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Water quality — Guidance on methods for sampling invertebrates in the hyporheic zone of rivers

bsi.

National foreword

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Water quality - Guidance on methods for sampling invertebrates in the hyporheic zone of rivers

Qualité de l'eau - Lignes directrices relatives aux méthodes d'échantillonnage des invertébrés dans la zone hyporhéique des rivières

Wasserbeschaffenheit - Anleitung zu Methoden für die Probenahme von Invertebraten (Wirbellosen) in der hyporheischen Zone von Flüssen

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European foreword

This document (EN 16772:2016) has been prepared by Technical Committee CEN/TC 230 “Water analysis”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2016, and conflicting national standards shall be withdrawn at the latest by October 2016.

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Introduction

WARNING — Safety issues are paramount when surveying rivers. Surveyors should conform to EU and national Health and Safety legislation, and any additional guidelines appropriate for working in or near rivers.

The term "hyporheic" is derived from two Greek words: *hypo* (under) and *rheos* (flow), and was first used by Orghidan in 1959 [1] to delineate the area of saturated subsurface sediments beneath and lateral to the wetted channel that contains a mix of surface water and groundwater. In the past 50 years, scientific understanding of the hyporheic zone has improved [2] and the term has been modified and expanded by hydrologists, hydrogeologists, chemists and biologists to reflect the importance of:

— the upwelling and downwelling of water into and out of the stream bed and the mixing ratio of surface water and groundwater;

— the nature and rate of biogeochemical processes resulting from upwelling of interstitial water or downwelling of surface water;

— the ecotonal nature of the hyporheic zone which provides important habitat for benthic taxa, specialist hyporheic organisms and groundwater fauna, including macroinvertebrates, meiofauna and microorganisms. Meiofauna includes microcrustaceans, rotifers and nematodes as well early instars of many aquatic insects.

In this standard the hyporheic zone is defined as the spatio-temporally dynamic ecotone between the surficial benthic substrate and the underlying aquifer. Within the hyporheic zone, water, solutes and biota are exchanged with the stream above, the groundwater below and the saturated sediments lateral to the channel. The term "hyporheic zone" is applied to the physical habitat while the term "hyporheos" coined by Williams and Hynes in 1974 [3] is used to describe the faunal community inhabiting it.

Over the past few decades, the importance of the hyporheic zone has been increasingly recognized, with the vertical dimension added to spatial concepts of lateral and longitudinal connectivity. Together with the temporal dimension this has created a four-dimensional understanding of river ecosystems [4, 5, 6]. As the hyporheic zone is an ecotone between surface water and groundwater, abiotic conditions may reflect a transition between the two. Table 1 provides a general comparison of the physical characteristics of each environment.

Table 1 — Physical characteristics of typical groundwater and hyporheic environments compared with surface waters

Physical characteristic	Groundwater	Hyporheic
Light	Constant darkness	Constant darkness
Current velocity	Much lower	Lower
Annual and daily temperature range	Much smaller	Smaller
Substrate stability	Much higher	Higher

Approaches to river conservation and management recognize the need for a better understanding of the interactions between surface water and groundwater when undertaking investigations in the field. As the ecotone between the two, the hyporheic zone plays a vital part in ecosystem functioning in many rivers, including a critical role in the flow of energy, cycling of nutrients and organic compounds, as well as pollution attenuation. The hyporheic zone contributes to overall river biodiversity. It also provides a nursery for young life-stages of some fish and invertebrates and a potential refuge for benthos during adverse environmental conditions, such as flooding, low flows, chemical pollution, stream-bed drying or freezing. The hyporheic zone may therefore enhance the recovery of the benthic community following disturbance.

An increased interest in the hyporheic zone has resulted, in part, from international legislation, such as EC directives: the Habitats Directive [7], the Water Framework Directive [8], the Groundwater Directive [9] and the Nitrates Directive [10]. Although investigations into the hyporheic zone are not explicit within these directives, they do require national regulatory authorities to adopt a more integrated approach to the management of river catchments as a whole. Consequently, an understanding of the hyporheic zone, including its functions and the potential threats to these, is vital in order to comply fully with the requirements of these directives.

Investigations of the hyporheic zone may also be needed more generally for catchment management, river restoration, site-based investigations or for research. Consequently, the purpose of any study should be carefully considered when selecting the most appropriate method for sampling the hyporheos, especially if the collection of water quality and associated sediment data is also required. In addition, the methods described in this standard may require modification to reflect local conditions.

1 Scope

This European Standard provides guidance on methods for sampling invertebrates in the hyporheic zone of wadable rivers. It describes each method, including details of the equipment involved and its use in the field. Guidance is given on developing a sampling strategy and selecting an appropriate survey technique for the purpose of investigation.

NOTE Benthic macroinvertebrate sampling is covered by other published standards (see Bibliography). Selected literature with references in support of this document is given in the Bibliography.

2 Terms and definitions

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

2.1
aquifer
underground zone of water-bearing permeable rock or unconsolidated material from which groundwater can be extracted

2.2
benthic
relating to the surface substrate

2.3
benthos
community inhabiting the surface substrate of rivers

2.4
biofilm
coating on a substrate composed of microorganisms, extra-cellular polysaccharides, other materials that organisms produce, and particles trapped or precipitated within the matrix

2.5
biomass
total mass of living organisms per unit surface area or volume

2.6
catchment
basin
area from which precipitation or groundwater will collect and contribute to the flow of a specific river

2.7
diversity
taxonomic richness of a community and the distribution of individuals across taxa

2.8
downwelling
movement of water in a downward direction, typically from the surface stream to the hyporheic zone or groundwater

2.9
ecotone
transition area between two adjacent ecosystems

2.10
exposed river sediments
particles, typically comprising cobbles, gravel, sand and silt, deposited by flowing water but exposed as water levels fall

2.11
groundwater
water that is within the saturated zone below the water table

2.12
hyporheic flow
flow of water through the hyporheic zone

2.13
hyporheic zone
spatio-temporally dynamic ecotone between the surficial benthic substrate and the underlying aquifer

2.14
hyporheos
faunal community inhabiting the hyporheic zone

2.15
interstitial
referring to the spaces between substrate particles

2.16
macroinvertebrate
invertebrate that is easily visible without magnification (0,5 mm)

[SOURCE: EN ISO 10870:2012, 2.8]

2.17
meiofauna
invertebrates that pass through a 500-µm or 1-mm sieve but are retained on a 45-µm- or 63-µm sieve

2.18
permeability
capacity of a porous medium, either rock or unconsolidated material, to transmit water

2.19
pool
habitat feature characterized by distinctly deeper parts of the channel that are usually no longer than one to three times the channel's bankfull width, and where the hollowed river bed profiles are sustained by scouring

[SOURCE: EN 14614:2004, 2.24]

2.20
porosity
proportion of a given volume of rock or unconsolidated material that is occupied by pores

2.21
reach
major sub-division of a river, defined by physical, hydrological, and chemical character that distinguishes it from other parts of the river system upstream and downstream

[SOURCE: EN 14614:2004, 2.25]

2.22
riffle
fast-flowing shallow water with distinctly broken or disturbed surface over gravel/pebble or cobble substrate

[SOURCE: EN 14614:2004, 2.28]

2.23
riparian zone
area of land adjoining a river channel (including the river bank) capable of directly influencing the condition of the aquatic ecosystem (e.g. by shading and leaf litter input)

[SOURCE: EN 14614:2004, 2.29, modified — the NOTE was not adopted]

2.24
stream ordering
methods for classifying rivers and streams related to the complexity of the drainage basin, generally with progressively higher order numbers usually assigned to streams with greater discharge lower down the catchment

[SOURCE: EN 14614:2004, 2.37]

2.25
substrate
material making up the bed of a river

[SOURCE: EN 14614:2004, 2.40]

2.26
upwelling
movement of water in an upward direction, typically from the groundwater or hyporheic zone to the surface stream

3 Survey objectives

The objectives of the survey should be clearly defined before selecting which method to use for sampling the hyporheic zone, because the suitability of each method varies according to the purpose of study. Table 2 summarizes each sampling method according to its suitability for particular objectives. This includes consideration of:

— attributes and variations in hyporheic fauna, substrate and the interstitial environment;

— whether the method can be applied instream and/or in the riparian zone;

— whether data collected are fully quantitative or semi-quantitative.

All methods can be used to describe diversity, taxon richness, abundance and biomass, recognizing their known limitations.

Table 2 — Overview of sampling methods described in this standard and their suitability for particular surveys

	Karaman-Chappuis pit	Bou-Rouch pump	Vacuum pump	Standpipe trap	Williams corer	Colonization devices	Freeze coring
Migration/dispersal	No	No	No	Yes	No	Yes	No
Spatial heterogeneity	No	Yes	Yes	Yes	Yes	Yes	Yes
Temporal variability	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Interstitial sediment transport	No	No	No	Yes	No	Yes	No
Substrate characteristics	No	No	No	No	No	No	Yes
Used on submerged substrate	No	Yes	Yes	Yes	Yes	Yes	Yes
Used in riparian zone	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Quantitative	No	No	No	No	Yes	Yes	Yes
Semi-quantitative	No	Yes	Yes	Yes	Yes	No	No

4 Sampling strategy

The design of a sampling strategy will vary according to the purpose of the investigation. Sampling site location may be influenced by pre-existing monitoring networks or previous investigations. The following should be considered:

— sampling method;

— number and location of sampling sites;

— number of replicates per site required to characterize site heterogeneity (e.g. upwelling and downwelling, substrate characteristics);

— sampling frequency;

— sampling depth;

— seasonal variability;

— abiotic data requirements;

— spatial and temporal scale of investigation.

Scale is important when examining the hyporheic zone, as various processes occur at different spatial scales. For example, at a stream-bed (patch) scale the size, shape, sorting and stability of unconsolidated material are the primary determinants of porosity and permeability. These factors have a major influence on community composition over relatively short distances and methods have been developed to address this [11]. At a broader scale, lateral connectivity (e.g. between the riparian zone and out to the wider floodplain) is a key consideration. Hyporheic flow paths occur at multiple scales, from the stream bed to the catchment.

Case studies are presented in Annex A, giving examples of suitable sampling strategies for three different types of investigation.

5 Sampling methods

5.1 General

This standard describes only those techniques that are suitable for use in wadable rivers; other approaches are available for sampling in deeper waters. Some techniques (e.g. freeze coring) require a recovery time after the equipment has been installed, allowing organisms to recolonize the sample area. Some techniques work better in fine-grained substrates while others can be used in coarser substrates. Surveyors should carry out a preliminary investigation of the substrates before selecting an appropriate technique.

Each method is described in the following sections while Table 3 compares their ease of use, costs, recovery time and the possibility of obtaining repeat samples. Further details on all of these methods can be found in the PASCALIS sampling manual [12] and the Hyporheic Handbook [13].

Table 3 — Practical considerations when selecting sampling methods

	Karaman-Chappuis pit	**Bou-Rouch pump**	**Vacuum pump**	**Stand-pipe trap**	**Williams corer**	**Coloniz-ation devices**	**Freeze coring**
Minimum number of operators [a]	1	1	1	1	1	1	2
Equipment cost (in Euro, 2016)	< 200	< 1 000	< 350	< 200	< 1 000	< 500	< 10 000
Installation time	~15 min	~15 min	~15 min	~2 h	~15 min	~1 h	~30 min
Time to collect one sample	~5 min	~10 min	~10 min	~10 min	~5 min	~10 min	~45 min
Portability	High	Medium	High	Medium	High	Medium	Low
Typical recovery time after installation	None	None	None	2 weeks	None	Variable	Variable
Repeat sampling possible	No	No	Yes	Yes	No	Yes	No

[a] Installation of equipment for some methods will require additional help, i.e. for the standpipe traps, colonization devices and freeze coring.

5.2 Karaman-Chappuis pit

5.2.1 Description and operation

This is a rapid, qualitative method for obtaining a hyporheic sample from exposed river sediments, particularly in gravel and sand [14]. A pit (~50 cm diameter) is excavated to such a depth that its base is below the water level. Interstitial flow into the pit is maintained by extracting water, using a hand pump

or bailer, which is then filtered (using a mesh size appropriate to the study) to collect the invertebrates dislodged by the flow. In addition, small amounts of substrate from the bottom of the pit should be collected and carefully examined to obtain gastropods and bivalves. Pits should be dug on gravel bars or as close as possible to the river, while avoiding the risk of contamination by river water and benthic invertebrates. The method cannot be used during high flows as suitable sample sites will be inundated. Vegetated areas with compacted substrate should also be avoided.

This method is suitable for preliminary investigations or as a roaming technique, as it is cheap, quick and easy to use. A disadvantage of this method is that sampling is limited to the shallow portion of the hyporheic zone (~30 cm depth) and to areas of exposed river sediment.

5.2.2 Species sampled

A range of macroinvertebrates and meiofauna can be collected using this technique. Pits should be excavated and the sample taken quickly in order to capture all animals present.

5.2.3 Environmental variables

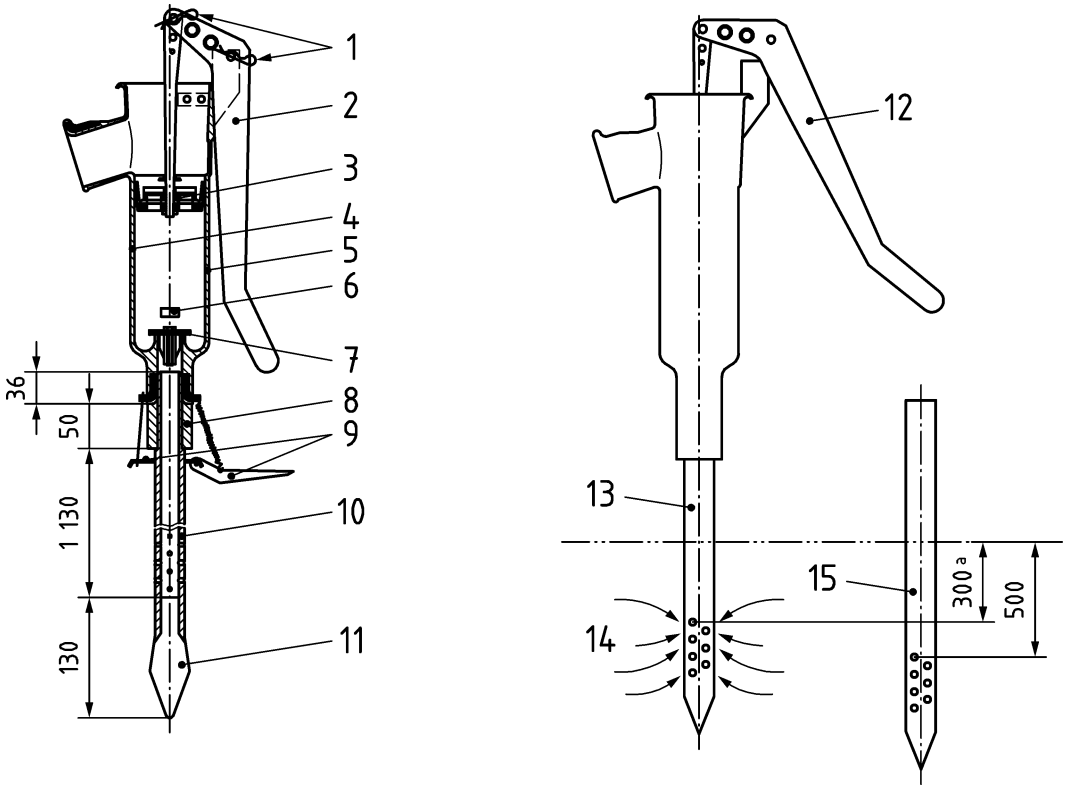
A full suite of water chemistry analysis can be undertaken provided that there is no ingress of surface water into the excavated pit. Physical and chemical parameters (e.g. dissolved oxygen and water temperature) should be measured immediately after pit excavation. It may be possible to make estimates of permeability by timing infiltration into the excavated pit.

5.3 Bou-Rouch pump

5.3.1 Description and operation

The principle of the method is to create a disturbance and maintain a flow around a pipe, sufficient to dislodge hyporheic organisms for extraction by pumping [15]. A hand-piston pump is fixed on top of a portable stainless steel standpipe that is hammered into the stream bed at various depths (typically 20 cm to 60 cm but occasionally up to 2 m) (see Figure 1). A standard volume should be collected for all samples; usually a minimum of 5 l although samples up to 10 l may be extracted. Sampling efficiency can vary depending upon the volume of water extracted and the nature of the substrate. The method can be used in submerged conditions as well as in exposed river sediments such as gravel bars. Approximately 3 to 5 replicates should be collected at each site, depending on habitat heterogeneity, with a distance of at least 1 m between them.

Dimensions in millimetres



Key

1	safety pin	9	bolt
2	lever	10	screen
3	upper piston valve	11	tip
4	liner	12	hand piston pump
5	body of the pump	13	mobile standpipe
6	lower piston	14	hyporheic zone
7	lower valve	15	standpipe with screen with 5-mm diameter holes
8	cap for hammering	a	sampling depth

Figure 1 — The Bou-Rouch pump for sampling invertebrates in the hyporheic zone of rivers [15]; hole diameter and standpipe length can be varied

An advantage of the Bou-Rouch pumping technique is that it causes relatively little disturbance to the river. Samples can be taken immediately after the installation of equipment so it can be used rapidly and is suitable for roaming surveys as the equipment is relatively portable. Pipes can also be left *in situ* for long periods if desired. The main disadvantage is that the method is not strictly quantitative as the sampled volume of hyporheic substrate cannot be measured and may vary depending upon the strength of force applied to the hand-piston pump during operation. Also, the location from which organisms are drawn in is unknown; pore-spaces and flow pathways are complex in the hyporheic zone so organisms may be sucked in from the proximity of the standpipe or from a greater distance.

5.3.2 Species sampled

Owing to its high extraction rate the pump samples swimming organisms and those linked to the substrate. Note that the sample passes through the pump itself which can damage the collected

organisms. Both macroinvertebrates and meiofauna are captured. However, smaller and more passive species may be preferentially sampled.

5.3.3 Environmental variables

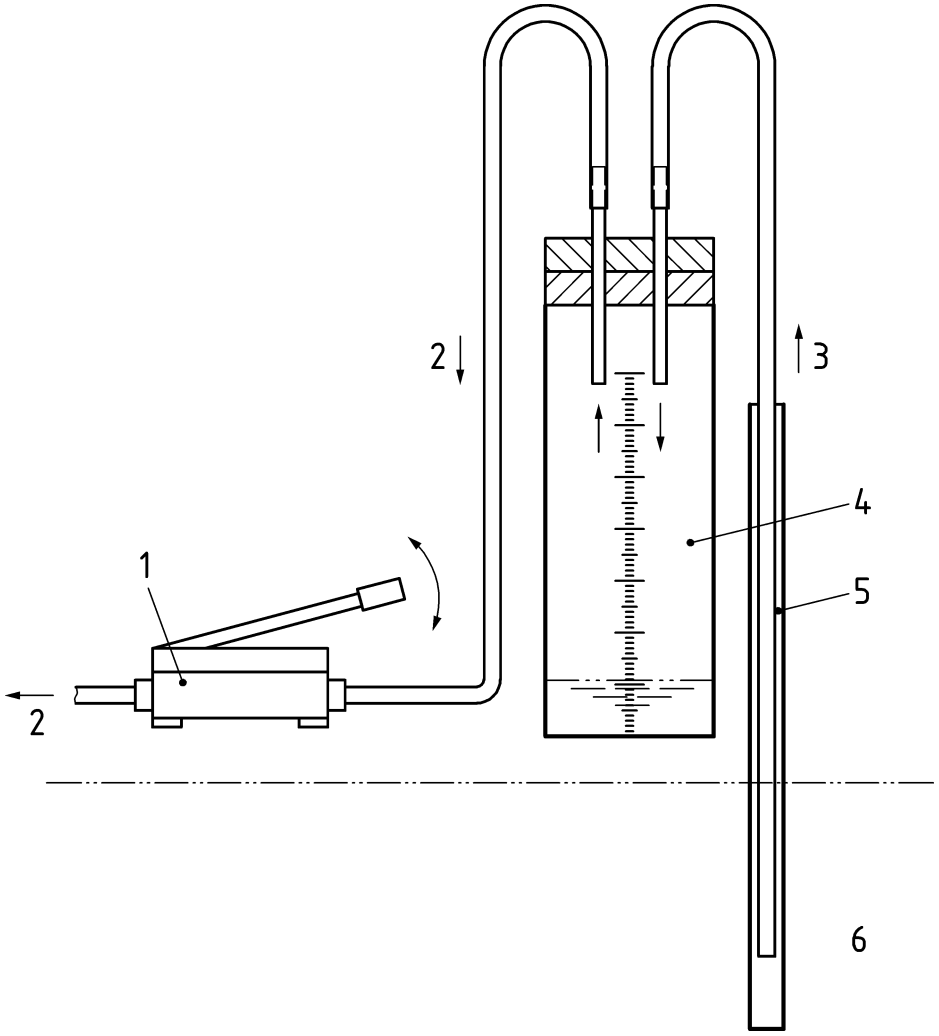
Temperature, dissolved oxygen, conductivity and pH can all be measured within the pipe, before water is extracted. Water can also be extracted for further chemical analyses, but water used to prime the pump should be purged first. A semi-quantitative measurement of dissolved/particulate organic matter and fine sediment can also be made. Where pore spaces are completely saturated, a measure of permeability and porosity can be obtained by quantifying the pumping rate, i.e. noting the time taken to pump a certain number of litres.

5.4 Vacuum pump

5.4.1 Description and operation

The vacuum pump method uses a similar principle to the Bou-Rouch, except that it creates a vacuum inside a closed bottle to extract and collect the sample (see Figure 2) [16]. An open-ended PVC pipe, with or without perforations (typically 5-mm diameter), is placed onto the end of a stainless steel T-bar, which is then hammered into the substrate to the required depth (typically 20 cm to 60 cm but occasionally up to 1 m). Pipes act as sampling wells, which can remain *in situ* for the duration of a study. If used in coarse substrates the addition of an outer metal tube can protect the PVC pipe from damage during insertion. Pumping (either manual or automatic) creates a vacuum which maintains an interstitial flow around the pipe that is sufficient to dislodge hyporheic organisms for extraction. It is a self-priming system that does not damage the organisms because they do not pass through the pump itself. A standard volume should be collected for all samples, usually a minimum of 5 l although samples up to 10 l may be extracted. If the pipe is submerged a seal should be made around the top during pumping to prevent benthos from entering.

The method is most suitable for use instream, but it can also be used in exposed river sediments such as gravel bars, as long as the interstices are saturated. Approximately 3 to 5 replicates should be collected at each site, depending on habitat heterogeneity, with a distance of at least 1 m between them. Pipes can be left *in situ* for repeated surveys as long as bungs are used to seal the top of the pipes between surveys to avoid contamination by benthos.



Key

1 manual pump
2 direction of air movement
3 direction of sample movement
4 vacuum jar for sample collection
5 open-ended PVC pipe
6 hyporheic zone (from which the sample originates)

Figure 2 — A vacuum pump for sampling invertebrates in the hyporheic zone of rivers; diagram not to scale [12]

As with the Bou-Rouch pump, an advantage of vacuum pumping is that it causes minimal disturbance to the river bed. The method is quick and easy to use making it particularly suitable for roaming surveys and investigations into spatial dynamics (e.g. riffle-scale studies). As sampling wells can be left *in situ* indefinitely, repeated sampling from the same location can be carried out providing temporal sequence data for long-term studies. The main disadvantage is that the method is not strictly quantitative as the sampled volume of hyporheic sediments cannot be measured. Also, the location from which organisms are drawn in is unknown; pore spaces and flow pathways are complex and often patchy in the hyporheic zone so some organisms may be sucked in from the proximity of the pipe while others from a greater distance.

5.4.2 Species sampled

The pump samples swimming organisms as well as those linked to substrate particles, including macroinvertebrates and meiofauna. However, smaller and more passive species may be preferentially sampled.

5.4.3 Environmental variables

These can either be measured directly in water extracted from the pipe, or samples can be taken to the laboratory for further chemical analysis. These measurements should not be taken from the first aliquot extracted, to avoid contamination from water that may have been in the pipe for a long time. A quantitative measurement of dissolved organic matter and a semi-quantitative measurement of particulate organic matter and fine sediment can also be made. Where interstices are completely saturated, a measure of permeability and porosity can be obtained by quantifying the pumping rate, i.e. noting the time taken to pump a certain number of litres.

5.5 Standpipe trap

5.5.1 Description and operation

Standpipe traps (perforated metal or plastic tubes) are installed permanently within the river bed with a short internal cylindrical bung for closing off the holes (see Figure 3) [17]. They are inserted into the substrate at least 1 m apart, at various depths (typically 20 cm to 60 cm but occasionally up to 1 m) and remain *in situ* for the duration of the study. Once in place, the traps are opened (by removing the bung) and exposed to the hyporheic zone for a defined duration allowing organisms to enter the pipe via the holes. Exposure times may range from hours to days or weeks. A protective lid is placed on top of the pipe to prevent contamination, particularly by high flows or riparian inputs (leaves and other organic and inorganic particles). The contents of the traps are removed using a syringe. Once a sample has been taken, the pipes are cleaned of fine sediment and the holes closed until preparation for the next sampling occasion.

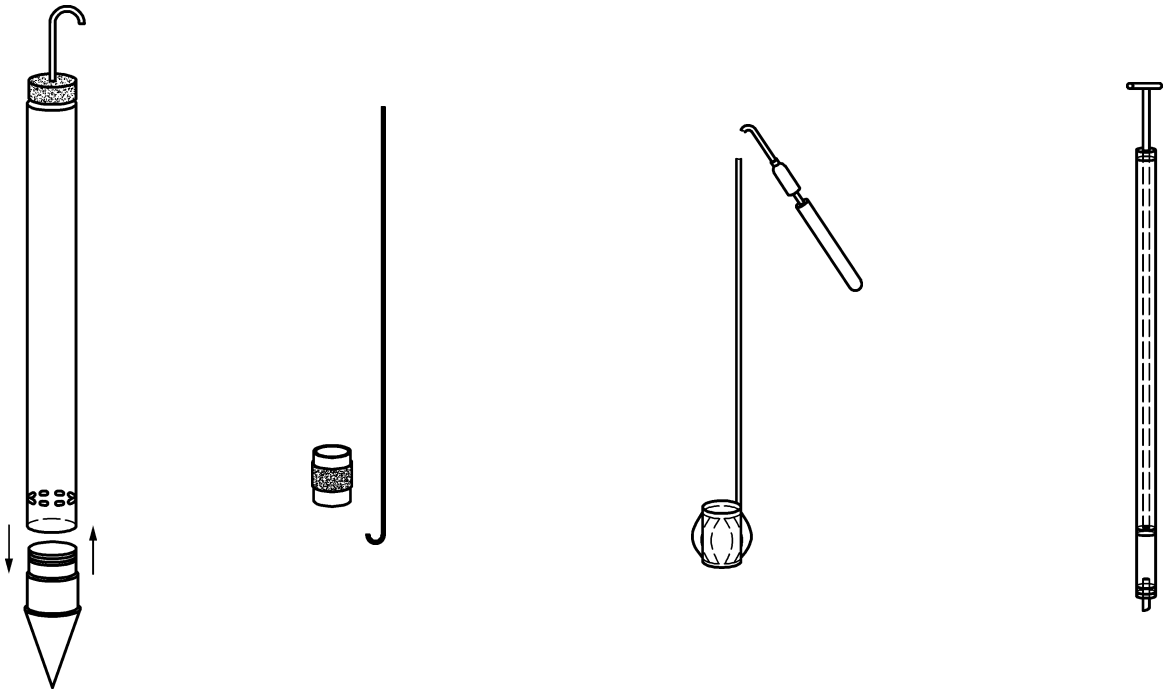
Advantages of this technique are its low cost, ease of operation and its use in repeat sampling and long-term monitoring. The relatively small sample size (typically 0,5 l) may be a disadvantage depending upon the purpose of the study.

5.5.2 Species sampled

A range of macroinvertebrates and meiofauna can be collected using this technique.

5.5.3 Environmental variables

These can either be measured directly in water extracted from the pipe, or samples can be taken to the laboratory for further chemical analysis. A quantitative measurement of dissolved organic matter and a semi-quantitative measurement of particulate organic matter and fine sediment can also be made..



a) **b)** **c)** **d)**

Key

a PVC pipe with ring of holes (each hole 5 mm × 15 mm) and timber tip that seals off the pipe from below

b bung that tightly closes the holes (in between sampling occasions) and metal hook to pull bungs out from inside the pipes

c inflatable rubber collar that can slide down the pipe to tightly close the holes to extract the sample

d syringe-like pump that extracts the interstitial water (sample)

Figure 3 — Standpipe trap [17], [18]

5.6 Williams corer

5.6.1 Description and operation

This technique involves the physical extraction of a small core of substrate from depths of 20 cm to 60 cm below the river bed (see Figure 4) [3,19]. The standpipe has an internal diameter of 2,5 cm, a solid conical tip and two openings (10 cm in length) shielded by two welded wings near the tip. The core-rod fits inside the standpipe so that when in the open position its opposite windows coincide with the two openings in the standpipe. Samples can be taken at various depths by turning the device in an anti-clockwise direction. This causes the wings to scoop gravel into the chamber of the core-rod. The corer is then closed, sealing off a 25 mL sample of substrate for removal from the river bed and subsequent analysis.

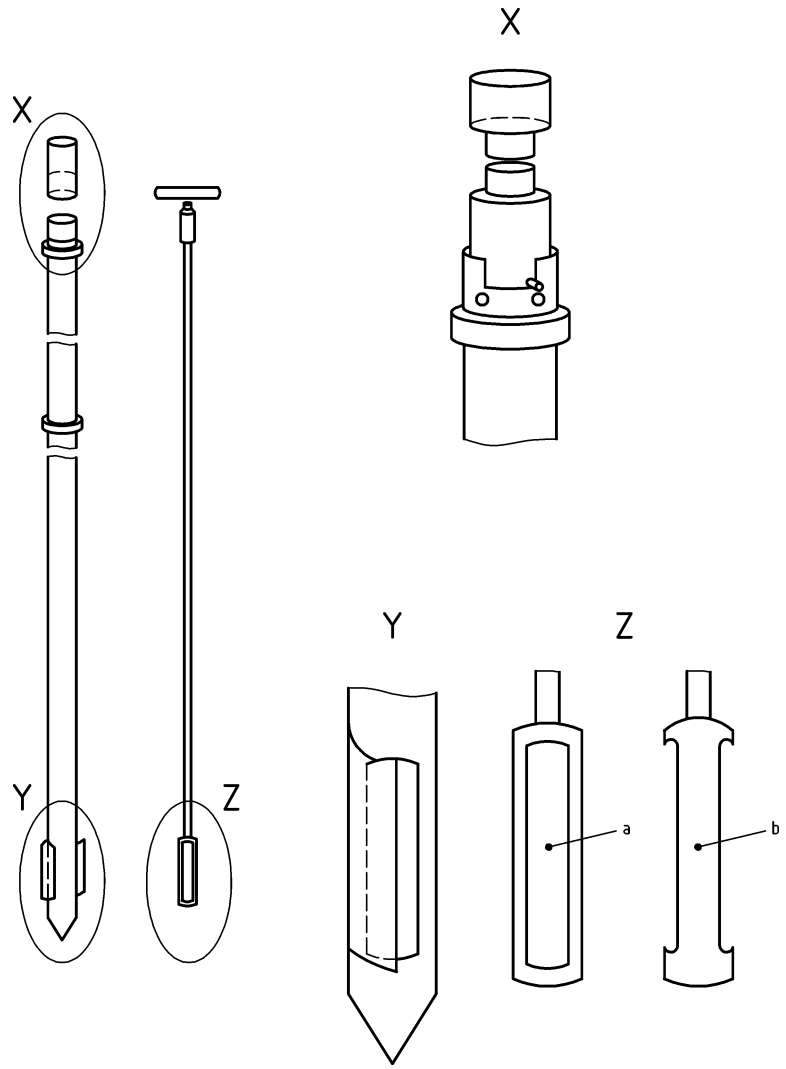
An advantage of the Williams corer is that it is light and relatively portable, allowing a series of replicate, quantitative samples to be taken without the need for recovery time. Consequently, it can be used during roaming surveys. The sampler works well in substrates that comprise sand, silt and fine gravel. The small sample size may be a disadvantage depending upon the purpose of the study.

5.6.2 Species sampled

Smaller macroinvertebrates and meiofauna.

5.6.3 Environmental variables

These can be measured in water extracted from the corer using a syringe.



Key

X details of locking device

Y details of conical tip with two openings

Z details of base of the core rod with windows;

a indicates open window, allowing sample collection;

b indicates plate to close window following sample collection

Figure 4 — The Williams corer [5]

5.7 Colonization devices

5.7.1 Description and operation

This technique involves installing chambers (such as those in Figure 5a) and Figure 5b)) containing artificial or natural substrates into the river bed, for colonization by hyporheic invertebrates [20]. Note that inserting the devices requires excavation and back-filling of the river bed. The design of these chambers is highly variable and they may take many different forms, e.g. baskets, mesh bags and perforated pipes. Replicate devices are left *in situ* for a pre-determined time, typically 2 to 4 weeks, although this duration will vary depending on local hydraulic and stream-bed characteristics and the purpose of the study [11]. This period allows a biofilm to develop and organisms to colonize the chambers. The devices are then removed and the invertebrates extracted. Chambers may be positioned

at depths between 20 cm and 1 m and sample volumes may vary according to the size and type of equipment used.

a) Colonization chambers comprising three stacked 15 cm long gravel-filled sub-chambers with tubing for extraction of water (Photo: Jane Grant)

Key

1 tube for extraction of *in situ* water samples

2 lid

3 63 µm mesh net lining the chamber which prevents removal of organisms during water extraction

4 data logger

5 substrate

6 access hole into colonization chamber

b) Individual colonization chamber filled with gravel (Photo: Mark Dunscombe)

Figure 5 — Examples of colonization chambers

Organisms may be washed out when removing the equipment from the river, and therefore sleeves or covers should be pulled up over the chambers to reduce loss of catch. This method may be unsuitable in fish spawning areas as installation of the chambers may disturb deposited eggs.

5.7.2 Species sampled

Both macroinvertebrates and meiofauna are captured.

5.7.3 Environmental variables

Colonization chambers can be modified (before installation in the river bed) by inserting a tube into the chamber substrate. Chemical analysis can then be undertaken from water samples retrieved from the tube with a syringe. The rate of sediment transport can also be measured by analyzing the rate of ingress of fine particles into the chambers.

5.8 Freeze coring

5.8.1 Description and operation

A standpipe with a solid metal tip is hammered into the stream bed to a specified depth depending upon the purpose of investigation (see Figure 6) [21,22]. This typically needs to be left *in situ* for 2 to 3 days to allow recolonization in the surrounding substrate, although this duration will vary depending on local hydraulic and stream-bed characteristics. During this time, the top of the standpipe is covered with a bung to prevent rainwater entering. On the day of sampling, cryogenic fluid (usually liquid nitrogen, but sometimes carbon dioxide) is introduced into the pipe to freeze the surrounding substrate. The pipe is protected by a flow deflector (typically a metal or plastic cylinder) to ensure the upper layers of the substrate are frozen. The sample size depends upon the volume of cryogenic fluid used, but 10 l to 25 l liquid nitrogen per sample is typical for steel cores while 5 l to 10 l is typical for copper cores. The volume required varies according to water velocity, temperature and substrate type. The water in the substrate freezes progressively outwards from the pipe and, when sufficient time has elapsed, the pipe and frozen core are winched out of the surrounding unfrozen gravel. The core can be left to defrost or chiselled from the pipe into sections as required (see Figure 6b)). Between 3 and 5 replicates should be taken per site in non-overlapping areas as freezing and extraction of the core affects the surrounding substrate.

Freeze coring should be undertaken in combination with electro-positioning to limit the risk of organisms escaping from the freezing zone and therefore improve the number of invertebrates obtained. An alternating current (650 V, 50 Hz) is applied between electrodes in a depth-field around the core [23].

a) Frozen core being extracted

b) Frozen core defrosting over sectioned tray

Figure 6 — Freeze coring (Photos: Jessica Durkota)

Disadvantages of this technique include the cost of delivering the coolant and the number of operators required to transport, install and manage the equipment safely and effectively (a large tripod and winch are required in order to lift the sample out of the stream bed). Note that this method causes considerable disturbance to the habitat.

5.8.2 Species sampled

This method provides quantitative samples of the macroinvertebrates and meiofauna. However, soft-bodied organisms (e.g. rotifers and oligochaetes) can rarely be identified after the freezing process.

5.8.3 Environmental variables

Water chemistry analysis (excluding dissolved oxygen and temperature) can be undertaken in the laboratory once the core has defrosted. A detailed analysis of the three-dimensional sediment structure, particle size distribution and organic matter content is also possible.

6 Sample processing

After collection, samples should be preserved using a fluid appropriate to the organisms being studied or kept cool and examined as soon as possible. A stain may be added to assist processing of invertebrate samples.

In the laboratory, samples should be processed to remove invertebrates. Samples may be gently washed through a sieve stack appropriate to the purpose of the study, e.g. from 1 mm or 500 µm mesh size for macroinvertebrates down to 63 µm or 45 µm for meiofauna. For fragile taxa that may not survive preservation, such as Gastrotricha, Rotifera and Microturbellaria, live samples should be refrigerated and examined within 3 d of capture. Samples containing a large amount of organic matter should be processed immediately.

Annex A
(informative)

Examples of sampling strategies for three different types of investigation

A.1 Case Study 1 – Assessing regional biodiversity and species richness

Regional variations in community composition are often strongly associated with geology and climate and their effect on flow permanence. For example, it is likely that an investigation of this nature will cover more than one geological type across several river catchments. More than one sampling method may be needed to characterize regional and local variability.

Ideally, the sampling strategy for such an investigation should include stream ordering as the first step, to ensure that the work covers a range of stream orders within each catchment. Within each river, it is recommended that at least two reaches are surveyed, in which at least two riffle-pool sequences (including upwelling and downwelling areas) are sampled. Where unconstrained floodplains occur these should be sampled where appropriate. Samples should be collected at a frequency that takes into account hydrological variability and faunal life cycles.

A.2 Case Study 2 – Assessing impacts on fish spawning sites

The hyporheic zone and hyporheos may be surveyed to determine impacts (e.g. of fine sediment inputs) on fish spawning areas. Species studied may include those listed on the EC Habitats Directive such as Atlantic salmon (*Salmo salar*) or river lamprey (*Lampetra fluviatilis*). Investigations into physical and chemical variables (e.g. dissolved oxygen concentrations, temperature, fine sediments) and invertebrates will inform the assessment.

Surveys should cover all sections of the river where fish populations may have been affected as well as control sites. All previously recorded spawning sites should be surveyed as well as other areas with suitable gravels, up to a depth of 30 cm. Sample sites may also be co-located with other monitoring points, so that data can be correlated with long-term records of water chemistry, river discharge and flow velocity.

Various methods can be used to assess the composition of the hyporheos and for the measurement of physical and chemical parameters. These methods can be used in association with cage-pipes containing eggs to assess the variability of survival rates across the study area. Method selection should consider potential disruption to the stream bed especially during the fish spawning season.

A.3 Case Study 3 – Assessing the impacts of pollution

The survey strategy should be determined by the type of pollution, which may be point-source or diffuse. It is important to distinguish human impacts from natural environmental variability. These investigations should compare impaired versus non-impaired sites, such as upstream and downstream of a pollution point-source.

Where impacts are localized, multiple sampling sites should be located upstream and downstream of the point of impact. Additional sites should also be located on a comparable watercourse to serve as controls. For diffuse-source pollution a more complex strategy is required with sufficient samples to characterize the impact comprehensively.

Samples should be collected at a frequency that takes into account hydrological variability and faunal life cycles. Sample sites may also be co-located with other monitoring points, so that data can be correlated with long-term records of water chemistry, river discharge and flow velocity.

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