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Flow-induced fragmentation and mixing of eDNA for river biodiversity assessment

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Introduction

River restoration is an established method for the rehabilitation of river ecosystems in order to combat the current declines of freshwater biodiversity (Wohl et al., 2005; WWF, 2022). The urgency of restoration is recognized internationally, as the IUCN has proclaimed 2021-2030 to be the 'Decade on Ecosystem Restoration' (Cooke et al., 2022). So far only few restoration projects have been evaluated based on monitoring data (England et al., 2021), and there is a need for monitoring techniques to assess restoration practices.

The analysis of environmental DNA (eDNA) has gained popularity in the last decades, as it allows for rapid standardized biomonitoring across the tree of life, requires a reduced dependence on taxonomic expertise for species identification, and it is cheaper than traditional monitoring methods. Depending on the organism, eDNA is shed by its host in forms such as mucous, shed skin cells, and faeces. After release, eDNA is exposed to a wide spectrum of environmental variables that may impact its state, transport capacity, fate, and the subsequent inference made by the practitioner (Barnes and Turner, 2016). Our objective is to study how eDNA quantities are affected by flow and sediment transport in river ecosystems.

Methods

A set of laboratory eDNA concentration experiments was performed inside an annular flume (depth = 19.7 cm; Ø = 3.7 m) under different flow conditions (Fig. 1). The flume was filled with a mixture of potable water and effluent culturing water of wildtype *Danio rerio* (zebrafish) to introduce eDNA, which was subsequently rotated to induce a constant flow velocity. Each experimental run lasted for 168 hours, and was repeated for four rotation velocities. The flume was cleaned with bleach, and subsequently rinsed with potable water before each experimental run in order to remove trace amounts of zebrafish DNA. Prior to the addition of eDNA, vertical flow velocity profiles were measured inside the tank using a Nortek Vectrino ADV. Sampling and filtration equipment, and worksurfaces used during the sampling procedure were cleaned with bleach, ethanol, and demineralized water. Water samples were collected at multiple time points in triplo during each experimental run. 300 ml of each water sample was immediately filtered on site (pore size = 1.2 µm). Filters were stored at -80 °C awaiting extraction to avoid sample decay. Samples were extracted using the DNA/RNA Mini Prep Plus Kit (Zymo Research, Irvine, CA),

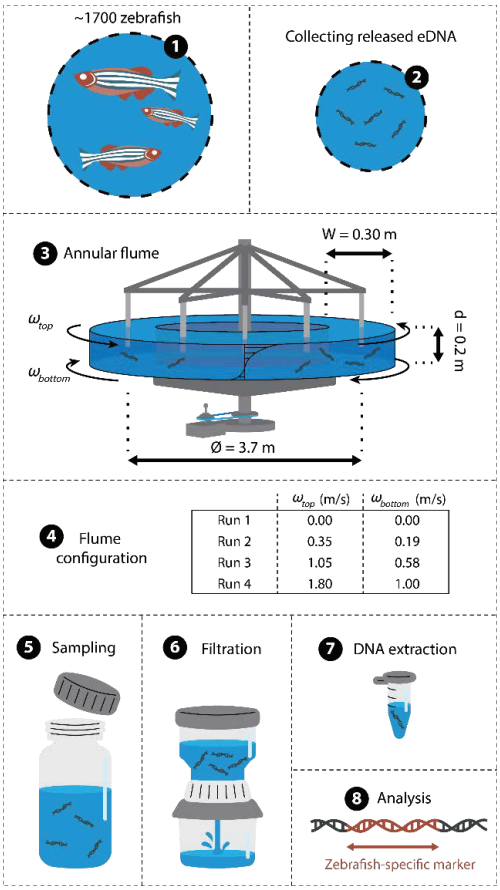

Figure 1. Schematized workflow followed through the set of laboratory experiments.

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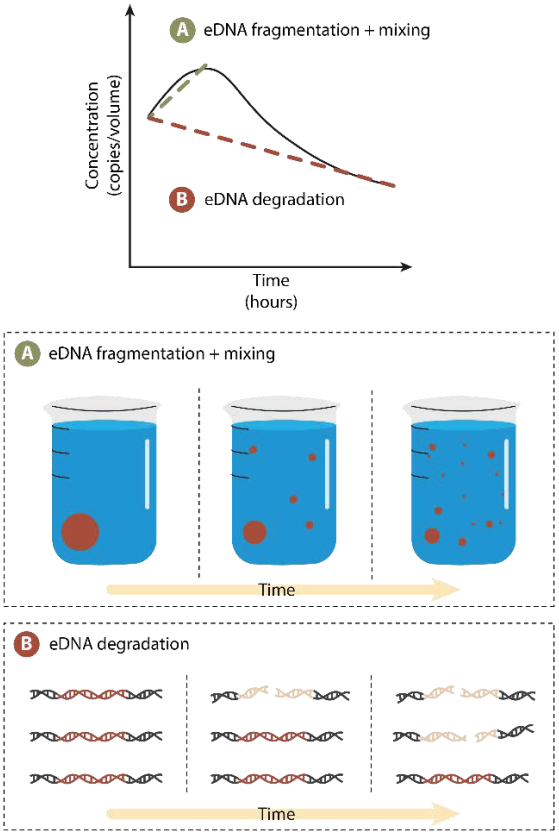

Figure 2. Schematized eDNA concentration over time, and the suggested mechanisms steering the trend.

following the extraction protocol as described in Marshall et al. (2021). eDNA concentrations were quantified in duplo by measuring copy numbers of a 73 base pair fragment on the cytochrome *c* oxidase I (COI) gene using a primer-probe assay designed by Zhao et al. (2021) and the QX200 Droplet Digital PCR (ddPCR) platform (Bio-Rad, Hercules, CA).

Results & discussion

Across all trials, the chosen COI marker consistently remained detectable throughout the entire experiment, with eDNA degradation rates notably lower than those typically observed in eDNA time-series data. In the absence of indicators of contamination, we attribute these low degradation rates to a combination of factors. Firstly, eDNA degradation rates increase exponentially with increasing fragment size (Jo, 2023). The selected DNA sequence of 73 base pairs is relatively short, with low degradation rates as a result. Secondly, the filters selected for eDNA larger than 1.2 µm. As a result, smaller-sized 'free' extracellular DNA particles, associated with high degradation rates, were discarded.

The low eDNA degradation rates allowed us to distinguish between two mechanisms that affect detectable eDNA quantities (Fig. 2): (A) particle fragmentation followed by mixing, and (B) eDNA degradation. We attribute the fragmentation-mixing mechanism to turbulent flow structures, which result in smaller, and more evenly abundant eDNA particles in the experimental volume, increasing the probability of eDNA detection. The impact of the fragmentation-mixing mechanism increased with flow rate, resulting in an initial increase of detectable eDNA quantity during an experiment. The second mechanism, eDNA degradation, resulted in a steady decrease of the total detectable quantity of eDNA over the course of the experiments.

Our data and the described mechanisms are in line with field data published by Wood (2021) and Van Driessche (2023), which took note of fragmentation and mixing phases. In the case of the field experiment of Van Driessche (2023) a consistent peak in the detected eDNA concentrations downstream of a source population of fish was partly attributed to the aforementioned mechanisms.

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