eDNA sampling strategy for aquatic environments

Protocol

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Table of Contents

1. eDNA sampling in watercourses, ponds, and lakes 3

1.1 Sampling method 3

1.2 Watercourses 3

1.3 Ponds and small lakes 4

1.4 Larger ponds and lakes 4

1.5 Water volume and filtration 5

2. Scientific background 6

2.1 Spatial distribution and habitat selection 6

2.2 Sampling density 6

2.3 Water volume 7

2.4 International standards 7

2.5 Reliability and interpretation 7

References 8

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1. eDNA sampling in watercourses, ponds, and lakes

Environmental DNA (eDNA) is not evenly distributed in water. Its concentration varies between locations, depending on the distribution of organisms, water movement and local environmental conditions. In rivers and streams, eDNA can be transported hundreds of metres or even several kilometres downstream (Deiner & Altermatt, 2014; Bedwell & Goldberg, 2020). In ponds and lakes, eDNA may remain concentrated close to its source (Li et al., 2019), although factors such as wind, water depth and seasonal mixing can influence its spatial distribution (Hervé et al., 2022).

An effective sampling strategy must therefore ensure adequate spatial coverage while accounting for habit characteristics and the ecological preferences of the target species.

At Sylphium molecular ecology, we apply a standardised sampling approach that is based on existing guidelines and published research. Our method involves collecting spatially distributed subsamples and combining them into composite samples. The sampling effort is adapted to the size, characteristics, and habitat diversity of each waterbody.

1.1 Sampling method

Each composite water sample generally consists of 20 subsamples of approximately 50 mL, collected from spatially distributed locations, resulting in a total filtered water volume of approximately 1 litre.

1.2 Watercourses

For continuous watercourses, we collect **one composite sample per 500-metre section**. This sampling density follows the RAVON guidelines for linear waterbodies longer than 8 kilometres (Herder, 2025).

Within each 500-metre section, 20 subsamples of approximately 50 mL are collected at intervals of roughly 25 metres. Instead of following these fixed intervals rigidly, we select sampling locations based on habitat suitability, prioritising areas where the target species is most likely to occur, such as vegetated margins.

Where suitable habitats are unevenly distributed, sampling effort is concentrated in favourable areas. Sections lacking suitable habitats may be excluded from sampling. In narrow watercourses, subsamples collected from both banks are combined into a single composite sample.

Any sections excluded from sampling, as well as deviations from the standard sampling strategy, are documented.

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1.3 Ponds and small lakes

For ponds and small lakes up to approximately 1 hectare, we typically collect **one composite sample per waterbody**.

This approach is based on established eDNA sampling protocols for great crested newts, which involve collecting water from 20 locations around a pond (Biggs et al., 2015; Harper et al., 2025).

The 20 subsamples are distributed around the shoreline to ensure covering as much of the waterbody as possible. Priority is given to sampling areas where the target species is most likely to occur, such as vegetated margins, shallow areas and breeding sites. Open-water areas may also be included where relevant to the ecology of the target species.

Where suitable habitats are unevenly distributed, sampling effort is concentrated in favourable areas while maintaining adequate spatial coverage. Disturbance and collection of bottom sediment are avoided during sampling.

1.4 Larger ponds and lakes

In larger waterbodies, a single composite sample may not adequately represent the spatial variation in eDNA concentrations.

For ponds and lakes of approximately 1 to 10 hectares, we therefore use approximately **one composite sample per hectare** as a practical starting point. Each composite sample consists of at least 20 subsamples collected from a designated sampling zone.

Sampling zones are distributed across different shoreline sections and habitats. Composite samples are analysed separately, rather than pooled across the entire waterbody. This reduces the risk of diluting low concentrations of eDNA from species with localised distributions.

In deeper lakes, additional sampling in open water or at different depths may be necessary, particularly for species that do not primarily inhabit shoreline habitats (Zhang et al., 2020; Hervé et al., 2022).

The number and distribution of composite samples can be adapted according to the survey objectives, habitat diversity and ecological preferences of the target species.

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1.5 Water volume and filtration

For each composite sample, we aim to filter at least 1 litre of water, collected from approximately 20 spatially distributed locations.

Water is filtrated on-site using an eDNA filter capsule. If the capsule becomes clogged before the target volume is reached, additional capsules are used to ensure that the required volume of water is filtered.

When multiple filter capsules are used for a single composite sample, the DNA extracts are pooled in the laboratory and analysed as one sample. This approach allows the target water volume to be processed even under turbid conditions, while maintaining the original spatial sampling design.

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2. Scientific background

The sampling method draws on several studies investigating how eDNA is distributed in aquatic environments and how sampling design affects species detection.

2.1 Spatial distribution and habitat selection

Because eDNA concentrations can vary considerably within a waterbody, collecting water from multiple locations reduces reliance on any single sampling point (Rees et al., 2014; Hauger et al., 2020).

For ponds, Biggs et al. (2015) demonstrated the effectiveness of collecting subsamples from 20 locations around the shoreline when surveying for great crested newts. Li et al. (2019) also showed that eDNA in fish ponds can remain relatively localised, supporting the need for spatially distributed sampling.

2.2 Sampling density

For watercourses, the distance of 500 metres between composite sampling sections is based on the RAVON guidelines (Herder, 2025). Dividing each section into 20 subsampling locations provides a consistent way to distribute sampling effort along the watercourse.

For ponds, the use of 20 subsamples follows established great crested newt protocols (Biggs et al., 2015; Harper et al., 2025).

Larger lakes require a different approach. Zhang et al. (2020) found that analysing spatially separated samples individually detected more fish species than pooling them. In a study of 101 British lakes, Sellers et al. (2024) found that 10 to 20 spatially distributed samples generally captured most of the fish species richness, while rarer species were more likely to be missed with fewer samples.

These findings support collecting multiple independent samples when surveying larger waterbodies, particularly where detecting less abundant species is important.

Our approach of approximately one composite sample per hectare is a practical sampling density, rather than a fixed optimum established by these studies.

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2.3 Water volume

The volume of water filtered can also affect detection. Mächler et al. (2016) compared volumes ranging from 0.25 to 2 litres when surveying aquatic macroinvertebrates and recommended filtering at least 1 litre under the conditions studied.

Westra et al. (2024) also used composite samples of approximately 1 litre in Dutch surveys for Eurasian water shrews, combining at least 28 subsamples along 100-metre sections.

Together, these studies provide a basis for using 1 litre as a practical standard volume. However, the amount of water needed for reliable detection may differ between species and waterbodies.

2.4 International standards

Our sampling approach follows the principles of EN ISO 17805:2026, *Water quality - Sampling, capture and preservation of environmental DNA from water*, particularly Clauses 5.1 and 5.2.

The standard addresses spatial representativeness, habitat variation, target-species ecology, water depth and the collection of composite samples.

It does not prescribe specific sampling densities such as one sample per 500 metres or one sample per hectare. These are practical choices based on existing guidelines and the available scientific literature.

2.5 Reliability and interpretation

No sampling method can guarantee the detection of a species whenever it is present. Detection probability depends on several factors, including the amount of DNA released by the organism, its distribution in the water, seasonal activity, and environmental conditions.

A positive eDNA result indicates the presence of DNA from the target species in the sampled water. However, it does not necessarily confirm the exact location of the organism, particularly in flowing water where eDNA may be transported considerable distances downstream. Conversely, a negative result does not necessarily indicate the absence of the target species. Low eDNA concentrations, seasonal variation and local habitat conditions may all reduce the probability of detection.

Our sampling strategy is designed to minimise the effects of these sources of variation by ensuring adequate spatial coverage, prioritising suitable habitats and maintaining consistent sampling volumes. This provides a standardised, scientifically informed and reproducible approach to eDNA surveys across a range of aquatic environments.

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