A new flow path: eDNA connecting hydrology and biology

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Abstract
Environmental DNA (eDNA) has revolutionized ecological research, particularly for biodiversity assessment in various environments, most notably aquatic media. Environmental DNA analysis allows for non-invasive and rapid species detection across multiple taxonomic groups within a single sample, making it especially useful for identifying rare or invasive species. Due to dynamic hydrological processes, eDNA samples from running waters may represent biodiversity from broad contributing areas, which is convenient from a biomonitoring viewpoint.
Security of the quality and quantity of water supply and biodiversity loss are intertwined challenges (Vörösmarty et al., 2010) and constitute some of the most pressing current and future concerns faced by society (Roy et al., 2023). Due to anthropogenically induced global change, challenges to conservation of water resources involve all compartments of the hydrological cycle (de Jong, 2015; Griebler & Avramov, 2015; van Tiel et al., 2023), while freshwater biodiversity decline is higher than in most other ecosystems (Brosse et al., 2022; Tickner et al., 2020). Commonalities in research questions between hydrology and biology are reflected by conceptual connections, as water chiefly controls habitat distributions and dispersal routes (Ho et al., 2023; Muneepereakul et al., 2019; Rinaldo et al., 2020; Vannote et al., 1980), while at the same time vegetation and ecosystem processes dictate the amount and timing of evaporation and thus water quantity (Guswa et al., 2020).

Organisms shed genetic material (i.e., environmental DNA, or eDNA) that is stored and transported within water bodies (Figure 1). However, its release and production, mixing, and degradation in standing and running waters are not yet fully understood (Barnes & Turner, 2016; Harrison et al., 2019). At the same time, hydrology has quantified many sources, pools and fluxes of water to understand its movement around the planet, yet uncertainties remain with respect to the timing and flow paths of water across landscapes (Aulenbach et al., 2021; Benettin et al., 2022; Dethier et al., 2022; Dwivedi et al., 2022; Gentile et al., 2023; Hartmann et al., 2020; Michelon et al., 2023). Because genetic material is stored and transported by streams, rivers, lakes and groundwater, understanding its dynamics in water is relevant to many open research questions within both biology and hydrology (Figure 2). These field-specific research questions are inherently interconnected and could be potentially addressed through an interdisciplinary synthesis of eDNA and hydrological approaches and methods.
Environmental DNA encompasses DNA extracted from environmental sources, including intact living or dead organisms (e.g., whole microbes) and shed material (e.g., sloughed cells, tissues, organelles, or gametes; Pawlowski et al., 2020). This eDNA can exist in multiple states: the membrane-bound state, referring to eDNA encapsulated in cellular or organellar membranes; the dissolved state, referring to extracellular DNA without interactions with other particles; and the adsorbed state, referring to extracellular DNA bound to particles in the environment (Kirtane et al., 2023; Mauvisseau et al., 2022). Over the last two decades, eDNA-based methods have transformed the way biological communities are analyzed, allowing rapid identification of whole communities from environmental samples (Deiner et al., 2017; Taberlet et al., 2012). Notably, eDNA has gained widespread popularity over traditional techniques including kick-net sampling and electrofishing because it allows detecting species from multiple taxonomic groups within a single sample and without the need for physical specimen collection (Blackman et al., 2022), which also improves rare and invasive species identification (Deiner et al., 2017). The prospects and challenges of eDNA methods have already been subject to extensive review (Pawlowski et al., 2021; Takahashi et al., 2023). Nonetheless, there are criticisms about relying exclusively on eDNA observations for aquatic ecosystem assessment (Roussel et al., 2015), which include potential complications of molecular methods for metagenomics that can reduce reliability of quantitative species distributions (Burian et al., 2021). Therefore, eDNA analysis is often seen as complementary to conventional techniques (Nagarajan et al., 2022). In riverine ecosystems, eDNA analysis can be particularly valuable, as it can capture a broader biodiversity signal at both spatial and temporal scales, extending beyond a single sampling point (Figure 3; Carraro et al., 2020, 2023). This is achieved thanks to the dynamic flow of water, akin to a “conveyor-belt” mechanism transporting DNA downstream (Deiner et al., 2016). Consequently, eDNA analysis allows for the integration of a much larger scale of biodiversity than what might be observed at a single sampling location. Despite its advantages, interpreting eDNA samples from flowing ecosystems can also be challenging, as the precise location or position of the signal detected is often unknown and remains difficult to pinpoint due to the changing hydrological processes to which DNA is subject.

**Figure 1**: Both hydrological fluxes (blue text and dashed arrows) and biological processes (green text and dotted arrows) influence the distribution of DNA within aquatic environments (black double-helix DNA symbols). The hydrological cycle begins with precipitation entering aquatic systems through overland or subsurface flow paths. Water then flows within streams and rivers, with additions from tributaries, and is eventually discharged out of a catchment or evaporated back to the atmosphere. Within this context of moving water, biological materials are also carried into aquatic systems through superficial and subsurface routes as well as being produced and released by biological processes within the water column. This material is transported and mixed though both advection and dispersion, and may eventually be removed or degraded through photochemical, microbial or adsorption processes.
FIGURE 2 Overview of research questions across the fields of hydrology and biology in which environmental DNA (eDNA) could provide new insights. Generally speaking, eDNA could assist in advancing the specific and overarching research interests of both hydrology (i.e., understanding the hydrological response of watersheds) and (freshwater) biology (i.e., assessing biodiversity patterns and trends). More specifically, eDNA could constitute a “unidirectional” bridge between these two disciplines in contexts where one of the two aspects is well resolved (“diverging interests”): For instance, if hydrological processes such as water flow paths and travel times are well known (which is e.g., the case for water flow in river systems), eDNA could be coupled with hydrology-based models to infer the spatial distribution of freshwater species (Carraro et al., 2018, 2020; Carraro & Altermatt, 2024). In other contexts, the use of eDNA could be seen as a “bidirectional” bridge between hydrology and biology, specifically when quantitative aspects of the dynamics of eDNA in stream water are investigated (“shared interests”). For instance, composition of biological communities assessed via eDNA could be used to simultaneously disentangle different water sources in a catchment and provide information on its biodiversity (Mächler et al., 2021; U Rycki et al., 2020).

Hydrology is concerned with the study of the physical processes governing the movement of water across the hydrosphere, but especially in the subsurface, where water flow paths, storage, and release dynamics are not yet fully understood (Benettin et al., 2022; Blöschl et al., 2019; Kirchner, 2003; Tromp-van Meerveld & McDonnell, 2006). In general, the discipline is continually expanding available metrics and tools to assess and quantify flow paths and storage, and their respective spatial and temporal scales. To this end, several conservative and non-conservative tracers have been used, each with specific advantages and drawbacks (Leibundgut et al., 2009). However, the key limitation of current tracer methods is that they suffer from seasonal limitations (e.g., fluctuations in stable isotopes are overwhelmingly determined by seasonal patterns, potentially overshadowing the sought signal) or geological limitations (e.g., the applicability of geo-originating tracers is constrained by their heterogeneity within a given study site). In particular, key features of a good hydrological tracer are its non-toxicity and the possibility to use multiple, distinguishable tracers simultaneously. Since eDNA meets these requirements, recent works have explored the potential of eDNA as a hydrological tracer (Figures 4–6; Florent et al., 2022; Good et al., 2018; Mächler et al., 2021; Pollitt et al., 2022; U Rycki et al., 2020; U Rycki et al., 2022).

Here, we offer an overview of some key aspects of eDNA and associated technologies and applications, particularly as they relate to hydrological science, complemented by a discussion of key hydrological understandings as they relate to the science of eDNA collection and analysis. Due to the breadth of scientific disciplines connected with the study of eDNA in aquatic environments, we constrain our discussion to naturally occurring DNA, produced and collected in the environment, and exclude discussion of artificial (free or encapsulated) DNA; see Foppen (2023) for a review on this topic. This paper is organized as follows. Following this introduction, in Section 2, we discuss the dynamics and interactions of water and eDNA in the environment. Specifically, we consider a control volume of water in a stream and the eDNA contained within it (Figure 1); we discuss the mechanisms by which water and eDNA each enter the control volume (production and release) and subsequently interact, are transformed, and exit the control volume (transport, mixing, and removal). We particularly focus on rivers and streams, acknowledging the influence exerted by water exchange with substrate, subsurface, groundwater, and upstream water bodies on the stream water column. In Section 3, we identify and attempt to resolve some important issues for researchers involved in or considering studies concerned with eDNA collected in the water column. Finally, in Section 4, we present specific research needs that, if resolved, would meaningfully strengthen the emerging synergy between hydrological and eDNA studies, offering a foundation of common language and understandings on which to build transdisciplinary collaborations, thus opening new paths and opportunities to advance both fields.
2 | PROCESSES AND CYCLES

2.1 | Production and release of water and DNA

2.1.1 | Production of water

The amount of surface and subsurface water produced by catchments is determined by a number of factors including the geology and geomorphology, the climate, soil properties, vegetation cover, land use, and other anthropogenic factors. The quantity of precipitation over the growing season and its intra and interannual variability arise from a combination of variability in the frequency, intensity, and seasonality of precipitation (Good et al., 2016), which drives, to a large degree, the biogeography of vegetation at continental scales (Good & Caylor, 2011; Guan et al., 2018). Generally well understood through overarching climatological and hydrological paradigms of Budyko (1974) and Dunne (1983), water and energy limitations provide first-order controls over runoff amounts, with low-aridity (defined as the ratio of potential evapotranspiration to precipitation) landscapes producing more streamflow and high-aridity landscapes returning more water to the atmosphere as actual evapotranspiration.

FIGURE 3 Examples of spatial patterns of predicted alpha diversity (expressed in terms of genus richness) for different taxonomic groups and seasons obtained via the eDITH model (Carraro et al., 2023). This model enables assessing taxon spatial distributions based on spatially repeated eDNA measurements and integration of eDNA transport along stream networks. Source: Adapted with permission from Carraro et al. (2023).
The routing of precipitation into overland flow or subsurface return flow (Figure 1) is broadly determined by the ratio of precipitation intensity to soil infiltrability, with higher values of this ratio driving overland flow and lower ratios driving subsurface flow (Trancoso et al., 2016). The routing and transit times of water moving through the subsurface is controlled by slope, geology and flow path lengths (Butler et al., 2023; McGuire et al., 2005), while the presence of glaciers, snowpack, and other frozen waters within a watershed lengthens the timescales of water moving through catchments, with areas strongly influenced by cryospheric waters experiencing more pronounced summer return flows (i.e., baseflow; Segura et al., 2019). The combination of all these characteristics determines how, where, and when water moves through catchments.

2.1.2 | Production of DNA

All DNA is produced within living cells and organelles, such as the nucleus, mitochondria and chloroplasts. In the case of macro-organisms, this DNA produced within the cells of the organism is subsequently released into the environment (see Section 2.1.4). This DNA can only degrade over time. However, eDNA also can include microbial DNA, which consists primarily of living but also some dead organisms. The living organisms can reproduce, subsequently producing new DNA. Microbial communities contribute to a significant proportion of the eDNA pool and comprise a myriad of species of prokaryotes (Bacteria and Archaea), microbial protists (Burki et al., 2020) and fungi, in addition to genetic material of viral origin. Contrary to macro-organismal eDNA, microbial communities are composed of individual cells or cell-aggregates (e.g., colonial life forms) that might quickly respond to changing environmental conditions (Nguyen et al., 2021). Also, the coalescence of microbial community composition is not longitudinally uniform (Mansour et al., 2018).

Environmental conditions influence microbial community development, thereby affecting the composition of eDNA produced by the system. Streamwater microbiomes, for example, are composed primarily of microbes that originate in upstream soil and groundwater environments (Crump et al., 2007, 2012; Sorensen et al., 2013) and are influenced by climate and geomorphology, particularly in headwater catchments (Figure 4; URycki et al., 2020). Temporal and spatial changes in temperature, light, and physicochemical conditions (e.g., salinity) can foster microbial growth and shift community composition, for example by promoting algal blooms, which might in turn significantly change the community composition of other microbes (Van der Gucht et al., 2005; Winter et al., 2007). Also, the discharge of trace elements or...
heavy metals can abruptly restructure microbial communities (Jones et al., 2020). Changes in microbial community composition are furthermore observed on seasonal timescales (Crump & Hobbie, 2005; Hahn, 2006). Seasonal changes on land, such as autumn senescence and leaf-drop in temperate ecosystems, can quickly shift the microbial community toward fungi, which can constitute up to 95% of the microbial biomass during early leaf decay, leading to proportionally higher DNA production (Grossart et al., 2019). Heavy rain events are also known to quickly shape microbial communities of surface and groundwaters, not only in composition (Sugiyama et al., 2018), but also in functions, for example, degradation, reduction, and oxidation of compounds (M. Wu, Wang, et al., 2021). Abrupt changes in microbial community composition such as algal blooms are of particular relevance to the use of macro-organismal derived eDNA, for example, targeting fish or macroinvertebrates, as the increased microbial cells can hinder sampling due to filter clogging and impact the eDNA degradation rates (Barnes et al., 2014; Mauvisseau et al., 2022; Q. Wu, Sakata, et al., 2021).

2.1.3 | Release of water

Water enters the fluvial network either as a release from the subsurface, from upland tributaries, as overland flow, or directly as precipitation (Figure 1). Water is lost from a river via evaporation, seepage into groundwater, uptake by vegetation, or downstream flow into, eventually, lakes and oceans. These fluxes occur over a wide range of temporal (i.e., seconds to millennia) and spatial scales (i.e., centimeters to many kilometers) and are governed by substrate, geology, climate, and land cover, among others (Ward et al., 2019). Much of the water that is gained and lost to the fluvial network is exchanged into and out of the hyporheic zone. The hyporheic zone, which is the area directly underneath the streambed in which stream water interacts dynamically with shallow groundwater, is a critical compartment of fluvial systems, as it is the zone which is thought to be responsible for the largest turnaround of nutrients and other solutes (Boano et al., 2014; Poole et al., 2008; Wondzell & Swanson, 1999). As such, even small variations in flow, solutes and substrate conditions can dramatically change the dynamic storage offered by the hyporheic zone (Gomez-Velez et al., 2017). Many processes in fluvial systems happen on a diurnal cycle, particularly those driven by inputs of solar radiation, such as plant water uptake, algal growth, glacier and snow melt, and activation of heat-driven processes in the aquatic biota (Kirchner et al., 2020).

Besides fluvial networks, there are various other pathways by which catchments can transport and release water, and thereby biological and chemical constituents. A primary pathway for water storage and movement within and out of a catchment is through the subsurface. The speed and direction of subsurface flow of water, solutes, and particles is thereby strongly controlled by subsurface heterogeneity (both in the unsaturated and saturated zones). As such, water moves more rapidly along preferential pathways, for example, formed by fractures, conduits, highly conductive buried channels in alluvial sediments, or soil macropores, compared to the surrounding porous media (Bradford et al., 2017; Schilling et al., 2022). Physical and chemical properties of different soil, sediment, and bedrock types also influence the rate of infiltration and flow velocity. Thus, the age distribution and composition of water and its constituents in a fluvial network are the result of the integration of myriad highly variable physical and biological characteristics and processes (Cepeley et al., 2020; Michelon et al., 2023; Sprenger et al., 2019).

2.1.4 | Release of DNA

The amount of macro-organismal eDNA present in water bodies is determined by the organism type, its body size, its abundance, and the developmental and physiological stage of the organism among other factors (Andruszkiewicz et al., 2021; Harrison et al., 2019; Stewart, 2019). Specific organismal characteristics such as the surface-biomass ratio as well as the surface texture and dermal tissue type of the organism determine the release of cellular material and dissolved DNA fragments (Wood et al., 2020). Therefore, shedding rates of eDNA from organisms depend very much on the organism type (e.g., fish shed more eDNA than crustaceans, despite having equal biomass; Andruszkiewicz et al., 2021), but vary also within taxonomic groups (Van Driessche et al., 2023). Not only the organism itself, but also abiotic factors such as water temperature and pH can influence the shedding rates of organisms, likely due to changes in metabolic activity (Harrison et al., 2019; Jo et al., 2019; Stewart, 2019). The co-occurring spatial and temporal variability of taxon distribution, seasonality, environmental conditions, and metabolic activity present in complex river networks affect macro-organisms’ DNA production, and thus eDNA sources exhibit corresponding spatial and temporal variability. Pulse release events, triggered by factors such as organism behavior (e.g., stress reactions or hatching),
increased metabolic rates, seasonal activities (e.g., reproductive cycles), and predation (including tissue damage and fecal deposition), further contribute to non-uniform eDNA release patterns (Bylemans et al., 2017; Nørgaard et al., 2021; Ostberg & Chase, 2022; Thalinger et al., 2021). These patterns therefore must be considered in both biological and hydrological studies utilizing macro-organism eDNA.

2.2 Transport, mixing, and removal of water and DNA

2.2.1 Transport and mixing of water

The physical processes that transport and mix water along (surface and subsurface) flow paths are generally well understood, and a myriad of models of varying degrees of complexity to describe these processes exist (Panicoti & Putti, 2015). Mixing and transport of solutes and particles is also reasonably well understood, but the large computational requirements to mathematically describe the fully integrated surface-subsurface transport of water and (reactive) solutes and particles on the catchment scale currently restricts the use of such complex models to experimental approaches (Xu et al., 2022). Essentially, dynamics of transport and mixing of water and solutes in a stream are described by the advection–diffusion equation, which is ubiquitous in the study of environmental fluid dynamics (Imberger, 2013). Specifically, it implies that suspended particles and solutes, while being advected by fluid motion, are subject to diffusion following Fick’s law, according to which the mass flux is proportional to the opposite of the concentration gradient. Depending on the value of the diffusion coefficient \( D \), Fick’s law can describe processes that act at very different spatial scales, such as molecular diffusion (related to random molecular motion; \( D \approx 10^{-9} \text{ m}^2 \text{s}^{-1} \)), turbulent diffusion (due to stochastic variations in the velocity field due to turbulent motion; \( D \approx 10^{-2} \text{ m}^2 \text{s}^{-1} \)), and hydrodynamic dispersion (interaction between transverse diffusion and shear flow, that is differential velocities across a channel’s cross-section owing to shear stress effects; \( D \approx 10^1 \text{ m}^2 \text{s}^{-1} \); Fischer, 1979; Rinaldo et al., 2020). An additional dispersion effect (termed geomorphological dispersion) occurs at a larger spatial scale, namely the whole fluvial network, and is related to the effects of the different lengths of the drainage paths leading to a measurement cross-section (Rinaldo et al., 1991; Rinaldo et al., 2020).

2.2.2 Transport and mixing of eDNA

Transport and mixing of eDNA in water highly depends on the respective state of eDNA. Intracellular and intra-organellar or particle-adsorbed eDNA is hypothesized to be transported through water bodies like particles, whereas dissolved eDNA behaves like other dissolved substances (Mauvisseau et al., 2022; Pont et al., 2018). Particles are subjected to sinking and resuspension processes as well as turbulent mixing (Andruszkiewicz et al., 2019). For dissolved substances, advection and diffusion are the major transport processes, with sinking playing a minor role. The heterogeneous nature of eDNA is one of the major challenges in the interpretation of its behavior in aquatic environments (Mauvisseau et al., 2022). Furthermore, the type of water body also plays an important role. While transport in rivers tends to be longitudinal and driven by advection, lentic systems (lakes, ponds, marine) are usually stratified and transport is predominantly lateral and vertical, and strongly impacted by diffusion (Jeunen et al., 2020; Littlefair et al., 2021). In groundwater, advection and diffusion/dispersion usually play an equally important role, and due to the relatively large size of the different eDNA particles in comparison to the pore spaces through which water is transported, transport becomes highly complex and difficult to track and quantify (Hunt & Johnson, 2017; Tufenkji, 2007). Addressing coupled water and eDNA reactive transport on the catchment scale with fully integrated surface-subsurface models is therefore currently not possible. However, given the small size of free-floating eDNA particles that can be found in the water column of streams (either in dissolved or membrane-bound state), a reasonable first order approximation of their transport behavior is to assume reactive advection–diffusion dynamics (Figure 1).

The extent to which eDNA can mix in the transverse direction with respect to streamflow is controlled by turbulent diffusion and is highly dependent on the longitudinal shape of the channel, as well as roughness and irregularities at the channel bottom. In a wide, straight river reach with a regular riverbed, a source of eDNA at a river bank was not identified in samples at the other bank even several km downstream of the source location (Laporte et al., 2020), which demonstrates the poor lateral mixing in channelized rivers. However, most rivers are characterized by irregularities such as meanders, boulders, pools, riffles, or weirs, which cause heterogeneities in the velocity fields and thus strongly enhance lateral mixing and longitudinal dispersion. Importantly, by assuming a constant release of a tracer over time,
shear flow dispersion is often negligible with respect to advection (Fischer, 1979). While eDNA release patterns are in principle variable in space and time, the time scale of water motion in rivers (considering, e.g., a characteristic water velocity of 1 m s\(^{-1}\)) is usually much faster than changes in production and shedding rates. Hence, as a first approximation, it could be reasonable to assume that eDNA dynamics in water follow pure advection dynamics, however mediated by decay (see Section 2.2.3).

An important aspect to also consider are the inputs of water and therefore eDNA into the fluvial network via the subsurface, from overland flow, direct precipitation or atmospheric deposition. These inputs have a critical impact on the eDNA that is found within a stream water column, and the composition of eDNA is usually a mixture of eDNA from different geographic origins (Deiner et al., 2016; Machner, Schütz, et al., 2021). The origins, composition and transport pathways of the water that makes up a stream water column are moreover highly variable in space and time (Sprenger et al., 2019), inevitably also changing the spatial and temporal origins and composition of eDNA. During the spring snowmelt season, for example, the water in a fluvial system can be made up of a highly dynamic mix of snowmelt overland flow, precipitation and subsurface return flows originating from short as well as long flow paths. Conversely, during summer low flows, the principal component of water in a stream is groundwater return flow, particularly that of longer flow paths. The contribution of eDNA from terrestrial ecosystems, on the other hand, is dependent on rainfall intensity capable of transporting eDNA directly into the fluvial network via overland flow (Bae et al., 2022). Changes in the physicochemical conditions in different hydrological compartments can also have an impact on eDNA, as they are capable of rapidly changing the microbial community composition. From the perspective of modeling water, eDNA transport and mixing in a fluvial network, however, these inputs can be accounted for via relatively simple flux boundary conditions (Cho et al., 2016; Schilling, Park, et al., 2019). The major challenge lies rather in characterizing these inputs of water and eDNA, which are both spatially and temporally highly variable.

### 2.2.3 Removal of water

The bulk of water in the stream channel exits the catchment at the stream outlet; the volume of water passing through the outlet (or any point in the stream) in a given period of time, known as streamflow or discharge, reflects the integration of many complex variables and their interactions across the catchment. Streamflow is shaped largely by climate (e.g., Guo et al., 2008), and also by the catchment characteristics that govern the movement of water throughout the catchment, such as subsurface composition (Atwood et al., 2023; Beven & Germann, 1982; Dingman, 2015), land cover (Guo et al., 2008; Oudin et al., 2008), elevation and topographic organization (McGuire et al., 2005), and finally by the size and position of the sub-catchment within the larger basin; headwater streams and tributaries generally experience more variable streamflows and quicker responses to rainfall events than lower reaches. Anthropogenic influences such as groundwater and surface water withdrawals also shape a stream’s flow regime. The governing flow regime is not only important in terms of water quality and quantity for societal needs such as municipal and agricultural uses, but also for the ecological function of the system, including nutrient transport and habitat suitability, especially at critical life stages (e.g., salmonid fry; Poff & Ward, 1989; Richter et al., 1996). As such, differences in hydrological function often result in differences in ecological function and biological communities, including microbial communities (Figure 5; Savio et al., 2019; Urycki et al., 2022; Virgin et al., 2024; Zeglin, 2015). Understanding differences in hydrological function over space and time, for example between different catchments (Figure 4; Read et al., 2015; Urycki et al., 2020) or in response to anthropogenic disturbances, may help explain differences in community structure and therefore also of eDNA in a stream water column. Streamflow is therefore a fundamental and informative metric to understand both the hydrological processes (McMillan, 2021) and also the eDNA signals.

### 2.2.4 Removal of DNA

Aside from flow-driven transport, removal of eDNA from the water column in surface water bodies such as lakes and streams can be described through two processes: sedimentation and degradation (Figure 1). Sedimentation of eDNA involves the settling of the adsorbed and membrane-bound states of eDNA, and is a function of hydraulic conditions and particle size of the eDNA (Jo & Yamanaka, 2022; Turner et al., 2015; Van Driessche et al., 2023). Water matrix characteristics such as suspended particles may also influence the sedimentation rate of eDNA (Brandão-Dias et al., 2023; Snyder et al., 2023). However, sedimented DNA can also be resuspended and act as an input with changing hydraulic conditions such as increased turbulence (Shogren et al., 2017). The degradation rate of eDNA also depends on the state
of eDNA, where the adsorbed DNA may remain protected from degradation for extended periods of time (Barnes et al., 2014; Mauvisseau et al., 2022), while the membrane-bound and dissolved states of eDNA are readily degraded via enzymatic (intracellular and extracellular) and photochemical oxidation (Mauvisseau et al., 2022). While UV light exposure can be easily measured, the level of enzymatic activity is challenging to quantify as it is also impacted by temperature, salinity and organic pollutants (Eichmiller et al., 2016; Strickler et al., 2015; Yang et al., 2019). The enzymatic activity is a function of the concentration of nucleases (intracellular and extracellular), and environmental conditions for optimal activity such as temperature, pH, UV light exposure, and presence of cofactors. Acidity (i.e. low pH) has been shown to promote and accelerate the degradation of eDNA (Lindahl, 1993; Seymour et al., 2018). Degradation rates also depend on the source organism likely due to the variability in the structural stability between cell types (Zhao et al., 2021).

In the case of flow-driven eDNA transport, under the assumption of first-order decay dynamics, the eDNA concentration \( C \) at a distance \( x \) from a source can be modeled as \( C = C_0 \exp(-kx/u) \), where \( C_0 \) is the concentration at the source location, \( u \) the water velocity, and \( k \) [T⁻¹] a decay rate (Carraro et al., 2018, 2020; Sansom & Sassoubre, 2017). In principle, the decay rate \( k \) subsumes photochemical and microbial processes that degrade eDNA. With an emphasis on gravitational settling as the key mechanism for removing eDNA from the water column, the behavior of eDNA in streams has often been modeled via an approach borrowed from fine particulate organic matter dynamics (Pont et al., 2018; Shogren et al., 2017), in which eDNA particles are assumed to be subject to a depositional velocity \( v_d \) in addition to the flow velocity \( u \). In such a case, the above first-order decay equation still applies, with the decay rate expressed as \( k = v_d/d \), with \( d \) being the channel depth. In practice, it is difficult to disentangle the effects of settling, photochemical, and microbial degradation in the decay of eDNA. Hence, the use of a single lumped decay rate parameter might suffice (Box 1).

### 3 | CONSIDERATIONS FOR APPLYING HYDROLOGICAL AND EDNA TECHNIQUES

#### 3.1 | Hydrological considerations for biologists

#### 3.1.1 | Sampling strategy

When preparing to sample eDNA in an aquatic environment, researchers must understand catchment characteristics that influence the quality and composition of the water they sample. The first metric to consider when planning to
sample stream water for eDNA is the streamflow hydrograph, that is, the dynamics of discharge over time, as this metric reflects the most important catchment characteristics such as precipitation frequency and intensity, runoff generation mechanisms as well as surface-subsurface interactions. A related metric of interest, which can be derived from the streamflow hydrograph via hydrograph separation, is the base flow index (BFI). The BFI describes the ratio of total river flow to baseflow (or groundwater return flow). The BFI can inform about species habitats, the variability in microbial community composition, and the presence of certain functional genes (Clark et al., 2022; Gustard et al., 1992; Schoonover, 2005).

Another metric to consider is drainage area, as it is the master variable controlling other geomorphological and hydraulic variables such as slope, stream depth and width (Leopold & Maddock, 1953; Tarboton et al., 1989), and thus ecological characteristics (Jacquet et al., 2022). In fact, along a hydrological continuum, community composition is heterogeneous, with bacterial alpha and beta diversity found to be decreasing downstream (Crump et al., 2007; Crump et al., 2012; Savio et al., 2015). Knowledge of the hyporheic zone and larger scale stream–aquifer interactions is also important, because the macro- and microorganismal communities located within and underneath the streambed are different from those found within the stream water column. Consequently, changes in streamflow, groundwater levels and stream–aquifer interactions can rapidly shift the composition of eDNA in the stream water column (Brunke & Gosner, 1997; Marmonier et al., 2012). Although it is difficult to measure, another important metric to understand the origins, flow paths, and composition of stream water is the transit time distribution (TTD) of the water, or the expected distribution of the ages of water parcels in a sample, which reflects the flow paths that resulted in the suspended material and geochemical properties of the sampled water (Brookfield et al., 2021; McDonnell et al., 2010; Sprenger et al., 2019). The groundwater flow paths do not necessarily correspond with the topography of the surface and TTD can vary both spatially and temporally within a reach (Brunke & Gosner, 1997; Lauber & Goldscheider, 2014). While not an exhaustive list, catchment level metrics similar to these should be included in selecting sampling locations to ensure a representative sample is taken. While sampling eDNA, it is recommended that researchers measure and assess both the abiotic (e.g., temperature, dissolved oxygen concentrations, salinity, sediment composition, and pH) and biotic conditions (e.g., primary production rates, extracellular enzyme concentrations) of the reach, as these conditions have often been shown to impact eDNA persistence (Barnes et al., 2014; Holden, 2005; Stewart, 2019; see Section 2.2.4 Removal of DNA, see Table 1 for general considerations in the sampling process).
3.1.2 | Hydrological modeling

Hydrological models use existing data and observations to predict conditions in places and times without data, such as in the future, under different conditions, in other places, or at other resolutions. Models can be useful to test theories, fill data gaps, and make predictions (Horton et al., 2022). When deciding whether and which hydrological model to include in an eDNA study, researchers should select based on the model’s appropriateness for the specific system and the research question, rather than a researcher’s familiarity with the model (Addor & Melsen, 2019). The inclusion of a hydrological model in an eDNA study moreover requires an understanding of the hydrological processes that govern the study site, the spatial dimensions of interest, and how to utilize existing hydrological data, as the processes that govern a study site guide the choice of an appropriate hydrological model (Horton et al., 2022; see Table 1 for general modeling considerations).

For instance, static hydrological modeling inputs (e.g., catchment characteristics, base river networks, and dendritic distance) help inform the background and covariates of biological samples taken in a riverine system (Carraro et al., 2015; Carraro & Altermatt, 2020; Read et al., 2015; URycki et al., 2020). This suggests that the inclusion of dynamic, stochastic hydrological models may also increase the understanding of biological samples. Areas where snow and glaciers contribute significantly to the hydrological regime require a model that incorporates the cryospheric

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Domain-specific eDNA and hydrology methods summarized to encourage synergy and promote interdisciplinary cooperation.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>eDNA methods</strong></td>
<td><strong>Hydrology methods</strong></td>
</tr>
<tr>
<td><strong>Planning</strong></td>
<td>(1) <strong>Sampling methods</strong>: gauges, handheld sensors, loggers, remote sensing.</td>
</tr>
<tr>
<td>(1) <strong>Select methodology</strong>: quantifying method, directed to only one target species (qPCR or ddPCR) or a non-quantifying but directed to a specific community method (metabarcoding).</td>
<td>(2) <strong>Target metrics</strong>: Hydrological processes (e.g., discharge, transit time distributions, groundwater contribution), chemical characteristics, catchment/reach characteristics.</td>
</tr>
<tr>
<td>(2) <strong>Select target species</strong> (invasive species, rare species, endangered species, etc.) or <strong>target community</strong> (bacteria, fungi, diatoms, macroinvertebrates, etc.) depending on the approach.</td>
<td>(3) <strong>Measurement locations</strong>: Locations representative of the reach, the catchment, and the hydrological gradient. More sites increase the amount of information.</td>
</tr>
<tr>
<td>(3) <strong>Select number, replicates and distribution of sampling sites</strong>: Changing between lentic, lotic and marine studies.</td>
<td>(4) <strong>Measurement times</strong>: Diurnal variation, seasonal changes, precipitation events, repeated sampling events.</td>
</tr>
<tr>
<td><strong>Field</strong></td>
<td>(5) <strong>Sampling</strong>: equipment such as bottles for spot sampling, pumps, filtration methods, auto samplers, potentiometers.</td>
</tr>
<tr>
<td>(4) <strong>Sampling</strong>: depends on capture methods. The following exist: membrane filtration, biofilm, passive sampling on adsorbent material, sediments.</td>
<td>(6) <strong>In situ variables</strong>: measurements such as temperature, surface tension, color, and turbidity.</td>
</tr>
<tr>
<td>(5) <strong>Preservation</strong>: ethanol, RNA later or other preservatives are the most commonly used.</td>
<td>(7) <strong>Storage</strong>: Stabilizers, temperature control, and protection from outside elements.</td>
</tr>
<tr>
<td><strong>Lab</strong></td>
<td>(8) <strong>Water analysis</strong>: Water and sediment samples can be analyzed to measure variables such as electrical conductivity, major ion concentrations pH, water isotopes, dissolved gases, nutrients, or suspended solids.</td>
</tr>
<tr>
<td>(6) <strong>Extraction methods</strong>: commercial Inhibitor-free DNA extraction kits are broadly used.</td>
<td></td>
</tr>
<tr>
<td>(7) <strong>Libraries</strong>: the use of library preparation strategy and sample labeling depends on the precision needed for analysis and economic considerations.</td>
<td></td>
</tr>
<tr>
<td>(8) <strong>Sequencing technology</strong>: Nanoball sequencing technologies or by synthesis methods.</td>
<td></td>
</tr>
<tr>
<td><strong>Data analysis</strong></td>
<td>(9) <strong>Remote sensing</strong>: characterize catchment and reach characteristics using surface temperature, soil moisture, water quality as well as estimate hydrological fluxes, evapotranspiration, and runoff.</td>
</tr>
<tr>
<td>(9) <strong>Bioinformatic pipelines</strong>: Analysis pipelines have different strengths and limitations that may affect results, making standardization a priority to achieve reproducibility.</td>
<td>(10) <strong>Modeling</strong>: Hydrological models can be used to estimate the flow path, transit times, and water quality across temporal and spatial scales where traditional sampling may not be possible.</td>
</tr>
<tr>
<td>(10) <strong>Downstream analysis</strong>: exists some graphical user interfaces based on structure biological indexes or community composition, and exists an opportunity to adapt traditional water quality index to molecular water quality.</td>
<td></td>
</tr>
</tbody>
</table>

*For more information on eDNA method(s): (1) see McColl-Gausden et al., 2023; (2–6) see Bruce et al., 2021; (7) see Zizka et al., 2019 and Bohmann et al., 2022; (8) see Anslan et al., 2021; (9 and10) see Hakimzadeh et al., 2023.

*For more information on hydrology method(s): (1–8) see Madrid & Zayas, 2007; (9) see Carraro et al., 2020, Read et al., 2015, and URycki et al., 2020; (10) see Beven & Young, 2013 and King et al., 2022.
processes either into its calculations or at least as boundary conditions (Barnett et al., 2005; Schilling, Park, et al., 2019). Work that involves hydrodynamic/sediment transport and production requires a model that describes the type of sediment movement and considers its spatial dimensionality; for example, suspended loads in three dimensions vs. chemical transport in one dimension (Papanicolaou et al., 2008). In a coupled ecological-hydrological system, an ecohydrological model can be used to model the feedback between biology and hydrological processes (Schilling et al., 2014; Schilling, Park, et al., 2021; Tague et al., 2019). Research in areas where hydraulic infrastructure (e.g., dams, reservoirs, and diversions) contributes to water flow requires the usage of anthropogenic hydrological models (Schaeffli et al., 2019).

The spatial and temporal scale of a study site or region should also be considered when selecting a hydrological model. Models range from the mesoscale to the micro-scale and inform a wide range of system behaviors, from long-term hydrological trends to instantaneous hydrodynamic processes (Felder et al., 2018). Climate change impact and land-use change studies generally use simplified or process-based models such as “WaSIM-ETH” to model hydrological processes for basins of sizes <1 km² to up to more than 100,000 km² (Bormann & Elfert, 2010; Middelkoop et al., 2001). Water flow models that simulate the soil–plant-atmosphere-interface, such as “a Root,” can operate at scales of <30 cm³ (Schneider et al., 2010). Sediment transport models vary in their spatial resolution from 2 to 5 m grid cells in the “UnTRIM Bay-Delta” to 2–6 km grid cells in the “Delft3D-FLOW” model (Bever & MacWilliams, 2013; Hu et al., 2009). For systems where stream–aquifer interactions play a crucial role, fully integrated surface-subsurface flow and transport models such as “HydroGeoSphere” or “HYDRUS” are the tools of choice, and owing to their high versatility, they can be used to model flow and transport from the soil column to the catchment scale (Delollitter et al., 2022; Paniconi & Putti, 2015). A multi-model approach utilizing a suite of models with differing spatial resolutions would maximize the hydrological information that could be used alongside eDNA research, but even the inclusion of a single hydrological model may help inform biological data.

Simulation, forecasting, hindcasting, projection, and prediction are all terms used to describe a model’s use case (Beven & Young, 2013). A simulation model uses base inputs shaped by some characteristic equations without the use of past simulation outputs to reproduce the behavior of a system. Forecasting models are like simulation models, but they consider the outputs of a system up to the point from which the behavior of a system is to be predicted. Hindcasting models consider past inputs and outputs of a system and aim to reproduce a system’s past behavior during periods for which no data is available. Projection models are simulations of a system’s future, using predicted future input data such as the ones resulting from climate change scenarios. Prediction is an ambiguous modeling term in hydrology that can describe a use-case of all the previous models. Data limitations and research objectives need to be fully understood before choosing a model.

Throughout hydrological modeling, epistemic uncertainty (model error as a result from wrong assumption made about a process) can be found in measurement errors, incorrect model selection, the misrepresentation of spatial and temporal data, inappropriate boundary conditions, misidentification of statistical assumptions, and treating dynamic processes as static (Beven & Young, 2013; Efstratiadis & Koutsoyiannis, 2010; King et al., 2022). Due to the large heterogeneity of environmental parameters and processes and our inability to fully characterize that heterogeneity, and considering the many potential sources of epistemic uncertainty, all hydrological models should be calibrated against relevant hydrological measurements in order to reduce their uncertainty. At the very least, surface hydrological models should be calibrated against available streamflow measurements, and subsurface hydrological models against groundwater level measurements. However, generally, the more diverse (and for the study relevant) data are used to calibrate a flow model, the better such a model’s outcomes will be (Pool et al., 2017; Schilling, Cook, & Brunner, 2019). Most importantly, when using hydrological model simulations or predictions to guide the analysis and interpretation of eDNA, one should always quantify and consider the uncertainty of the different model outputs in order to avoid potentially biased or misleading interpretations.

3.1.3 | Hydrological data sources

As streamflow is the overarching focus of hydrologic inquiry, and because it integrates a range of complex climatic and geomorphic variables and their interactions, the amount of streamflow at a point (the “outlet,” in volume per time, i.e., m³/s) is one of the most widely available hydrologic observations. Typically, streamflow is monitored by national and regional authorities and data are also provided directly by the responsible governmental agency. In addition streamflow can be measured indirectly in the field via an indirect methods such as the tracer dilution method (Day &
Day, 1977), and continuously via measuring the stage (water height) in a location with a fixed cross-sectional area (weir) which is then converted to volume via frequent measures of velocity or volume indirectly (i.e., tracer-dilution method). Uncertainty exists with all measurements (Levin et al., 2023). Daily, monthly and annual mean streamflow; frequency, duration, magnitude, and timing of extreme high and low flows (Richter et al., 1996); baseflow index; and streamflow variability over different timescales (Poff & Ward, 1989) are some of the statistics that describe the flow regime of a catchment and that are also often provided alongside discharge time-series data. If not provided, relative contributions of surface runoff and baseflow, the latter generally considered to originate from groundwater, can also be estimated from discharge time series using either tracer-based or non-tracer-based baseflow separation methods (Arnold & Allen, 1999; Chapman, 1999; Eckhardt, 2005, 2008; Xie et al., 2020). A large number of web-based tools are available to obtain streamflow and catchment-based data, and conduct simple or sometimes even more complex analyses. For example, the StreamStats map-based web application, produced and hosted by the United States Geological Survey (USGS), outputs a suite of catchment characteristics and streamflow summary statistics for any user-defined point within a stream in the United States, based on a series of geospatial datasets and decades of stream gage records (Ries et al., 2008; US Geological Survey, 2016). Analogously, such tools also exist for European countries, for example the HydroMaps web application by the Hydrological Atlas of Switzerland (HADES, 2023). Examining even just a few key streamflow characteristics such as flow variability and flood characteristics, in addition to the discharge time series themselves, can provide useful insight into the hydrological functioning of a system and therefore support eDNA sampling, analysis, and interpretation.

### 3.2 Biological considerations for hydrologists

#### 3.2.1 Preliminary steps

In order to use eDNA observation as a hydrological tool, researchers should first consider which analysis method they need to use. In this sense, one can use a quantifying method, directed to only one target species, such as quantitative polymerase chain reaction (qPCR) or droplet-digital PCR (ddPCR) or a non-quantifying method directed to a specific community (metabarcoding; see Table 1 for eDNA general considerations; The mechanistic explanations for the non-quantifying nature of metabarcoding are elaborated on in Section 3.2.3). Researchers may choose to target single species. For instance, a microbe specific to human feces can help to trace sources of fecal contamination (Shanks & Korajkic, 2020), or the presence of invasive species can be detected (Ardura et al., 2015; Rishan et al., 2023; Thomas et al., 2020). The analysis output will provide concentration data (e.g., copies of target DNA fragment/liter) similar to most conventional tracers used in hydrology studies. Changes in community composition may also be able to indicate hydrological changes. In such cases, a change in the concentration of eDNA of one species may not have a strong observable effect, but changes in multiple species’ presence or absence (i.e., community composition) may indicate environmental changes. For instance, rainfall events caused an increase in the mammal and bird eDNA after extreme rainfall events (Staley et al., 2018). Community-level analyses are usually achieved through metabarcoding, which involves: (1) amplification of a barcode region of DNA, (2) sequencing the produced amplicons via high-throughput sequencing technologies, and then (3) taxonomically assigning the amplicons with the help of reference databases (Creer et al., 2016; Deiner et al., 2017). Environmental DNA analysis methods are highly sensitive to contamination during sampling and analysis. Thus, best practices dictate use of negative controls (i.e., DNA free water) to be used at the sampling, extraction, and PCR steps to detect potential contamination (Bruce et al., 2021).

#### 3.2.2 Sampling and preservation

Due to the heterogeneous distribution of eDNA, successful target detection hinges on precise sampling in terms of location and timing, as with conventional ecological survey methods. Researchers should determine the optimal sample quantity to characterize their study system, and it is recommended to collect replicate samples to assess within-site variability (Altermatt et al., 2023). Most eDNA capture methods use active sampling and membrane filtration (Takahashi et al., 2023). The filter pore size should be determined based on the expected size of the target eDNA material and the desired water volume. For instance, most microbial eDNA studies used 0.45 μm pore size, although the pore sizes used range from 0.2 to 45 μm (Takahashi et al., 2023). Filter pore size also impacts the volume of water that can be filtered,
as small-pore filters clog more readily with eDNA as well as other debris in the water column. Alternatively, passive sampling relies on adsorbent material suspended in the water column to capture eDNA from the environment over a longer period (Bessey et al., 2021; Kirtane et al., 2020). This approach reduces the need to be at the “right place at the right time” as the eDNA is captured through time. Currently, available materials saturate rapidly, reducing their potential as passive samplers, although the yield and diversity of passively captured eDNA have been reported to be on par with active sampling methods (Bessey et al., 2021; Chen et al., 2022; Kirtane et al., 2020).

Preservation of collected samples is a key step in the use of eDNA-based methods and is aimed at protecting DNA from degradation, and microbes from growing and producing new DNA that was not initially in the sample (Dully et al., 2021; Guerrieri et al., 2021; Sales et al., 2019). Methods for sample preservation vary widely, but using ethanol, freezing directly, and using preservatives (e.g., RNAlater, Longmire’s solution; see Table 1 for general considerations of eDNA preservation methods) are the most common (Box 2).

### 3.2.3 DNA extraction and molecular analysis

Once collected, DNA from the samples needs to be extracted and isolated from the other cell material and debris present in the filter. Typically, this process includes cell lysis with proteinases and surfactants, followed by a purification step to separate the DNA from other cellular components. Various methods of DNA extraction have been developed and are available as commercially marketed DNA extraction kits. Most eDNA studies have utilized the DNeasy Blood and Tissue kit (QIAGEN, Hilden, Germany) and the PowerWater DNA isolation kit (QIAGEN, Hilden, Germany) (Bruce et al., 2021; Takahashi et al., 2023; Wang et al., 2021). These methods focus on extracting membrane-bound DNA, while alternative approaches can be employed for dissolved or adsorbed DNA states (Kirtane et al., 2023).

Extracted DNA then undergoes PCR, whereby primers are used to target and amplify DNA using a thermal cycler. The choice of appropriate primers is a critical aspect of this step as primers determine the sensitivity and specificity for amplifying the target taxa. For species-specific analyses, such as qPCR or ddPCR, highly specific primers and Taqman probes are essential to ensure that only the intended species’ DNA is amplified during the PCR. Conversely, PCR for metabarcoding aims at broader taxonomic range detection but still maintains specificity within a given phylogenetic clade. For example, primers targeting cytochrome oxidase-1 (COI) are preferred for metazoans, ribulose bisphosphate carboxylase (rbcL) and Internal Transcribed Spacer (ITS) for plants, and 16S ribosomal RNA for bacteria (Deiner et al., 2017; Hering et al., 2018; Ruppert et al., 2019). However, the amplification stage can be confounded by artifacts

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**BOX 2 Participatory research and eDNA**

Involving the public in environmental water research, including but not limited to monitoring and sampling campaigns, can benefit researchers by increasing the quantity of data collected, as well as stakeholder involvement and outreach, which is often necessary for funding. From the other point of view, in a best case scenario, citizen science can empower communities to hold institutions and organizations responsible for their impacts and management of the water resource and can even lead to information co-creation, where scientific understanding is advanced by the perspective of stakeholders and citizens (Aronoff et al., 2021). However, citizen science can sometimes be considered exploitative, as it overwhelmingly is motivated by increasing data collection without increasing costs, while keeping citizens in the non-knowledge generating role of data collectors rather than information co-creators (Haklay et al., 2018). This is often justified because the experience of contributing to research is considered to be reward enough without subsequent sharing of data analysis, interpretation, and scientific insights. These issues have led to the idea that collaborative, participatory research will provide better results. In the realm of eDNA observation, concerns about sample integrity and the expenses associated with DNA analysis require a collaborative model more obviously than lower-risk tools. In the case of eDNA, samples can be easily contaminated or degraded, and extraction kits and DNA sequencing are expensive, so full collaboration is necessary to ensure success, for example via a defined protocol (Walker et al., 2021). Further examples of successful participatory research leveraging eDNA can be found in biodiversity monitoring (e.g., Altermatt et al., 2023; Larsen, 2024).
from potential PCR bias and intramolecular recombination during reactions, making quantitative assessments difficult and possibly even resulting in identification of different sets of organisms than those actually in the sample (Lamb et al., 2019; Nagai et al., 2022). In meta-analyses, the selected polymerase was shown to have a crucial effect on the resulting molecules obtained for sequencing, particularly chimeric forms (Lamb et al., 2019; Nagai et al., 2022). Nonetheless, modeling approaches may help determine the true community structure for more reliable and quantitative results (Shelton et al., 2023). In metabarcoding, primers may also require additional sequences to add unique individual identification-tags (4–10 base pairs long) and sequencing adaptors depending on library preparation strategies (Bohmann et al., 2022; Bruce et al., 2021). Following PCR, the amplicons are then combined to create a library, which is subsequently purified and sequenced using Next Generation Sequencing (NGS) techniques (Anslan et al., 2021; Bruce et al., 2021).

3.2.4 | Bioinformatic analysis and pipelines

Raw sequencing data needs to be processed to ensure quality, minimize sequencing errors, and finally, to be taxonomically annotated, which is generally achieved through several steps in an analytical pipeline (e.g., Hakimzadeh et al., 2023). In general, pipelines result in a count of either Exact/Amplicon Sequence Variants (ESVs/ASVs) or Operational Taxonomic Units (OTUs, i.e., highly similar sequence clusters) present per sample. First, raw sequence reads are demultiplexed as, due to high sequencing capacity, samples are usually pooled prior to sequencing. Every sample gets an individual identification-tag during PCR processing. This tag is used to demultiplex the samples. Then forward and reverse reads are merged into single sequences and primer sequences are trimmed. In between those steps (position varies between the different pipelines), several quality filtering methods are used to obtain high quality data, for example, the removal of chimeras and NUMTs (nuclear mitochondrial pseudogenes), denoising (to lower sequencing bias, especially on ESV level) or post-clustering curation (e.g., lulu filtering; Frosliev et al., 2017) on OTU level. Finally, ESVs or OTUs can be assigned taxonomically (Wangensteen & Turon, 2017). Choosing the appropriate pipeline for a given dataset is challenging due to the wide variety of software, algorithms, and operating systems and the variation among them (Prodan et al., 2020). However, bioinformaticians have developed pipelines for nearly every need (Hakimzadeh et al., 2023), in form of newly designed algorithms or precompiled pipelines based on open source tools (e.g., Anacapa; Curd et al., 2019; SLIM, Dufresne et al., 2019; PEMA, Zafeiropoulos et al., 2020; or eDNAFlow, Mousavi-Derazmahalleh et al., 2021, and many more). Precompiled pipelines are generally adapted to different operating systems and employ ready-to-use interfaces, thus requiring less bioinformatics experience. Choosing an appropriate pipeline is important, especially with challenging approaches, like detection of rare taxa, and biological validation should be considered (Hakimzadeh et al., 2023).

The completeness and accuracy of reference databases are a fundamental part of the analysis of metabarcoding data. Taxonomic assignment tools (e.g., BLAST, Altschul et al., 1990) assign sequences to a taxonomic group, often as specific as species. It is therefore important that reference databases are properly curated (Blackman et al., 2023), for example, that sequences are accompanied by geographical information, such as location, to increase the reliability of taxonomic assignment. Furthermore, species should be represented by multiple sequences and sequences should be phylogenetically placed (Blackman et al., 2023). If no information about the target organisms exists, taxonomic assignment might fail and result in a list of unassigned sequences (Arranz et al., 2020; deWaard et al., 2019; Ekrem et al., 2007). One of the first databases developed and available to the public was GenBank, which is still one of the most widely used databases, currently holding over 2.9 billion nucleotide sequences (Burks et al., 1985; Sayers et al., 2023), and is the data repository for most eDNA studies. However, the submission of reference sequences to GenBank is not verified for accuracy in their taxonomic annotation, leading to the incorporation of misannotated sequences, resulting in the erroneous annotation of environmental sequences in studies using uncurated databases like GenBank (Claver et al., 2023). This issue has led to an increase in custom reference database curation, mainly focusing on either organisms group or genetic regions (Table 2).

3.2.5 | Downstream analysis

After metabarcoding or qPCR analysis, it is necessary to identify the proper downstream analysis for the data set. First, data quality needs to be checked with respect to the study design. For qPCR, certain quality control requirements need
to be fulfilled to validate the results. This includes a qPCR amplification efficiency of 100% ± 10% and reporting of the Limit Of Detection (LOD) and Limit of Quantification (LOQ; Klymus et al., 2020) that aid in the interpretation of qPCR data. Between the LOD and LOQ, it is just possible to detect the presence or absence of a species, but variation in the assay measurement is too high to quantify the amount of target fragments. Quantification is just possible above the LOQ. For metabarcoding data, read-based rarefaction curves are a good proxy, if sequencing depth was enough to capture all species (e.g., Macher, Beermann, & Leese, 2021). The following statistical analysis depends on the research question. In general, some open-source graphical user interfaces are adapted to metabarcoding results to help with downstream analysis (Macher, Beermann, & Leese, 2021). Community structure, biological indices of alpha diversity (e.g., richness), or community composition (e.g., Jaccard presence-absence) can be determined using these interfaces (Hakimzadeh et al., 2023). In some cases, it is useful to use specific indexes for different organisms (Pawlowski et al., 2018). The adaptation of traditional water quality indices to molecular water quality is broadly extended to analyze the community in applied research and can give insights to flow pathways and hydrological processes by comparing these indices from different locations. Some examples of these biological indices are microgAMBI in bacteria (Aylagas et al., 2021), various indices in lake zooplankton (Yang & Zhang, 2020), the Specific Pollution Sensitivity Index (IPS) in freshwater diatoms (Rivera et al., 2018), and Danish Stream Fauna Index (DSFI) for river macroinvertebrates (Kuntke et al., 2020).

<table>
<thead>
<tr>
<th>Database</th>
<th>Targeted region</th>
<th>Organism</th>
<th>Last update</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greengenes</td>
<td>16S rRNA</td>
<td>Bacteria and Archaea</td>
<td>2017</td>
<td>DeSantis et al., 2006</td>
</tr>
<tr>
<td>Greengenes2</td>
<td>16S rRNA</td>
<td>Bacteria and Archaea</td>
<td>2024</td>
<td>McDonald et al., 2024</td>
</tr>
<tr>
<td>EzBioCloud</td>
<td>16S rRNA</td>
<td>Bacteria and Archaea</td>
<td>2023</td>
<td>Yoon et al., 2017</td>
</tr>
<tr>
<td>SILVA</td>
<td>16S/23S rRNA</td>
<td>Bacteria and Archaea</td>
<td>2023</td>
<td>Quast et al., 2012</td>
</tr>
<tr>
<td></td>
<td>18S/28S rRNA</td>
<td>Bacteria and Archaea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR2</td>
<td>18S rRNA</td>
<td>Protists</td>
<td>2023</td>
<td>Guillou et al., 2012</td>
</tr>
<tr>
<td>UNITE</td>
<td>ITS</td>
<td>Fungi</td>
<td>2023</td>
<td>Kõljalg et al., 2005</td>
</tr>
<tr>
<td>MaarjAM</td>
<td>Small subunit (SSU) rRNA</td>
<td>Fungi (arbuscular mycorrhizal)</td>
<td>2023</td>
<td>Õpik et al., 2010</td>
</tr>
<tr>
<td>PhytoREF</td>
<td>Plastidial 16S rRNA</td>
<td>Photosynthetic eukaryotes</td>
<td>2023</td>
<td>Decelle et al., 2015</td>
</tr>
<tr>
<td>PLANiTS</td>
<td>ITS</td>
<td>Plants</td>
<td>2023</td>
<td>Banchi et al., 2020</td>
</tr>
<tr>
<td>Diat.barcode</td>
<td>rbcL</td>
<td>Diatoms</td>
<td>2022</td>
<td>Rimet et al., 2019</td>
</tr>
<tr>
<td>MIDORI2 (Genbank based)</td>
<td>Protein-coding (13) and rRNA (2) mitochondrial genes</td>
<td>Eukaryotes, including animals and plants</td>
<td>2023</td>
<td>Leray et al., 2022</td>
</tr>
<tr>
<td>BOLD</td>
<td>ITS</td>
<td>Fungi</td>
<td>2023</td>
<td>Ratnasingham &amp; Hebert, 2007</td>
</tr>
<tr>
<td></td>
<td>rbcL and matK</td>
<td>Plants</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>COI</td>
<td>Animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MetaZooGene Atlas and Database, or MZGdb (Genbank based)</td>
<td>COI, 12S, 16S, 18S, and 28S</td>
<td>Zooplankton, Benthic invertebrates, Fish and marine mammals, Phytoplankton</td>
<td>2023</td>
<td>Bucklin et al., 2021</td>
</tr>
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</table>
Environmental DNA has the potential to serve as a new source of information on both hydrological and biological questions. Documented patterns in eDNA are connected with the hydrological cycle at a variety of spatial and temporal scales, with multiple biotic and abiotic processes mediating these connections. Despite still being in its infancy, research at the intersection of these fields has shown promising results, and is expected to expand substantially in the near future along with the ever-growing availability of eDNA data, especially considering that eDNA is becoming a standard method for assessing aquatic biodiversity and the ecological status of water bodies (Pawlowski et al., 2021). For instance, differences in microbiome composition have been shown to reflect macroscale climatic and geomorphic differences in headwater streams (Figure 4; URycki et al., 2020). Accordingly, much hydrological information is encoded within the eDNA found in rivers and streams (Figure 5; URycki et al., 2022), though work remains to identify specific mechanisms and ecological processes underway. However, even given these limitations, efforts have been successful at predicting hydrological function with aquatic gene fragments (Good et al., 2018) and predicting biodiversity based on the flow of eDNA through hydrological networks (Figure 3; Carraro et al., 2023). Hydrology is concerned with the sources and pathways associated with water movement, and eDNA thus provides an additional approach for partitioning flows (Figure 6; Mächler et al., 2021), as well as patterns of groundwater circulation and water uptake by vegetation (Pollitt et al., 2022; Schilling et al., 2023).

Understanding the behavior of eDNA in the environment is an emerging science (Figure 1), and therefore, eDNA data should be interpreted with caution. An aspect requiring particular attention is the fact that eDNA is not a conservative tracer: DNA molecules shed by macro-organisms are subject to environment-mediated degradation, and a similar consideration applies to microbial eDNA, as microbial communities are living and therefore dynamic, often responding to environmental factors on very short timescales and potentially complicating their use as a quantitative tracer. However, the sensitivity of microbial communities to environmental conditions can be an advantage for some investigations, including those of certain hydrological processes. For example, this sensitivity could be used to observe a change in time, such as a river’s response to a precipitation event, or in space, such a tributary inflow point into a stream (Figure 6). Furthermore, microbial community profiles can give insights into the origin of water; for example, some environments, such as groundwater and deep soil waters, are better suited for highly specific organisms. Thus ecological knowledge of microbial community constituents could indicate the origin of water masses (Schilling et al., 2023). Although subject to degradation, eDNA has been shown to be transported over large distances in fluvial networks, thereby providing information on diversity and/or species distribution from across entire catchments (Deiner et al., 2016), while simultaneously indicating the flow paths of water through the same catchment. Because eDNA data can support investigations in biology as well as in hydrology, both fields will benefit from interdisciplinary collaborations. Development of an open access database of hydrology and eDNA research is one opportunity for a transdisciplinary effort. Tools like GAPeDNA (https://shiny.cefe.cnrs.fr/GAPeDNA/; Marques et al., 2021) collect eDNA data from existing nucleotide databases. More recently, eDNAexplorer (https://www.ednaexplorer.org/) one of the first eDNA project-based databases, was released. Cross-cutting datasets, like the National Ecological Observation Network (NEON; https://www.neonscience.org/), WHONDRS (https://www.pnnl.gov/projects/WHONDRS), or GROW (https://

![Figure 6](https://www.neonscience.org/)

**Figure 6** Example of how the quantity of discharge at a catchment outlet could be decomposed into water origin based on sampled eDNA. In this case the contribution of upstream sources was calculated using the overlap in ZOTUs (i.e., zero-radius operational taxonomic units) that were shared between the outlet and each of the upstream sources. Contribution of volume of discharge from springs is shown in blue, from tributaries in green, and from unknown sources in red. The annual hydrograph is shown for 2017; however, the volume outside of the sampling period is not computed and is shown in gray. Vertical lines indicate days when eDNA was sampled. Source: Adapted with permission from Mächler et al. (2021).
growobservatory.org/) offer eDNA datasets or information that includes or can be combined with hydrologic observations. However, challenges remain to ensure that eDNA-derived data fully align with the FAIR (Findable, Accessible, Interoperable, and Reusable) data principles (Berry et al., 2021; Takahashi & Berry, 2023). The rapidly increasing amount and availability of data and tools are quickly opening opportunities that expand this interface between hydrological and biological research. This quickly emerging interface places eDNA as the key bridge across disciplines, demanding effective interdisciplinary collaboration, and driving process understanding of the complexity of both our physical and biological environment.

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Dawn R. URYcKı: Conceptualization (equal); writing – original draft (lead). Anish A. Kirtane: Conceptualization (equal); writing – original draft (lead). Rachel Aronoff: Conceptualization (equal); writing – original draft (equal). Colton C. Avila: Conceptualization (equal); writing – original draft (equal). Rosetta C. Blackman: Conceptualization (equal); writing – original draft (equal). Luca Carraro: Conceptualization (equal); visualization (equal); writing – original draft (equal). Olivier Evrard: Conceptualization (equal); writing – original draft (equal). Stephen P. Good: Conceptualization (equal); visualization (equal); writing – original draft (equal). Diana C. Hoyos: Conceptualization (equal); writing – original draft (equal). Nieves López-Rodríguez: Conceptualization (equal); writing – original draft (equal). Demetrio Mora: Writing – original draft (equal). Yvonne Schadewell: Conceptualization (equal); writing – original draft (equal). Oliver S. Schilling: Conceptualization (equal); writing – original draft (equal). Natalie C. Ceperley: Conceptualization (lead); funding acquisition (lead); project administration (lead); writing – original draft (lead).

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The authors declare no conflicts of interest.

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Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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