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Predicting downstream transport distance of fish eDNA in lotic environments

# Running title

Predicting eDNA downstream transport distance

Author Didier PONT

**Institute of Hydrobiology and Aquatic Ecosystem Management (IHG)**

University of Natural Resources and Life Sciences

Gregor-Mendel-Straße 33/DG

1180 Vienna, Austria

Didier PONT: ORCID 0000-0001-5187-0135

Correspondence Didier Pont,

e-mail : didier.pont@boku.ac.at

# Abstract

Environmental DNA is an effective tool for describing fish biodiversity in lotic environments, but the downstream transport of eDNA released by organisms makes it difficult to interpret species detection at the local scale. In addition to biophysical degradation and exchanges at the water-sediment interface, hydrological conditions control the transport distance. We have developed an eDNA transport model that considers downstream retention and degradation processes in combination with hydraulic conditions and assumes that the sedimentation rate of very fine particles is a correct estimate of the eDNA deposition rate. Based on meta-analyses of available studies, we successively modelled the particle size distribution of fish eDNA (PSD), the relationship between the sedimentation rate and the size of very fine particles in suspension, and the influence of temperature on the degradation rate of fish eDNA. After combining the results in a mechanistic-based model, we correctly simulated the eDNA uptake distances observed in a compilation of previous experimental studies. eDNA degradation is negligible at low flow and temperature but has a comparable influence to background transfer when hydraulic conditions allow a long uptake distance. The wide prediction intervals associated with the simulations reflect the complexity of the processes acting on eDNA after shedding. This model can be useful for estimating eDNA detection distance downstream from a source point and discussing the possibility of false positive detection in eDNA samples, as shown in an example.

# Key-words

Environmental DNA, downstream transport, degradation rate, depositional velocity, particle size distribution, lotic environment

# 1 | Introduction

Following methodological advances in the 2010s, the use of environmental DNA (eDNA) to study the biodiversity of macroorganisms has become increasingly common, as evidenced by a spectacular increase in the number of publications in recent years (Takahashi et al., 2023). eDNA is now recognized as a powerful tool for biomonitoring fish (Yao et al., 2022). Compared with conventional fish sampling techniques, taxon-specific approaches and metabarcoding are generally more effective in detecting rare or evasive species and estimating the diversity of fish communities (McElroy et al., 2020; Wang et al., 2021). The concentration of eDNA copies in a sample is a significant indicator of the absolute abundance of a single target species (Rourke et al., 2021; Wilcox et al., 2016; Yates, Fraser, & Derry, 2019). Similarly, metabarcoding can describe the structure of an entire fish community in terms of relative abundance (Boivin-Delisle et al., 2021; Di Muri et al., 2020) or even provide an approximation of absolute abundance when the number of reads is normalized by eDNA copies concentration in the sample (Pont et al., 2023). However, the fate and transport of eDNA in aquatic environments are complex, modifying the relationship between species abundance and eDNA concentration (Rourke et al., 2021). The potential presence of false positives at one site may be due to downstream transport of eDNA (Wang et al., 2021). In addition to shedding variability (Thalinger et al., 2021), several processes control eDNA concentration in the water column: biophysical degradation and exchange at the water-sediment interface and transport (Harrison, Sunday, & Rogers, 2019; Shogren et al., 2017).

eDNA released by living macroorganisms comprise fragments of diverse lengths and nature (free DNA, cells, and tissue fragments) that tend to be degraded into smaller fragments at a rate that depends on the environmental conditions, the concentration of extracellular bacteria/nucleases, or the type of target gene (Bylemans, Furlan, Gleeson, Hardy & Duncan, 2018; Jo & Minamoto, 2021; Jo et al., 2017; Pilliod, Goldberg, Arkle, & Waits, 2014; Shogren et al., 2018). Additionally, eDNA adsorbs onto fine suspended particles and biofilms, protecting them from degradation (Barnes et al., 2014; Harrison et al., 2019; Shogren et al., 2018; Mauvisseau et al., 2022). Additionally, eDNA particles are subject to gravitational sedimentation and are transferred to the water-sediment interface (Turner, Uy, & Everhart, 2015).

In lotic environments, eDNA is transported downstream over a highly variable distance ranging from a few hundred meters to several tens of kilometers (Deiner & Altermatt, 2014; Jane et al., 2015; Pont et al., 2018) and the numerous studies conducted in experimental and natural streams have highlighted the major role of hydraulic conditions (Jo & Yamanaka, 2022; Pont et al., 2018; Van Driessche, Everts, Neyrinck, & Brys, 2023). The decline in eDNA concentration downstream of its source point is described as a first-order exponential decay (Shogren et al., 2017; Wilcox et al., 2016). Nevertheless, highly turbulent conditions at the water-sediment interface and/or sediment resuspension due to hyporheic flow can strongly alter the mass transfer coefficient from the water column to the bottom (deposition velocity) and compromise the effectiveness of such a simple model (Shogren et al., 2018; Tillotson et al., 2018).

Several models combine simple constant exponential decay with advection processes to simulate eDNA transport along a river (Nukazawa, Hamasuna, & Suzuki, 2018) or river system (Carraro, Hartikainen, Jokela, Bertuzzo, & Rinaldo, 2018). Other models include both the degradation rate and the exchange with the bottom interface (Fremier, Strickler, Parzych, Powers, & Goldberg, 2019; Nukazawa, Hamasuna, & Suzuki, 2018; Shogren et al., 2017; Shogren, Tank, Egan, Bolster, & Riis, 2019) but without computing the associated uncertainties. A hydraulic mechanistic model initially developed for solute transport in streams (Stream Solute Workshop, 1990) and later for very fine particles (VFPM; Cushing, Minshall, & Newbold, 1993; Minshall, Thomas, Newbold, Monaghan, & Cushing, 2000) was applied to evaluate the eDNA transport distance experiments (Jerde et al., 2016; Shogren et al., 2017; Wilcox et al., 2016). The eDNA uptake distance Sp (distance required to retain 63.21% of the particles in the riverbed; Stream Solute Workshop, 1990) provided by this mechanistic approach was significantly correlated with streamflow (Jo & Yamanaka, 2022), and the eDNA deposition velocity values were comparable to those of VFPM (Pont et al., 2018).

Our aim was to develop a model for predicting the eDNA transport distance and the associated uncertainty for users. We hypothesized that i) eDNA transport follows transport dynamics similar to those of very fine particulate matter (VFPM), ii) the settling velocity of VFPM is a correct estimate of eDNA deposition velocity (Minshall et al. 2000, Thomas et al. 2001), and iii) the decomposition process reduces the transport distance when hydraulic conditions allow a long uptake distance (long time for eliminating eDNA from the water column) and/or at high temperatures (Mauvisseau et al., 2022). Additionally, we conducted several meta-analyses of available studies related to these processes, focusing on fish eDNA experiments to avoid variability in eDNA between zoological groups (Zhao et al., 2021). We modelled i) eDNA particle size distribution, ii) the relationship between the settling velocity and VFPM size, and iii) the influence of temperature on eDNA decomposition rate. We developed an eDNA transport model considering downstream retention and degradation processes and computed the prediction interval associated with the simulation. We tested our major hypothesis by comparing our predictions of the eDNA transport distance with experimental observations (Fig.1).

Finally, we simulated the downstream detection distance of fish eDNA along a stretch of river recently monitored by a traditional fishing method and by eDNA to highlight the practical aspects of our model for end users (false positive detection, sampling strategy).

# 2 | Materials and Methods

## 2.1 | eDNA size distribution

A regular search was conducted on the Web of Science for published peer-reviewed literature (January 2008 to 2023) with the key search "TITLE: (environmental DNA) OR TITLE: (eDNA)" but excluding most references related to bacteria. This bibliographic database was completed when additional references on the same subject were found (1931 publications in total). We systematically examined all references to select publications related to fish eDNA particle size distribution (PSD) in aquatic environments. We considered only values obtained in the steady state before eDNA degradation. The metaDigitize package (Pick et al., 2022) was used to extract values from the plot. To standardize the experimental conditions between studies, we excluded the proportion of eDNA not retained on a filter with a pore size of 0.2 μm. When the minimum pore size was 0.4 μm (Wilcox et al., 2015), we added to the smallest pore size class (0.2-1.2 μm) the average frequency of eDNA particle size between 0.2 and 0.4 μm (3.65% of the total amount of eDNA particles) obtained by other authors (Jo, Arimoto, Murakami, Masuda & Minamoto, 2019, Zhao et al.,2021). When samples were pre-filtered to remove eDNA particles >60 µm (Barnes et al., 2021), we added to their largest size class the mean frequency of eDNA particle size >60 µm (5.40% of the total amount of eDNA particles) obtained by other authors (Wilcox et al., 2015; Turner et al., 2 014). We computed the mean cumulative frequency (n = 164) for each pore size condition in each of the 32 experiments conducted in the seven studies (Supplement 1).

We modeled the cumulative distribution of fish eDNA capture *F(x)* as a function of pore size using the complementary cumulative distribution function (CCDF) (Barnes et al., 2021; Turner et al., 2014):

with *F(x)* the expected proportion of eDNA captured by a *x* pore size, and *α* and *β* scale and shape of the CCDF Weibull distribution.

To control for variability in PSD between studies due to the analytical protocol, we used a non-linear mixed-effect model (NLMM), with the study as a random effect. The NLMM provides maximum likelihood estimates of parameters in nonlinear mixed-effect models using the stochastic approximation expectation-maximization (SAEM) algorithm (Comets, Lavenu & Lavielle, 2017; R Core Team, 2022; package saemix, function saemix.model, 10000 simulations). This model was compared to the model with the study as a random parameter and particle size constant using the Akaike information criterion (AIC) (Burnham & Anderson, 2002). The significance of the fixed parameters was tested using the Wald chi-square test (Comets, Lavenu & Lavielle, 2017), the normality of the residuals with the Shapiro test, and the goodness of fit of the selected model by comparing the observed and predicted values at the individual level.

## 2.2 | Settling velocity

Based on a regular Web of Science search for peer-reviewed published literature (January 2023) with the key search "ABSTRACT: (settling velocity) AND ABSTRACT: (size)" and additional articles, we compiled field observations on the relationship between settling velocity *Vs* and the size *Dm* of *in situ* suspended particles in aquatic environment. As eDNA particles were predominantly VFPM (Barnes et al., 2014; Wilcox et al., 2016), we only considered sedimentation velocity for particle sizes < 50 µm (see Supplement 2 for selection criteria). The MetaDigitize software (Pick et al., 2022) was used to extract 437 pairs of values (four studies) from the graphs describing the relationship between *Vs* and *Dm*. Classically (Xia et al., 2004), a power law was used to model the relationship between measured Vs (in mm.s-1) and *Dm* (in µm), with:

We fitted a linear mixed model (LMM) to predict *Vs* with *Dm* after log transformation of both variables. The model included a random effect to control for variability in *Vs* between studies owing to the analytical protocol (R package lmerTest, an extension of package lm4, function lmer, Kuznetsova et al., 2017). This model was compared to the model with the study as a random parameter and particle size constant using the Akaike information criterion (AIC) (Burnham & Anderson, 2002). The goodness of fit of the selected model was appreciated with the marginal and conditional pseudo-R-squared values associated with only the fixed effect and entire model, respectively (Package MuMin, Barton et al., 2022). 95% Confidence Intervals (CIs) and *P* values were computed using a Wald *t*-distribution approximation.

After checking for the normality of residuals (Shapiro test) and their homoscedasticity (scatterplot of residuals against fitted values), we estimated 95% prediction interval from the LMM using parametric bootstrapping method (Package lme4, function bootMer, 10,000 simulations; Bates, Mächler, Bolker, & Walker S., 2015), by simulating data from the fitted model and refitting the model. For the simulations, we assigned each observation (settling velocity) to diverse studies from the random-effect distribution to consider the entire range of possible effects. New estimated parameters corresponding to the quantiles 0.025, 0.25, 0.75, and 0.975 of the simulated distributions were extracted.

We computed the weighted average eDNA settling velocity, and its 95% and 90%, and prediction interval limits ([, ] and [, ] respectively). For each micron size class *i* (from 0.2 to 180 µm), the simulated settling velocity Vsi and its associated prediction intervals was weighted by the simulated eDNA particle frequency *fi*

with and

*Fi*: expected proportion of eDNA captured by pore size i (µm)

## 2.3 | eDNA degradation

To include a temperature-dependent degradation parameter in our fish eDNA transport model, we revaluated a recently published dataset of fish eDNA degradation experiments from 20 studies (Mauvisseau et al., 2022, Supplement S4). We also considered a first-order exponential increase in the rate of degradation (r per hour) with temperature (T in °C) but reshaped these data to obtain not only simulated values of *r* but also their associated prediction intervals.

After computing the average degradation values for each temperature condition in each of the 40 essays, we fitted an LMM with ln(*r*) as the dependent variable, studied as a random factor, and T as a fixed effect. We proceeded in the same manner as for modeling the settling velocity: checking the quality of the model, comparison with a null model (only random effect), and simulations with each observation assigned to the different studies. The lower and upper limits of the 95% and 90% prediction intervals ([r0.025-r0.975], [r0.05-r0.95]) were computed for a temperature range from 4 to 35 °C.

## 2.4 | eDNA transport model

We have assumed that the decrease in eDNA concentrations downstream of a source point is comparable to that of VFPM (Wilcox et al., 2016) and is related to hydraulic conditions and mass transfer to sediments (Stream Solute Workshop, 1990).

The solute concentration at a given distance x from the point of origin is a function of advection, diffusion, and transfer to and from the sediment (transient storage zone). For a 'reasonably' uniform channel, a constant water flow rate, uptake by sediments in the streambed is proportional to the concentration in water, and negligible release of the solute from the sediments relative to the concentration in the water occurs. The eDNA concentration at a distance x will reach a plateau (steady state) with

(Equation 1)

where C0 and Cd are the eDNA quantity at the source point and at a distance *d* (in m) downstream, *u* the mean water velocity (in m.s−1), *k* is an overall uptake rate coefficient, and K = *k/u* the loss rate constant (per m). k is the inverse of the average turnover time of a molecule in the water column.

The inverse of *K* is the uptake length *Sp* (in m).

(Equation 2)

with *h* the mean water depth (in m), w the wetted width (in m), *Q* the discharge (in m3.s-1), and *Vd* the mass transfer coefficient (in m.s-1). *Vd* is the vertical velocity at which a particle migrates through the sediment–water interface (depositional velocity). In contrast to *Sp* and *K, Vd* is expected to be independent of the hydraulic characteristics of the stream and allows for comparisons among streams of diverse sizes.

Our eDNA transport model included the eDNA time degradation rate *r* (per s) in addition to the loss rate constant K. *r* was computed for the temperature that prevailed during the experiment under consideration. The deposition velocity was estimated using the weighted average eDNA settling velocity ( mm.s-1). During the time *t* (in seconds) required for eDNA transport at distance *d*, eDNA was also degraded following a first-order decay scheme. Assuming a weighted average degradation rate *r* (per second), the two processes can be combined as follows:

During the particle transport process, the water velocity *u* is the link between space and time, with *t* = *d*/*u*.

(Equation 3)

And then

(Equation 4)

The lower and upper limits of the 95% prediction interval around the predicted value of *Sp* (Sp0.025 – Sp0.975) were obtained by considering the lower and upper limits of the prediction intervals approximately r (r0.025 and r0.975 respectively) and ( and respectively), assuming the normality of the residual distributions of the two regressions (degradation rate *versus* temperature and settling velocity *versus* particle size) and the independence of the degradation rate and settling velocity.

A similar computation was conducted for the 90% prediction interval limits (-.

## 2.5 | Model validation and Uptake length simulation

We compared the *Sp* values computed from fish eDNA transport studies in streams with the *Sp* values simulated by our model, considering the prevailing environmental conditions in the diverse experiments (river width and depth, flow rate, and temperature). From the same bibliographic database, we examined all publications related to fish eDNA transport experiments and selected those for which (i) individual raw data describing the relationship between eDNA concentration and distance from the eDNA release point were available, either as a dataset or graph, and (ii) at least the mean flow rate was reported. eDNA raw data and downstream distance were extracted from graph using the R library metaDigitise (Pick et al. 2019, R Core Team, 2020), except when numerically available (Wood et al. 2020, Wood et al.2021).

Since river width (in meters), river depth (in meters) and water temperature (in °C) were needed to simulate eDNA transport, they were estimated, where appropriate i) from Google Earth (https://earth.google.com/web/) for river width (Thalinger et al. 2021, Van Driessche et al. 2022), ii) from a power law between river depth (d in meter) and flow (Q in m3.s-1) based on a number of studies of world rivers: (d = 0.27\*Q0.3, Moody and Troutman 2002) for river depth (Jane et al. 2015, Nukazawa et al. 2018, Robinson et al. 2019, Thalinger et al. 2021, Wood et al. 2020), and iii) from the Weather US forecast web site (https://www.weather-us.com/en) for monthly mean air temperature (Jane et al. 2015, Jerde et al. 2016, Shogren et al. 2017).

All observed *Sp* values were recalculated to homogenize the raw data collection procedure (Supplement 3) using equation 2: Of the 47 experiments selected (10 publications), six experiments demonstrating no decrease in eDNA concentration downstream of the origin (negative K value) were discarded. The Sp values predicted using our model were computed using equation (4).

Linear regression of the observed Sp values against the predicted Sp values was conducted after the log transformation of the two variables. The strength of the relationship and normality of the residuals was assessed using Pearson's correlation coefficient and Shapiro's test, respectively. To verify the absence of bias between the observed and predicted values, the slope and y-intercept of the regression line were compared at 1 and 0, respectively. The normality of the residuals (Shapiro test) and homoscedasticity (scatter plot of the residuals against the fitted values) were checked.

We also computed the predicted value of *Sp* without degradation using equation 4, set *r* to zero and compared the slope and intercept of the regression of the observed value against the predicted value of *Sp* without degradation at 1 and 0, respectively.

We simulated Sp as a function of temperature (5 to 25 °C) and a flow gradient (0.01 to 100 m3.s-1) computed using the power-law relationships between river width, depth, and flow (Moody and Troutman 2002). The relative importance of decomposition and deposition rates was estimated by comparing *Sp* values with and without decomposition using the same dataset.

## 2.6 | Detection distance

We simulated the downstream detection distance of fish eDNA from along the Upper section of the Danube which has recently been the subject of monitoring from the source to the mouth (Pont et al. 2023). This study combined a metabarcoding approach with an estimation of the total abundance of eDNA amplified by a universal marker using a qPCR approach to calculate the concentration of specific eDNA per litre. A joint traditional electrofishing survey showed that some species (*Barbatula barbatula, Salmo trutta, Thymallus thymallus*) were only present in the first 300 km downstream from the source (Bammer et al., 2019) while these species were still detected from eDNA water samples in the next 200 km (see Supplement 4 for environmental and eDNA data). We considered that these latter positive detections were only due to downstream eDNA transport from the upstream part of the river and tested whether our model could correctly simulate the specific eDNA concentration measured along this longitudinal gradient. The most downstream electrofishing sample where these three species were caught was considered the source point (262 kilometres downstream from the source of the Danube) for their specific eDNA present downstream along the studied river reach (306 km long).

Average daily flow data Q were obtained from seven gauging stations (www.gkd.bayern.de/de/fluesse/, www.hvz.baden-wuerttemberg.de/) and averaged for the corresponding eDNA sampling period (29/06 to 04/07/2019). Other hydraulic parameters (*w*, *h*) and water temperature (*T*) measured at each eDNA sampling site were available in Pont et al. (2023).

We applied our model and simulated the decrease of specific eDNA concentration every kilometre *j* downstream from the eDNA source point (*j* = 0) after interpolating the hydraulic (*Q, w, h*) and thermal conditions (*T*) at each kilometre from field observations and previous literature. We applied Equations 3 and 4 and considered the dilution due to the increase of the river size at each kilometre.

(Equation 5)

with *Cj* and *Cj-1*the specific eDNA concentration per litre at km *j* and *j-1*, *Qj* and *Qj-1*the discharge at kilometre *j* and *j-1*, and *d* = 1000 m.

The three target species were also detected in a water sample collected on a major tributary (Lech River, 18 km upstream from the confluence with the Danube River) converging with the Danube at 91 km downstream from the source point. Then we partitioned the study river in two parts (before and after the confluence) and injected the additional eDNA for the three species at the first kilometre of the second part. To define the maximal detection distance of eDNA, we estimated the limit of quantification per litre (*LOQL*) by applying the formula (Lance et al. 2017, Wilcox et al., 2016):

with V = 66 the mean water volume (in litre) of the eDNA sample, DNAT = 200 µl the volume of the DNA elution produced by extraction and purification, DNAA = 3 µl the volume of aliquot of template DNA taken form DNAA for each of the 12 PCR, and the limit of quantification LOQ = 500 eDNA copies per litre when performing 12 replicates (Pont et al., 2013).

All analyses were conducted using the R Statistical Software (v4.2.0; R Core Team 2022).

# 3 | Results

## 3.1 | eDNA size distribution

The graphical display of convergence during the fit demonstrated that all parameters were stable after 300 iterations for a total of 500 iterations. The fixed parameters α and β of the CCFD Weibull distribution were correctly estimated with a standard error (SE) of 3.7 and 13.2 % of the value of the estimate, respectively: α = 9.200 (SE = 0.3577), β = 1.050 (SE = 0.1387). The two associated Wald chi-square tests were significant (723.3 and 57.3, respectively; P < 0.001). Pearson's R coefficient between the observed and predicted values at the individual level was 0.944 (*P* < .001) but the residuals were not normally distributed (Shapiro test, W = 0.939, p < 0.001).

The simulated eDNA-PSD demonstrated that 39.5% of eDNA particles had a size <= 5 µm, 28.5% between 5 and 10 µm, 23.7% between 10 and 20 µm, 11.2% between 20 and 50 µm, and less than 0.5% over 50 µm (Fig. 2). The D50 percentile value of the distribution was 7 microns (Fig. 2).

## 3.2 | Settling velocity

The LMM model of settling velocity with studies and eDNA particle size as random and fixed effects, respectively, had a lower AIC than the LMM model with only the study random effect (917.7 and 1063.3%, respectively). The model’s total explanatory power was substantial (conditional R2 = 0.53), and the part related to the fixed effects alone was 42% (marginal R2 = 0.23). The model's intercept Ln(*V0*), corresponding to Ln (*Dm*) = 0, was at -4.30 (95% CI [-4.90, -3.71], t (433) = -14.19, P < 0.001). The effect of Ln (*Dm*) on Ln (*Vs*) was statistically significant and positive (b = 0.42, 95% CI [0.36, 0.49], *t* (433) = 13.21, p < .001). The residuals were normally distributed (Shapiro test, W = 0.996, p = 0.324) and their variance demonstrated no tendency with the fitted values.

For eDNA particle size ranging from 0.2 to 180 µm, the settling velocity varied from 0.007 to 0.122 with 95% prediction intervals of [0.002–0.020] and [0.041–0.359] respectively (Fig. 3), and a value of 0.071 [0.041–0.359] for a particle size of 50 µm.

The weighted average eDNA settling velocity was 0.0314 mm.s-1, with 95% prediction interval limits at [-] = [0.011, 0.093] (Supplement 5).

## 3.3 | eDNA degradation

The LMM model of the eDNA degradation rate with studies and temperature as random and fixed effects, respectively, had a lower AIC than the LMM model with only the studies as random effects (96.149 and 124.951, respectively). The total explanatory power of the model was substantial (conditional R2 = 0.91), and the part related to fixed effects alone (marginal R2) is of 0.16 (Fig. 4). The model's intercept, Ln(*r0*) corresponding to temp = 0, is at -4.36 (95% CI [-5.03, -3.69], t (36) = -13.17, p < .001). The effect of T (0.07) was statistically significant and positive (95% CI [0.05, 0.09], t (36) = 7.00, p < .001). The residuals were normally distributed (Shapiro test, W = 0.996, p = 0.324) and their variance showed no tendency with the fitted values. For temperatures ranging from 5 to 30 °C, the degradation rate varied from 0.018 to 0.10, with 95% prediction intervals of [0.0019-0.169] and [0.011-0.946], respectively (Supplement 5).

## 3.4 | Model validation and simulation

The 41 observed Ln (*Sp)* values varied from 6 to 12,613 m. The transport distance predicted by our model (Fig. 5) significantly correlated with the observed Ln (*Sp)* values (R2 = 0.55, *F* (1, 39) = 47.38, p < .001). The intercept of the model, corresponding to predicted *Vs* = 0, was at 0.85 (95% CI [-0.63, 2.33], t (39) = 1.16, p = 0.254) and the slope of the regression line was significant at 0.83 (95% CI [0.58, 1.07], t(39) = 6.88, p < .001). The slope of the regression line and the intercept did not differ from one (t-test = 1.157, P = 0.078) and zero (t-test = 1.157, P = 0.127), respectively. The residuals were normally distributed (Shapiro-Wilk test: W = 0.987, p = 0.914). The predicted *Sp* values and their associated 95% prediction interval limits (*Sp0.025–Sp0.975*) varied from 10 m (3–30) to 11,613m (2,120–47,256) m (Supplement 5).

When the degradation rate *r* was set to zero in equation 3, the correlations between the observed and predicted values were comparable (R = 0.735, p< 0.001). The slope of the regression line (0.793) differed from one (t-test = 1.769, P < 0.05), while the intercept (0.990) did not differ from zero (t-test = 1.367, P = 0.090).

As a function of discharge and water temperature, the simulated *Sp* values varied from 438 m (Q = 0.01 m3.s-1, T = 5 °C) to 37.8 km (Q = 100 m3.s-1, T = 5 °C). At low flow rates, Sp values were comparable whatever the temperature (Q = 0.01 m3.s-1, T = 25 °C, Sp = 424 m), whereas high temperature had a strong effect on *Sp* when combined with high discharge (Q = 100 m3.s-1, T = 25 °C, Sp = 26.35 km). The upper and lower 95% prediction intervals limits *Sp0.025* and *Sp0.975* were [0.15–0.33] to [2.99–4.90] times respectively below and above the predicted values, increasing with discharge and temperature (Fig. 6A). The reduction in *Sp* due to the degradation process (Fig. 6B) was only a few percent for a low flow rate (1.1 to 4.1% at Q = 0.01 m3.s-1) but increased sharply with discharge and temperature: 14 to 41% at Q = 100 m3.s-1 for 5 and 25 °C respectively.

## 3.5 | Detection distance

The discharge increased from 72 to 314 m3.s-1 along the 306 km with a major input from the Lech river at km 91 (Fig. 7A). The uptake length and the degradation rate varied from 18.9 to 51.0 km (mean = 31.9 km) and from 0.016 to 0.019 mm.s-1 (mean = 0.017 mm.s-1). The simulated curve of eDNA concentration downstream from the source point is globally in agreement with the specific eDNA concentration for the three species (Fig. 7B to 7D), except at detection distance 173 km for *B. barbatula*. At a detection distance around 3 km from the source point, the specific eDNA concentrations are close to the LODL value (42 copies per litre), mainly due to the injection of specific eDNA by the LRiveriver: maximal transport distances of 287, 279, and 295 km for *B. barbatula, S. trutta,* and *T. thymallus* respectively.

# Discussion

## eDNA PSD

We tested an eDNA transport model based on equations developed to simulate solute transport in streams, including the eDNA degradation process and the VFPM sedimentation rate, as estimates of the eDNA deposition rate. Using previous studies and non-linear and linear mixed models (meta-analysis), we summarized what is known about eDNA PSD and the relationship between eDNA degradation and temperature. Our model correctly simulated the eDNA PSD; however, the non-normality of the residual distribution made it difficult to estimate the uncertainties. The simulations demonstrate that more than 70% of eDNA particles are smaller than 10 microns, which is consistent with previous studies (Barnes et al., 2021; Brandao-Dias et al., 2023). Some authors have found eDNA particles larger than 50 µm more frequently (a few percent of the total) than those predicted by our model (Turner et al., 2014; Wilcox et al., 2016), which may be due to the tendency of the Weibull model to underestimate the frequency of large particles (Zobeck, Gill & Popham, 1999). The eDNA PSD can vary between species (Zhao, van Bodegom, & Trimbos, 2021) but also depends on environmental conditions, such as organic seston abundance (Barnes et al., 2021). Furthermore, eDNA PSD is unstable over time, as larger particles decompose into smaller particles (Jo, Arimoto, Murakami, Masuda, & Minamoto, 2019; T. Jo et al., 2017) and further studies are required to better understand eDNA PSD variability (Barnes et al., 2021; Brandao-Dias et al., 2023).

## Settling velocity

The relationship between the size and sedimentation rate of the VFPM was correctly described by our LMM. The prediction interval width is not surprising, as it is known that the sedimentation rate measured in situ depends not only on the particle diameter, but also on its relative density with respect to the fluid and the dynamic viscosity of water (Dyer & Manning, 1999; Maggi, 2013, Mikkelsen & Pejrup, 2001). Additionally, in the natural environment, the aspect ratio (elongation versus roundness) and surface texture of particles are highly variable and, in addition to the theoretical considerations of Stockes' law (Dietrich, 1982), strongly influence the speed at which the particles are deposited (Williams, Walling, & Leeks, 2008). Additionally, fine organic and mineral particles tend to coagulate (flocculation process), which modifies their PSD and hydrodynamic characteristics (Droppo, Leppard, Flannigan, & Liss, 1997; Dyer & Manning, 1999 ; Maggi, 2013 ; Thonon, Roberti, Middelkoop, van der Perk, & Burrough, 2005).

After combining the results of our settling velocity model with those of the modeled eDNA PSD, we obtained our weighted average eDNA settling rate ( = 0.031 mm.s-1), which was close to the median eDNA deposition rate (0.038 mm.s-1) computed from a compilation of fish eDNA transport experiments (Jo et Yamanaka, 2022). The interquartile range of the observed values (n = 37, 0.019–0.101) in this last study was larger than [0.25, 0.75] but comparable if only transport experiments in natural streams were considered (n = 31, 0.017–0.059).

## Degradation rate

As demonstrated in previous studies, we observed a substantial increase in the eDNA degradation rate with increasing temperature (Jo, Arimoto, Murakami, Masuda, & Minamoto, 2020; Lamb, Fonseca, Maxwell, & Nnanatu, 2022; Mauvisseau et al., 2022), probably due to an increase in enzymatic activity (Collins et al., 2018; Jo, Murakami, Yamamoto, Masuda, & Minamoto, 2019). Nevertheless, temperature elucidated only 17.9% of the total variance of the model, less than the value (29.3%) given by Mauvisseau et al. (2022) with the same dataset but excluding the studies as a random effect. Other environmental conditions (pH, microbial load, UV radiation, and water salinity) and the type of target gene also influence eDNA decomposition rate to a lesser extent (Jo et al., 2020; Lance et al., 2017; Mauvisseau et al., 2022). Binding of eDNA to particles reduces its accessibility to nucleases and increases eDNA persistence (Nagler, Podmirseg, Ascher-Jenull, Sint, & Traugott, 2022). We did not consider the effect of particle size on eDNA degradation. Larger fragments degrade faster (Jo et al., 2017; Shogren et al., 2018), and the ratio of large and small target eDNA molecules is related to the spatiotemporal proximity of the eDNA source (Brandao-Dias et al., 2023).

Several studies have separately modelled a fast-decaying and a slow-decaying eDNA fraction (Cerco, Schultz, Noel, Skahill, & Kim, 2018; Shogren et al., 2018). Similarly, it has been suggested that different components of eDNA (from free nucleotides to particle-bound eDNA) degrade at different rates (Nagler et al., 2022). Degradation tends to homogenize the PSD over time between species, with the dominance of particles smaller than 10 μm at high temperatures (Brandao-Dias et al., 2023; Jo, Murakami, Yamamoto, Masuda & Minamoto, 2019). eDNA degradation remains a complex and variable process, depending on the nature of the eDNA particles and the environmental conditions.

## Model and validation

By including both the VFPM settling velocity as a proxy for the eDNA deposition velocity and degradation rate at a given temperature, our eDNA transport distance model correctly simulated the eDNA uptake distance (Sp) computed from the eDNA transport experiments. This result verifies the similar behavior of VFPM and eDNA during transport in flowing water (Pont et al., 2018). The relative influences of the degradation and deposition rates varied strongly with environmental conditions. Our model predicted an increase in the relative influence of eDNA degradation on *Sp* with discharge, which is comparable to previous experimental results. Biological degradation is negligible for discharge below one m3.s-1 (Wilcox et al., 2016) but accounts for 20 to 50 % compared to physical retention for a discharge of 18 m3.s-1 (Shogren et al., 2019). Degradation is a longer-term removal process than deposition (Fremier et al., 2019) and can only negatively affect eDNA concentration in the water column when hydraulic conditions allow a long uptake distance and a removal time of the corresponding eDNA, that is, from a few hours to a day or more. Additionally, the degradation was more significant at high temperatures under the same hydraulic conditions.

One of the major limitations of the hydraulic mechanistic model that we used (Stream Solute Workshop, 1990) was high bed shear, which prevented particle deposition, predominantly in association with coarse bed sediments and highly turbulent flow (Cushing et al., 1993; Minshall et al., 2000). This phenomenon may elucidate the lack of a decrease in the eDNA concentration downstream of the discharge point in several transport experiments (Jane et al., 2015; Van Driessche et al., 2023; Wood et al., 2021; Jo et Yamanaka, 2022). Advective transport of particles into the interstices of the streambed, followed by resuspension (hyporheic flow), allows for repeated cycles of alternating deposition and resuspension events (Minshall et al., 2000; Shogren et al., 2017). This transient storage affects the persistence of eDNA in the water column and is primarily stochastic (Fremier et al., 2019; Harrison et al., 2019). Furthermore, the transfer of eDNA particles to the bottom can be accelerated by the presence of a biofilm in the initial phase, and then released into the water column (Shogren et al., 2018).

The prediction interval around the Sp simulation is wide and reflects the complexity and variability of the degradation process and settling velocity, as we saw earlier. The upper and lower limits of their respective prediction intervals can only be accumulated under normal distribution conditions and the assumption of independence between the two variables. While the first condition is well satisfied, the second condition is not completely satisfied as the change in the eDNA PSD during transport due to degradation (Jo et al., 2017) can affect the sedimentation rate.

## Detection distance

Applied to a stretch of river where the eDNA of three species of fish was detected when these species were known to be absent, our model was able to correctly simulate the concentrations of specific eDNA copy numbers per litre measured previously, and therefore demonstrate that these species detections were false positives. In our case, the maximum transport detection distance is quite long due to the size of the river but also to the contribution of eDNA from a tributary with a fish eDNA content like that of the main river upstream of the studied river stretch. One application of our downstream eDNA transport model involves collecting environmental data related to hydraulic conditions and temperature (Harrison et al., 2019), not only at the river sampling site but also upstream at a spatial scale depending on the river size and the complexity of the river network (tributaries).

## Conclusion

In conclusion, our results demonstrate that the combination of a hydraulic mechanistic model with both a temperature-dependent degradation process and VFPM settling velocity as an equivalent of the deposition velocity provides an accurate assessment of eDNA transport distance. The extension of the prediction intervals associated with the simulation reflects the complexity of the processes acting on eDNA post shedding (Shogren et al., 2018). One of the major limitations of this model is related to extreme hydraulic conditions (high bed shear stress), which are probably more common in small, coarse-bottomed streams than in large rivers. Further research is required to elucidate this process.

Combined with the volume of water sample at a site, the amount of eDNA extracted, the aliquot used per technical replicate, the eDNA detection/quantification limit, and the concentration of a specific eDNA at a site (Lance et al., 2017), our model can be used to estimate the downstream detection distance of the fish species considered. It can also be useful for practitioners to optimize their sampling strategy (distance between eDNA samples) and to discuss the potential presence of false positives at a site (Wang et al., 2021). Unlike a traditional fish sampling method, the interpretation of eDNA data collected in a river reach involves also considering the upstream river network both in terms of environmental conditions and possible sources of eDNA.

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# **Data Accessibility and Benefit-Sharing** **section**

No original data are associated with the manuscript.

The benefits generated by this research come from the sharing of our results for the calculation of the detection distance of eDNA transported downstream from a source point. Our work could be useful for end users to optimize their sampling strategy and discuss their results (in particular the risk of false positive detection).

# Author Contribution

D.P. conceived the study, conducted the literature search, analyzed the data, and wrote the manuscript.

# Figure Legends

Fig. 1 Methodological flowchart

Fig. 2 Cumulative eDNA particle size distributions for the seven studies and NLMM model fitted to these data (Weibull CCFD) at the population (red curve) and individual level (black curves).

Fig. 3 Plot of the settling velocity of natural suspended particles against their size for the four studies, and fitted curves from parameters estimated from a non-linear mixed model at the population (red line) and 95 % prediction interval (blue lines).

Fig. 4 Plot of the eDNA decay rate against temperature for the 20 studies and fitted curves from parameters estimated from a non-linear mixed model at the population (red line) and 95 % prediction interval (blue lines).

Fig. 5 Predicted against observed eDNA upstream uptake distances (Sp) for the 41 experiments, with corresponding 95% prediction intervals (solid and dashed lines for predicted and observed values respectively). Red line: fitted linear model; dashed line with slope 1 and y-intercept 0. Prediction intervals of observed (vertical dashed line) and predicted values (horizontal solid line) for each experiment. For observed values, the prediction intervals are indicative as the residuals from regressions of observed eDNA concentrations against distance are not normally distributed in most experiments (36 of 41 experiments).

Fig 6. Data simulation to predict eDNA uptake length *Sp* from temperature and discharge (a). Relative importance (in %) of decay on predicted eDNA uptake at different temperature and discharge (b).

Fig.7: Detection distance downstream of eDNA. A: Flow (dark line) and wetted width (blue line) of the river section studied. B to D: observed (black dot) and simulated (red line) eDNA concentrations of three fish species in the Danube River, eDNA concentration in the Lech River tributary (empty square); Biomass per hectare of the three species of fish (blue line) evaluated by electro-fishing. Limit of eDNA quantification per litre of water sample (LOQL, horizontal dashed red line).

# Figures

## Figure 1

**eDNA particle size distribution**

NLMM

**VFPM Settling velocity**

***vs particle size***

LMM and prediction interval

**eDNA decay rate *vs* temperature**

LMM

(*r* ± prediction interval)

eDNA

settling velocity

( ± prediction interval)

**eDNA transport model**

SP = Q/(w.(r.h + ))

(± prediction interval)

Discharge (Q)

Water depth (h)

Wetted width (w)

**eDNA detection**

**distance**

**simulation**

(± prediction interval)

Specific eDNA initial

concentration

Water sample volume

eDNA extracted volume

eDNA amplified volume

**eDNA transport**

**experiments**

Uptake

distance (Sp)

**eDNA transport model**

**validation**

Temperature

eDNA

Detection Threshold

## Figure 2

0.0

0.2

0.4

0.6

0.8

1.0

0.2

0.5

1

2

5

10

20

50

100

eDNA particle size (µm)

Cumulated frequency

## Figure 3

2

5

10

20

50

0.005

0.01

0.05

0.1

0.5

Settling velocity (mm.s-1)

Particle size (µm)

## Figure 4

0

5

10

15

20

25

30

35

Temperature (°C)

k (per hour)

0.001

0.005

0.05

0.1

0.5

## Figure 5

Predicted Uptake Length (m)

Observed Uptake Length (m)

5

10

50

100

500

5000

50000

5

10

50

100

500

5000

## Figure 6



20

40

60

80

100

5

10

15

20

25

10

20

30

40

% *Sp* reduction

Discharge (m3.s-1)

20

40

60

80

100

Temperature (°C)

5

10

15

20

25

Uptake Length (km)

0

20

40

60

80

100

120

Discharge (m3.s-1)

Temperature (°C)

## Figure 7

0

50

100

200

300

100

200

300

Discharge (m3.s-1)

0

50

100

150

River width (m)

0

50

100

200

300

Ln (Number eDNA

copies per litre)

**Barbatula barbatula**

0

10

100

10000

0

100

300

Fish biomass per ha

0

50

100

200

300

Detection distance (km)

**Salmo trutta**

0

10

100

10000

0

5

10

15

20

25

0

50

100

200

300

Detection distance (km)

**Thymallus thymallus**

0

10

100

10000

0

200

600

Fish biomass per ha

Fish biomass per ha

Ln (Number eDNA

copies per litre)

Ln (Number eDNA

copies per litre)

A

D

C

B

# Supporting/Supplemental Information

Supplement 1. Selected published literature for meta-analysis (modeling) of eDNA particle size distribution.

Supplement 2. Selected published literature (meta-analysis) for modelling the relationship between settling velocity of very fine natural particles and their size.

Supplement 3. Selected published downstream transport experiments to validate the mechanistic-based model of eDNA downstream transport distance.

Supplement 4. Raw environmental and eDNA data used to compare observed and simulated detection distance of three species over a 300 km stretch of the Upper Danube.

Supplement 5. Summary of the main numerical results