked on the outside of the sample bottles.

Used formalin must to be treated as chemical waste and should at no time get into contact with bare skin. H_2O_2 and H_2SO_4 have a strong oxidising effect and should also not get in tough with bare skin. Make use of a fume cupboard whenever possible, especially when preparing microscopic

slides with mounting medium, as potentially carcinogenic fumes are released. It is recommend that prior to the application of a chosen method, a full risk assessment is made, as different preparation and preservation techniques present different hazards.

6 Equipment and materials

6.1 Field sampling equipment

See "Sampling protocol benthic diatoms".

To remove algae more efficiently from firm substrata a scalpel blade or knife can be used instead of a toothbrush.

6.2 Field form and marking of sample bottles

The field form for recording details of the sampling site is part of the STAR site protocol (v1.11, page 7). On this page mark the substratum type that has been sampled (Macrophyte/ -algae, Sediment (silt/sand) or Stone /man-made constructions). Also indicate the type of macrophyte/-algae that was sampled in the appropriate table. Space is allocated to add remarks.

At the end of this protocol an additional field form is included to quantify macroscopically visible algal units (scoring form CEN protocol TC 230).

Clearly mark sample bottles with the same site name, date and sample number that is mentioned on the field form.

6.3 Laboratory processing

- coverslips and microscopic slides (sampling chamber, e.g Palmer or Sedgwick-Rafter type, may be useful)
- pipettes
- vials for storing of samples

6.3.1 Preservatives

One of the following:

- Buffered 34% v/v (minimum) formaldehyde (HCHO) solution :
- Lugol's iodine:

There's a good case for doing these analyses rapidly on live samples (not preserved) – it means that motile taxa can be more readily distinguished and, in some cases, filamentous taxa can be "encouraged" to produce zoospores or oospores. However, they need to be performed relatively quickly. If only about 10% of the volume of bottles is filled, there should be plenty of oxygen available.

No preservative is necessary if the sample is to be processed within a few hours of collection, as long as steps are taken to minimize cell division (i.e. by storage in cool, dark place). Lugol's iodine can be used for short-term storage; however, it is not suitable for long-term storage, due to problems caused by sublimation.

7 Methods

The methods for sampling benthic algae can be divided in two categories:
- sampling of macroscopically visible benthic algae
- algae that can only be made visible with a microscope (microscopic algae)

7.1 Sampling of macroscopically visible benthic algae (following the draft CEN Protocol TC 230)

The "macroscopic benthic algae" should be studied by wading on a stretch along the riverbank of at least 10 m and as far out form the bank as safety regulations allows, depending on depth and current velocity. An aqua scope should be used if water current or water depth hampers the visibility/observation.

The macroscopic elements may have different appearances, e.g. jellyish brown (often diatoms), green filaments (usually green algae) or dark green tufts (green or red algae, or cyanobacteria). It is useful to collect some stones (5-10) with different appearance to get an impression of the algae growing on them. Knife, blade, forceps and stiff brush is used to collect the algae. Be sure that as many as possible of the macroscopically visible elements at the site are collected. The material is sorted in the plastic tray. Each of the macroscopically different elements is sampled in separate vials.

The quantification of macroscopically visible elements is estimated according to the percentage of the bottom covered area and is given in intervals of 5%. This is a difficult point and is done by integrated assessment based on the results of studies of the collected stones, of observations with aqua scope and the general impression at the sampling site. Observations along one or more "line transects" might help to quantify the macroscopic elements.

7.2 Sampling of microscopic visible benthic algae

The methods for collecting benthic algae from a variety of substrata is described in the Sampling protocol benthic diatoms (chapter 7). With those methods it is not possible to collect a representative sample of the non-diatom algae present in sediments. An alternative method is presented in this chapter. Another difference with the diatom protocol is that the samples should not be oxidised (paragraph 7.6: Laboratory treatment) as non-diatom (soft bodied) algae will be destroyed that way. Also, no permanent slides have to be produced as the algal suspensions are analysed in a Palmer or Sedgwick-Rafter counting cell or directly on a microscopic slide (see below). The collected samples need to be split-up on arrival in the laboratory, so that one part can be processed for diatom identification and counting (see "Sampling protocol benthic diatoms"). The remaining part can be used for identification and counting of non-diatom benthic algae. This part of the original sample should be preserved and retained in case problems are encountered during the preparation process and for later reference.

7.2.1 Laboratory treatment non-diatom benthic algae

Epipelon pre-processing

Shake the sample bottle containing the sediment sample vigorously for about 10 seconds. Slowly decant the suspension into a vial for permanent storage, thereby leaving most of the sediment in the original sample bottle. Add a preservative to the sample if this has not already been done at an earlier stage. Store samples in a cool dark place.

Identifying and counting non-diatom benthic algae

The algal samples need to be homogenised with a tissue homogeniser or blender. Mix the sample thoroughly and pipette into a counting cell (e.g. Palmer counting cell) or microscopy slide. Assess the density of algal cells at 400X magnification. Suspensions that have 10 to 20 cells per field provide good densities for counting. Dilute samples if cells overlap too much. Identify and count 300 cell units to the lowest possible taxonomic level at 400X magnification. Use the provided taxonlist for nomenclature (Chapter 9). It is a problem to distinguish cells of coenocytic algae (e.g. Vaucheriae) and small filaments of cyanobacteria in cell counts. 'Cell-units' can be defined as 10 µm sections of the thallus or filament. Do not identify diatoms to lower than genus level if subsequent counts of cleaned diatoms are undertaken. Make taxonomic notes and photographs of important specimens.

Vials with preserved original samples, should be labelled with at least the following information:

- Institute: name of the organisation that takes the sample
- River name
- Location name
- Location co-ordinates: (xx°xx.xx E / xx°xx.xx S)
- Substrate: Boulder/cobble/pebble, macrophyte, mineral sediment
- Date: day/month/year
- Collector: persons name
- Sample number

8 Counting and identifying

Complete the count sheet (an example is attached). 300 cells or 'cell-units' are identified and counted at 400X magnification. The slide is scanned in such a way that successive fields are examined and that duplicate counting of the same field is avoided. Make sure that the examined fields are not all located on one small area of the slide.

Identify the taxa to the lowest taxonomic level possible (for identification of freshwater benthic algae see : Pascher *et al.* 1982-1999; van den Hoek *et al.* 1998; Simons *et al.* 1999; Whitton 2000; John *et al.* 2002). For nomenclature and encoding, the proposed standard taxonlist (see Audit procedure) must be used. Taxa that are not included in this list can be added if they are encountered in the sampled (use nomenclature as proposed by Whitton *et al.* 1998a)

To allow for a comprehensive comparison of important taxa during the course of the project for your own reference and also for the audit procedure, photographic records of abundant taxa and those taxa that cannot be identified with a high degree of confidence should be made. These photographs have to be sent electronically (by email or CD-ROM) to the auditors with the following requirements:

- Minimum resolution 300 dpi.
- Add a scale bar.
- If more than one specimen is visible on the diagram, add an arrow to clearly indicate which specimen is referred to.
- State length and width in µm of the specimen. Optionally extra information can be added on the range of dimensions of the taxon.
- State the record number of the slide on which the specimen was recorded and the institute that collected the sample. Include the name of the river, sample site and type of habitat from which the sample was collected.
- Make sure that the aspect ratio of the electronic pictures is never altered.

Example:

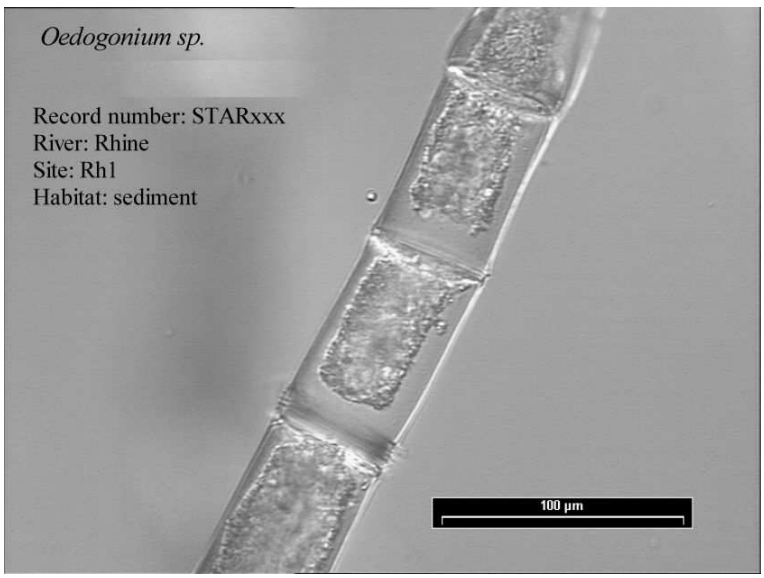

Figure 1 Example of micrograph

9 Audit procedure

Participating research groups will send preserved samples of 10 sites, or 5% of the amount of sampled sites, whichever is the larger, to the auditors at Alterra. As described in the sampling protocol, samples taken from a maximum of four microhabitats at each site are processed and analysed separately. From the sites that are selected for the audit, all four samples should be sent to the auditors.

The nomenclature of the taxa follows the 'Coded list of freshwater algae of the British Isles' (Whitton *et al.* 1998a; Whitton *et al.* 1998b). A selection of non-diatom benthic algae from this list will be attached to this protocol. This taxonlist comprises 256 freshwater benthic taxa that are recorded in the Netherlands (Simons *et al.* 1999). Additional taxa can be added, provided that the nomenclature follows that of the 'Coded list of freshwater algae of the British Isles' as closely as possible. Reference information on all taxa is part of the database.

A method to establish the similarity between samples, is the use of the Sørensen algorithm (Sørensen 1948). This methods not only compares the amount of similar taxa between two samples, it also takes the abundances of these taxa into account. The criteria to reject the hypotheses that two counts of the samples are different will be developed during two intercalibration exercises on samples collected during the workshop in La Bresse (April 2002). All participants will count and identify the samples taken from the same sites during this workshop and the results will be send to Alterra. This intercalibration exercise will enable us to establish the range of similarity that can be expected between the labs for two river systems in two different ecoregions.

A second audit method is based on the use of several metrics of biotic integrity such as species richness, total number of genera and total number of divisions. The indices calculated on the basis of the original results and those based on the audit results, will be compared using the Bray-Curtis similarity index.

10 References

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Quantification of macroscopically visible algal units

(scoring form CEN protocol TC 230)

Specified algal unit - description and degree of cover

A .....

B .....

C .....

D .....

E .....

F .....

G .....

Algae sampled from:

Additional information:

Sketch of sampling locality:

Benthic micro algae laboratory bench sheet

Benthic microalgae (non-diatom) project STAR	
Stream name:	*LAT :* *LONG:*
Station #:	*sample #:*
Date of sampling:	*slide #:*
Taxonomist:	*archive #:*
Habitat origin:	*remarks:*
Microphyte/Algae □ *Sediment* □	
Stone □	

taxon	*# of valves*	*total*
Total number of taxa:	*total*	

Date of analysis: ……. *Signature:* ……