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Water sampling for capture of macrobial environmental DNA in aquatic environments

Wasserprobenahme zum Nachweis aquatischer Umwelt-DNA

Techniques de prélèvement deau en vue de lanalyse de lADN environnemental dans les milieux aquatiques

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Water sampling for capture of macrobial environmental DNA in aquatic environments

Techniques de prélèvement d¿eau en vue de l¿analyse de l¿ADN environnemental dans les milieux aquatiques

Wasserprobenahme zum Nachweis aquatischer Umwelt-DNA

This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 230.

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (prEN 17805:2021) has been prepared by Technical Committee CEN/TC 230 “Water analysis”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

Introduction

WARNING — Persons using this document should be familiar with water sampling protocols to assess biological diversity. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate health and safety practices.

The monitoring of organisms is key to the assessment of the status of aquatic ecosystems and is required by national and international legislation such as the European Union Water Framework Directive (2000/60/EC). A range of methods have been described how to monitor organisms in aquatic environments, leading to a wide range of European standards (e.g. EN 14011:2003, EN 14757:2005, EN 15460:2007). These approaches, however, necessitate the capture and/or collection of the organisms of interest, which can be a laborious and time-consuming process.

The possibility to detect the presence of organisms and/or quantify relative abundance (e.g. [1]) in aquatic environments via the analysis of environmental DNA (eDNA) provides a novel means to monitor biodiversity across a wide range of taxonomic groups, including microorganisms, plants and animals ([2][3]). This approach allows to examine organismic diversity without the need to directly isolate and capture organisms and it is expected to play a key role for future biomonitoring aiming at temporally and spatially highly resolved species inventories [4]. Albeit the power of the eDNA approach has been repeatedly reported [5], there is a great need for standardizing the application of eDNA-based assessment of aquatic biodiversity ([6][7]). Note, however, that eDNA-based biomonitoring currently does not allow to obtain certain population parameters (e.g. individual size, sex) which can be obtained by traditional sampling techniques.

This document provides guidance how to collect and preserve eDNA from water samples, addressing the first and crucial step for any further downstream eDNA-based analyses of biodiversity. A specific technical report for the routine sampling of benthic diatoms from rivers and lakes adapted for metabarcoding analyses is CEN/TR 17245:2018.

1 Scope

This document specifies procedures for sampling, capture and preservation of environmental DNA (eDNA) in aquatic environments, stemming from organisms that are or have recently been present in a waterbody, have visited it or whose DNA has been introduced to the waterbody through some mechanism. This document also covers procedures for avoiding sample contamination and ensuring DNA quality, key properties of the filtering procedure and equipment and reporting standards.

This document does not include the collection of eDNA from biofilms, sediments or similar sample types and does not cover sampling designs.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

Note 1 to entry: Not all definitions listed below are necessarily applicable to all studies. Only those which are relevant to the aims and objectives of the study in question are required.

3.1
cross-contamination
unintended transfer of any source of and/or DNA from one sample to another sample

3.2
decontamination
procedure to remove any source and/or trace of DNA from material that might come into contact with the sample

3.3
enclosed filter unit
filtering device where the filter membrane is encapsulated and where the inflow and outflow can be closed for transport and storage

Note 1 to entry: The eDNA contained on the filter is typically extracted without removing the filter from the filter capsule greatly reducing the risk of contamination of samples

3.4
environmental DNA
eDNA
material stemming e.g. from dead or from living organisms and include single-stranded (ss) and double-stranded (ds) DNA fragments from nuclear and mitochondrial/plastid DNA of eukaryotes as well as plasmid DNA of prokaryotes

Note 1 to entry: Subsuming DNA from various sources such as unicellular or small multicellular organisms or tissue particles (e.g. shed cells, faeces) and gamets of multicellular

3.5
field equipment blank
sample obtained from processing target-DNA-free water through all the equipment used and covering all procedures involved in the eDNA sampling process to ensure that the equipment and procedures do not introduce contamination

3.6
housed filter unit
systems in which a filter membrane is protected within a solid housing during the filtration process

Note 1 to entry: The filters are removed from the housing for eDNA extraction. The housing can be opened and the filter removed for preservation and later processing

3.7
lysis buffer
buffer solution to preserve DNA present in the sample and to lyse/open cells as a first step of the DNA extraction

3.8
internal positive control
IPC
known fragment of synthetic DNA containing an amplifiable and quantifiable sequence that will not naturally occur in the sample

Note 1 to entry: The IPC can be added to the sample or the preservation/lysis buffer at a known concentration to verify the efficiency of DNA preservation, DNA extraction, DNA amplification and DNA identification.

3.9
open filter unit

filtering device from which the filter membrane has to be removed by hand for further processing including filtration towers (laboratory) and filtration backpacks

3.10
pre-filter
filter membrane, mesh or hose strainer with a larger pore-size than the main filter membrane (for capturing the eDNA) through which water is passed first to remove larger particles of sediment, plant material or algae to increase the volume of water that can be filtered before saturation of the main filter

3.11
sample contamination
process by which exogenous DNA is unintentionally introduced to the sample during the sampling process

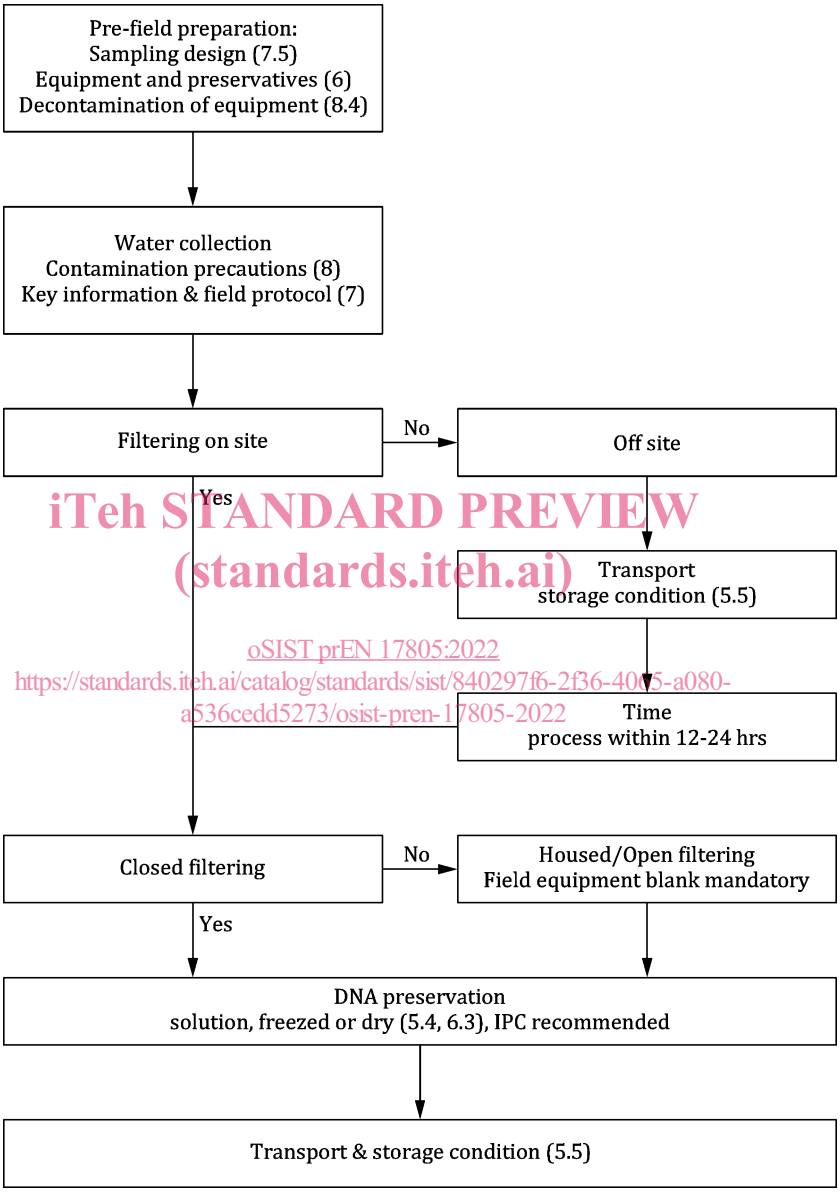
Note 1 to entry: DNA that is already present in the water before the eDNA sampling was undertaken is not considered as contamination

3.12
target DNA
any source and/or trace of DNA from the surveyed species/taxa

4 Principle

A representative water sample from the surveyed water body is collected according to an appropriate sampling design to capture and separate eDNA from the water sample. During the whole procedure (cross-)sample contamination with target-DNA is avoided and eDNA integrity is guaranteed.

An overview on the key steps and considerations for the eDNA water sampling process is provided in Figure 1.



NOTE Numbers in parentheses refer to respective clause/subclause.

Figure 1 —Key steps and considerations for the eDNA water sampling process

5 General Procedure

5.1 General

Water shall be collected to capture and separate eDNA via filtration or other processes. The probability of collecting eDNA from the targeted organism(s) is positively correlated with:

— the number of samples per waterbody;

— the spatial representativeness of the samples;

— the volume of water filtered;

— the optimum sampling time point/season with regard to the organism(s) eDNA shedding rates, abundances, metabolic activity and locomotion.

5.2 Sampling

Depending on the different applications/goals of each eDNA survey, the most appropriate sampling conditions and design shall be assessed based on case-by-case evidence to obtain water samples representative of the water body and the organisms which shall be monitored. These might include hydrological, meteorological, seasonal/temporal and biological/ecological variation.

This is particularly important in lentic (non-flowing) water bodies since eDNA is often unevenly distributed when the water is not well mixed. Representative sampling can be achieved by merging subsamples collected at different points in the water body, or alternatively by continuous sampling systems that move across the water body while drawing up water. When surveying deep water bodies and targeting deep water dwelling organisms, it may be necessary to collect water from depth.

To maximize the probability of capturing target DNA, the following shall be considered when planning where and when to collect samples and subsamples:

1) Features of the water body, including its size, depth, flow and the distribution of microhabitats as well as inlets/outlets of the waterbody.

2) Biology of all target taxa, including habitat preferences and lifecycle. Detection probability for individual species can be increased by timing sampling to coincide with times of intense activity (e.g. spawning). It is also important to consider whether target taxa are likely to be present in the water body at the time of sampling, especially in the case of amphibious or migratory species.

5.3 Equipment preparation prior to fieldwork

Prior to fieldwork the collecting vessels and equipment need to be cleaned to avoid contamination (for detailed instruction see 8.4).

5.4 Collecting the water and capturing the eDNA

Various systems are used for collecting and filtering water. Some involve initially gathering water into a collecting vessel where it is mixed and then filtered at the shore; other systems filter the water directly as it is drawn up from the water body. When the water is not filtered directly in the water body, the filtration can be carried out on the shore or in the laboratory

Water shall be collected and/or filtered to capture tissue fragments, cells and DNA. This may be achieved manually with syringes or using a hand or powered pump. If a pump is used and water passes through a pump tubing before reaching a filter then a new or decontaminated pump tubing shall be used for each sample.