

Supplementary material

S1. Environmental DNA metabarcoding data.

To complement the ddPCR analyses, eDNA metabarcoding was performed following the protocol of Van Driessche et al. (2024a, 2024b). In short, a ~142-bp fragment of the mitochondrial 12S rRNA gene was amplified using vertebrate-specific Riaz primers. PCR reactions (45 cycles) were performed in triplicate using inline-barcoded primers. Pooled libraries were purified with AMPure beads, quantified via Quantus Fluorometer (Promega), and sequenced on Illumina HiSeq3000 and NovaSeq platforms (Admera Health, USA). Bioinformatic processing utilized a dual-indexed demultiplexing script. Reads were merged (PEAR v0.9.11), trimmed (cutadapt v4.2), and quality-filtered (VSEARCH v2.22.1). Taxonomy was assigned using OBITools v3.0.1 against a curated 12S rRNA database for local fish.

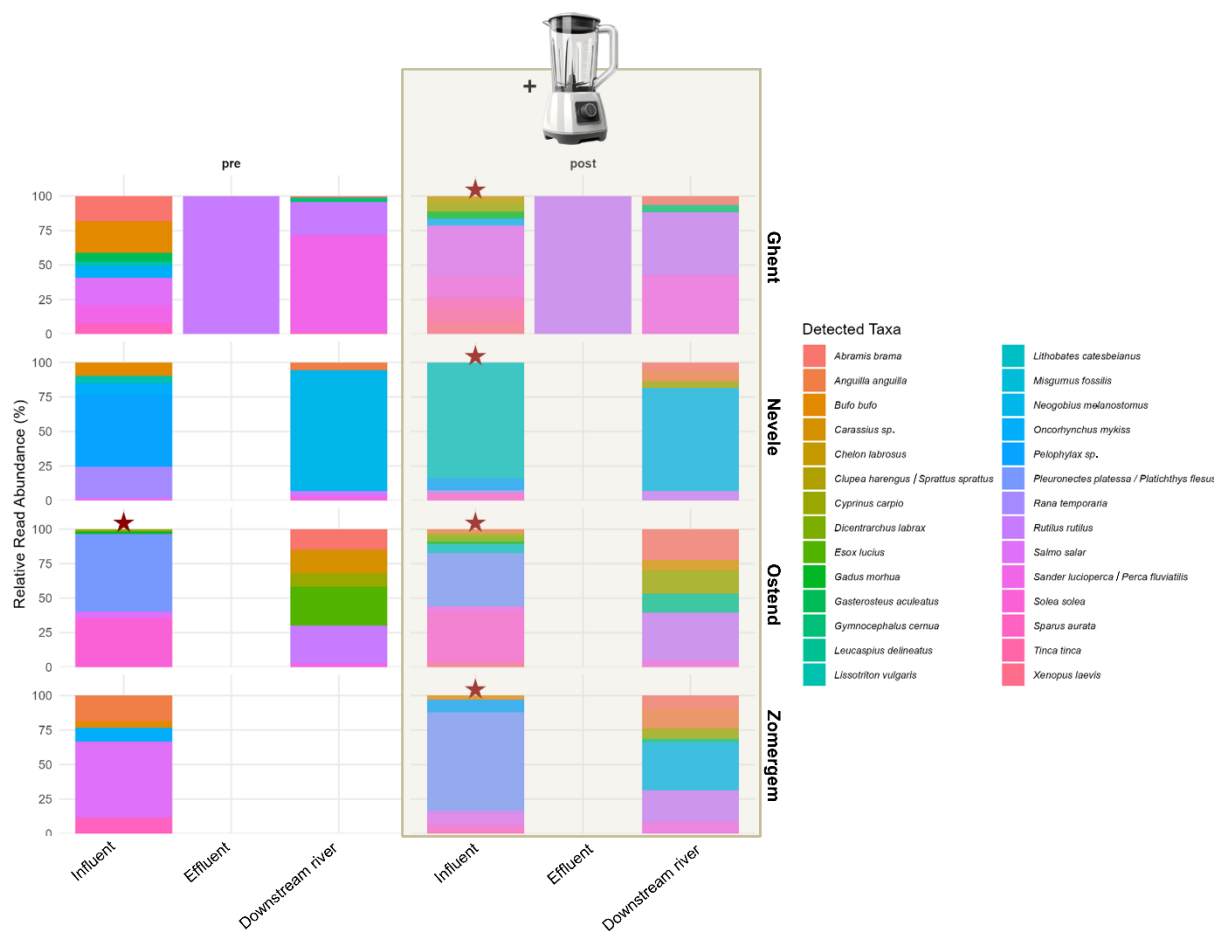


Figure S1. Spatiotemporal fish community composition across four wastewater treatment plants (WWTPs) based on eDNA metabarcoding. Relative read abundance is shown for three sampling points per WWTP (Influent, Effluent, and Downstream river), both before and after a controlled spike of six target species. Red stars indicated the detection of at least one of the six target species (*Misgurnus fossilis*, *Solea solea*, *Pleuronectes platessa*, *Xenopus laevis*, and *Lithobates catesbeianus*). Note that target species were detected across all influent post-spike samples, but were absent from all effluent and downstream river samples.