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Impacts of large and small barriers on fish assemblage composition assessed using environmental DNA metabarcoding

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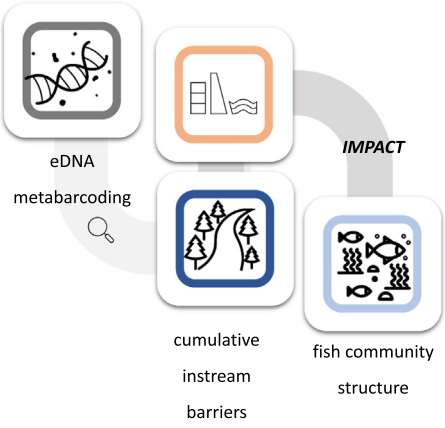
HIGHLIGHTS

- Based on eDNA, the Poutes dam that drove salmon close to extirpation is not the main cause of fish richness discontinuity in the Allier.
- Barrier density and cumulative height are the main drivers of fish species' presence/absence in the river Allier.
- Managing or removing small barriers can have a broader impact in the fish community than just focusing on large dams.
- eDNA-metabarcoding data provides an alternative to electrofishing sampling, particularly in large rivers.

GRAPHICAL ABSTRACT



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ABSTRACT

River fragmentation caused by instream barriers is a leading cause of biodiversity loss, particularly for freshwater migratory fish, the vertebrate group that has suffered the steepest decline. However, most studies have tended to focus on the impacts of large dams on only a few taxa. We estimated the cumulative impact of both large and small barriers on fish species richness and relative abundance along an altitudinal gradient in the main stem of the River Allier (France). Using eDNA metabarcoding, we identified 24 fish zero-radius operational taxonomic units (zOTUs), corresponding to 26 species distributed along the main stem of the river. Elevation explained the greatest amount of variation in fish distribution, together with average flow, barrier density and its interaction with cumulative barrier height. Based on eDNA, the largest discontinuity in species richness was not related to the location of Poutès, the largest dam in the system, but located downstream from it. Our results indicate that, in addition to the more obvious effects of large dams on migratory fish such as the Atlantic salmon, the cumulative effects of small barriers can have widespread impacts on fish species richness and relative abundance, which should not be overlooked. We suggest that, as for other fragmented rivers, acting on numerous small barriers might bring about greater benefits in fish species richness than focusing only on the largest dams.

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1. Introduction

Dams, weirs, and other instream structures can cause widespread impacts on fish assemblages by modifying fish habitats, turning flowing waters into semi-lentic systems (McKay et al., 2017) and by blocking fish movements (Buisson et al., 2008; De Leeuw and Winter, 2008; Taylor et al., 2008). Globally, freshwater migratory fish have declined by 96% over the last 50 years, the greatest decline of any vertebrate group (Deinet et al., 2020), in part due to increasing levels of river fragmentation (Belletti et al., 2020; Grill et al., 2019). Understanding changes in fish assemblage composition in rivers fragmented by barriers is key to developing corrective actions, like dam removal (Kornis et al., 2015). In this sense, the River Continuum Concept (Vannote et al., 1980) (RCC) can be used as a baseline to predict fish assemblage composition against which barrier impacts can be assessed. In addition, the Serial Discontinuity Concept can be used as a base line to make predictions on the recovery of regulated rivers, as a function of the downstream distance to the dam (Stanford and Ward, 2001). River barriers are predicted to have different impacts depending on species particular habitat use and tolerance (Welcomme et al., 2006). For example, barriers that cause impoundments might affect lentic and lotic fish species differently (Parasiewicz et al., 2018). Most of the attention on barrier impacts on freshwater fish has traditionally focused on the effects of medium to large dams (>5 m), particularly on migratory fish, ignoring the potential impacts of small barriers on fish habitat and species composition (Birnie-Gauvin et al., 2017). However, changes in habitat immediately upstream and downstream of small barriers can affect fish assemblages in a similar way to large dams (Alexandre and Almeida, 2010) and have potential selective effects, especially for the weakest swimmers (Jones et al., 2020b).

Here, we assessed the extent to which barriers affect the expected decrease in fish species richness with increasing elevation predicted by the River Continuum Concept in medium to large rivers. Unlike many other studies that used species or size-selective sampling techniques, we used environmental DNA (eDNA) metabarcoding with universal PCR primers (Deiner et al., 2017) to examine the effects of barriers on fish assemblage composition. eDNA methods can be more cost-effecttive than traditional electrofishing sruveys (Evans et al., 2017), particularly considering the rapid decrease in the cost of genomic sequencing (Tillotson et al., 2018). We combined eDNA metabarcoding and information on habitat preference of fish guilds (Parasiewicz et al., 2018) to contextualise changes in species richness and relative abundance and evaluate the impact of instream barriers on fish assemblages in the River Allier, the main tributary of the River Loire, one of France's largest rivers. The River Allier is one of the wildest rivers in Southern Europe, but its main stem is fragmented by several small barriers and a single large (17.7 m) hydroelectric dam (the Poutes dam) on the steepest section of the river. The Poutes dam is responsible for the near extirpation of the local Atlantic salmon population (Dauphin and Prévost, 2013) and has been the focus of a protracted environmental campaign and technical modifications to reduce its impact (Tétard et al., 2021).

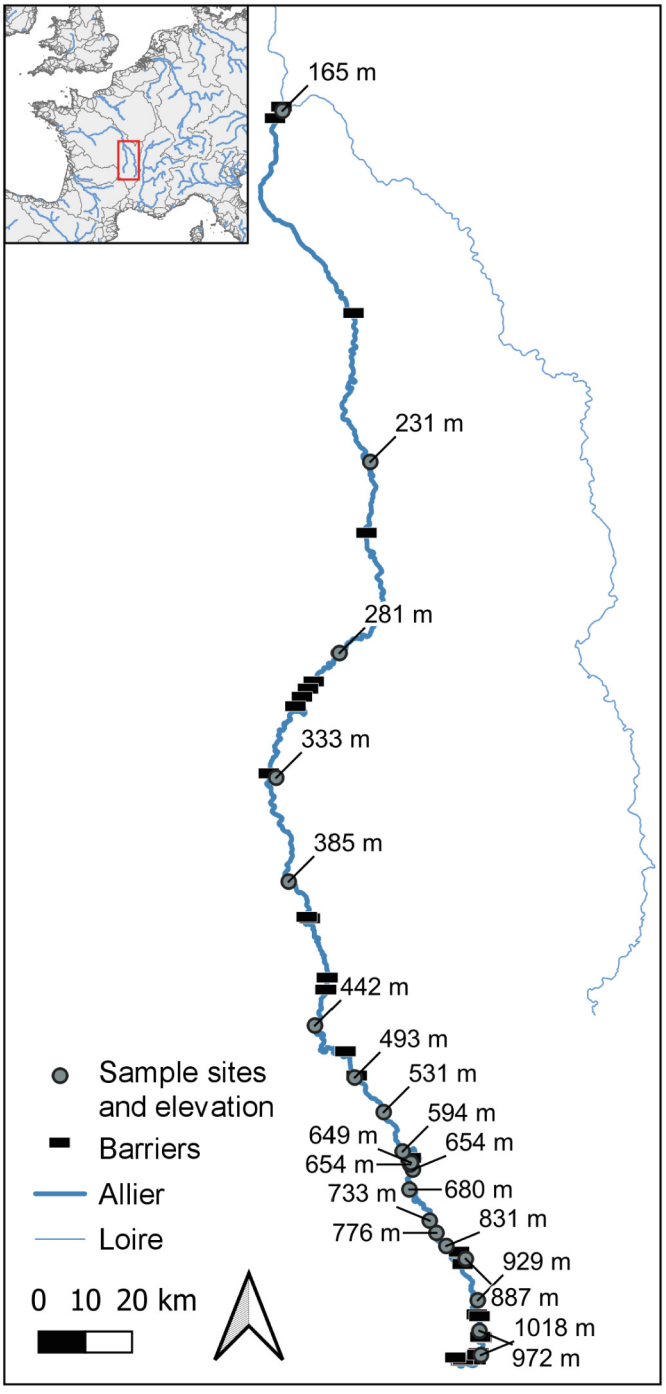

Fig. 1. Location of sampling sites for eDNA (grey circles), including altitude (m) and barriers (black rectangles) in the main stem of the River Allier.

2. Methods

2.1. Sample collection, DNA extraction and amplification

We sampled 20 sites along the main stem of the River Allier at altitudinal increments of ~50 m (ranging from 164 to 1018 m), covering over 400 km of river (Fig. 1). There are 29 artifical barriers in the main stem of the River Allier (Belletti et al., 2020), with a cumulative barrier height of ~64 m (Fig. 2a). The tallest barrier is the Poutès dam, 17.7 m high at the time of sampling and equipped with a pool and weir fish pass and a fish lift to allow upstream migration of adult Atlantic salmon, as well as an outflow for the downstream migration of smolts. Water temperature (°C), pH, ammonium concentration (NH4-N, mg/l) and dissolved oxygen (DO %) were measured using a YSI Professional Plus multiparameter meter (YSI Incorporated, OH) (Table S1). Unionized ammonia concentrations (NH3, mg/l) for each sampling site were estimated based on ammonium concentration, temperature and pH (http://home.eng.iastate.edu/~jea/w3-research/free-ammonia/nh3.html) and ranged between 0.001 mg/l and 0.031 mg/l. Average surface current velocity (m/s) at the time of sampling was measured using a Global Water flow probe (Xylem Inc.).

Triplicate water samples (1 l) were collected at ~20 cm below the water surface using 1 l Sterile bags (Whirl-Pak® stand -up Sample Bag), that were then refrigerated until filtration on the day of collection through 25 mm sterile 0.22 μm pore size polyethersulfone hydrophilic

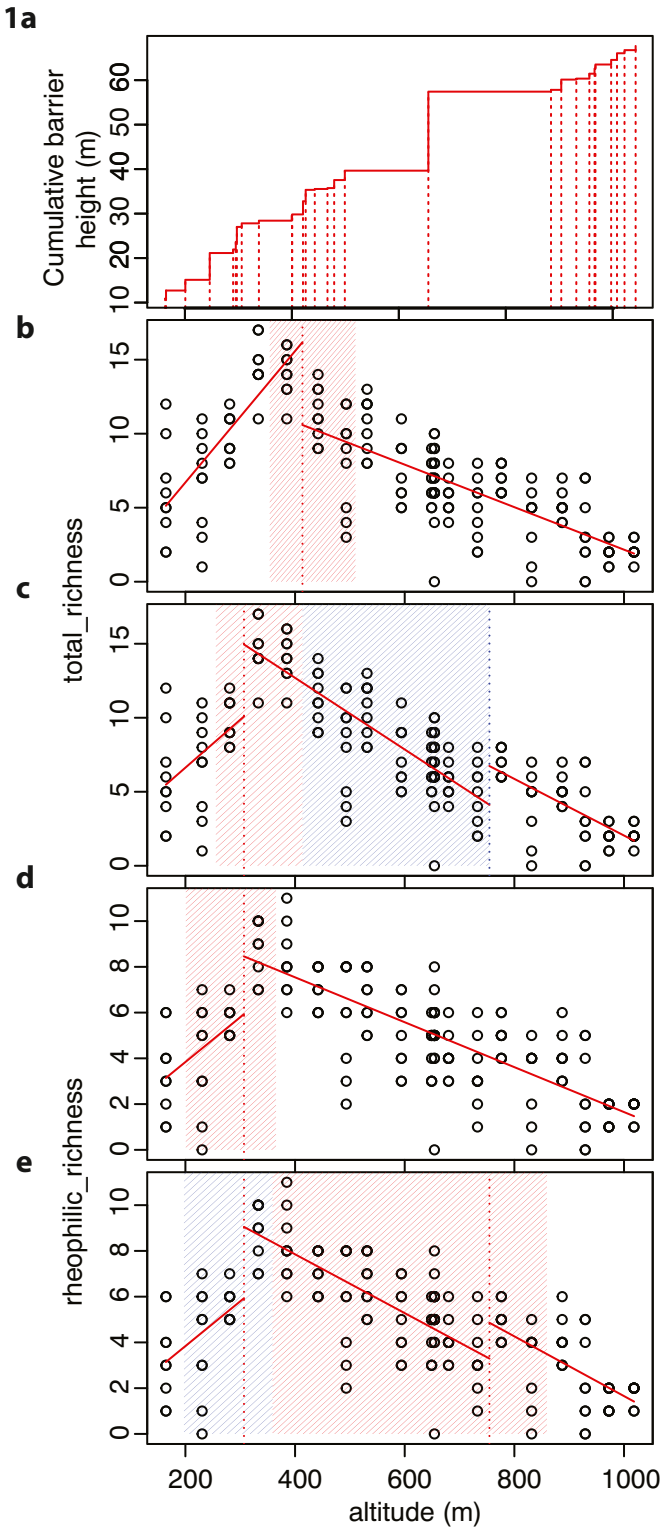

Fig. 2. Cumulative barrier height as a function of elevation (a) and piecewise linear models for total richness of fish communities (b, c) and richness of rheophilic fish (d, e) as a function of elevation, based on a single break point (two piece model: b, d) or two break points (three piece linear model: c, e). The solid red line in (a) represents cumulative height and vertical lines coincide with barriers. The solid red line in (b-e) are fitted linear models that minimise mean square error (MSE). Breakpoints minimise the MSE of a two-segment three-segment models and shaded rectangles delimit 95% confidence intervals determined by bootstrapping.

membranes (Millipore Express PLUS). Field blanks consisting of sterile water were processed in the same way.

DNA was extracted directly from filters using the DNeasy PowerLyzer PowerSoil® DNA Isolation Kit (Qiagen GmbH, Hilden, Germany), following manufacturer's guidelines, in a bleached and ultraviolet irradiated hood within a contained laboratory area exclusively dedicated to eDNA analyses. Extraction blanks were processed in parallel. We used the vertebrate-specific 12S—V5 mtDNA primers (Riaz et al., 2011), targeting a 106 bp region of the 12S mitochondrial gene. PCR master-mix preparation, and addition of eDNA to the PCR master-mix was undertaken in an ultraviolet irradiated hood exclusively dedicated to eDNA. Reaction 1 contained 12.5 μl of 2xPhusion High-Fidelity PCR Master Mix (Thermo Fisher Scientific), 0.4 μM of primers with 5' Nextera® tags, and 2.5 μl template DNA. Final thermal cycling conditions consisted of 98 °C for 30 s, then 35 cycles of 95 °C (10 s), at 52 °C (30 s) and 65 °C (30 s), followed by a final elongation step at 72 °C for 5 min. We performed three PCR replicates for each sample replicate to account for PCR stochasticity. A second round of PCR was used to append i5 and i7 tags: 25 μl of 2xPhusion High-Fidelity PCR Master Mix with HF Buffer, 0.2 μM of each Nextera XT Indexed primer (Illumina, San Diego, CA, USA) with conditions similar to above with 8 cycles with annealing at 63 °C. PCR products were purified using AMPure XP beads (Beckman Coulter, Brea, CA, USA) with a ratio of 0.85:1.0 beads to product. The pooled DNA library was quantified using QPR (NEBNext® Library Quant Kit, NEB, Ipswich, MA) and sequenced by Illumina MiSeq (Illumina, San Diego, CA, USA) using the pair-end MiSeq Reagent Kit V3 (600 cycle) (Illumina, San Diego, CA, USA) following the manufacturer's instructions.

Bioinformatic treatment of DNA sequence reads followed a standard pipeline using PEAR for alignment (Zhang et al., 2014), OBITools for file rearrangement (Boyer et al., 2016) and USEARCH (Edgar, 2010) for quality control and designation of zero-radius OTUs (zOTUs) (Edgar, 2016). To minimise the possibility of false positives, we only considered taxa that had 10 or more sequences. Taxonomy was assigned using the lowest common ancestor "weighted" algorithm in MEGAN (percent to cover = 80) (Huson et al., 2007) on locally BLASTed sequences (Altschul et al., 1990). We used the McNemar's symmetry test for paired binary outcomes ((P/A > Y/N) to test whether eDNA detected the same species as previous electrofishing samplings on three different sectors (T1- Haute Allier: corresponding to sampling sites 4-7, T2- Allier Moyen: corresponding to sites 8-16, T3- Allier Aval: corresponding to sites 17-18 (Federation Departamentale Peche, 2019).

2.2. Statistical analysis

Analyses were conducted in R v4.0.4 (R Core Team, 2019) using the packages *vegan 2.5-6* (Oksanen et al., 2007) and *mvabund* (Wang et al., 2012). Scripts are available in supplementary material (Supplementary material Fig. S1). To test whether fish species richness was inversely related to elevation, as expected from the RCC predictions, we carried out a breakpoint analysis using piecewise linear regression (Crawley, 2012) to detect abrupt discontinuities in species richness that might be caused by artificial instream barriers. To iteratively determine best fit, the following model was evaluated for each value of x, where model 1 is the case for a single breakpoint *c*, and model 2 is the generalised model for any n breakpoints:

$$S_i\tilde{x}_i * (x_i \le c) + x_i * (x_i > c)$$

$$S_i\tilde{x}_i * (x_i \le c_1) + x_i * (c_1 < x_i \le c_2) + \ldots + x_i * (c_{n-1} < x_i \le c_n) + x_i * (x_i > c_n)$$

S_i is the species richness at elevation *i* and x_i is the model evaluated at elevation *i*. The elevation of this 'best' breakpoint was compared to

the actual location of the Poutès hydroelectric dam to test whether this caused the greatest discontinuity. We then divided the data into rheophilic (i.e., lotic) and non-rheophilic (i.e., lentic) fish species to assess if barriers had a greater impact on rheophilic species richness. We used changes in Akaike Informatio Criteria (AIC) to assess model performance and calculated 95% confidence intervals by bootstrapping (999 resampling). A ΔAIC greater than 10 was considered to be an improvement in model fit (Burnham and Anderson, 2002).

Multivariate models based on parallel univariate generalised linear models were constructed with the *manyglm* function in the *mvabund* package (Wang et al., 2012) based on fish presence and the number of eDNA reads per replicate. The best model was selected by removal of independent variables to minimise AIC using *drop1*. Species presence/absence was modelled as a function of elevation, pH, NH4 concentration, average velocity, cumulative barrier density (cumulative number of barriers), cumulative barrier height and the interaction between the last two. Water temperature was removed as a predictor as it was correlated with elevation (Pearson's r = R -0.976, $P < 0.001$). Sequence read counts were used as model offsets (McMurdie and Holmes, 2014) because read count impacts the mean-variance relationship and PCR stochasticity is highly correlated with sequence read count (Smith and Peay, 2014). The volume of water filtered was also treated as an offset (we were unable to filter 1 l of water through all filters, with only 0.9 l passing through three of them, and < 0.9 l passing through another three), because it might influence the probability of species occurrence. Significance was determined by permutation (4999 resamplings), with permutations constrained to triplicated replicates permuting only inside each biological sample. A similar multivariate *manyglm* test as well as parallel univariate models were run for sequence read counts as a proxy for relative abundance (biomass).

3. Results

There were 19,255 ± 947 (SEM) reads returned per PCR replicate of each sample. Of these, 9368 ± 610 were assigned to fish from the Allier. These were grouped into 24 zOTUs, which were assigned to species except for two zOTUs where the short 12S rRNA locus targeted could not distinguish between *Alburnus alburnus* and *Alburnoides bipunctatus* nor between *Sander lucioperca* and *Perca fluviatilis* (Supplementary material Table S1). Fish species unlikely to occur in the Allier (killifish, lumpfish, wrasse and cod) were easily identified. They occurred randomly and only in one replicate PCR in one sample from a site, in very low concentrations (0.21% of all fish reads) and thus were removed from further analyses. This highlights the advantage of using PCR replicates. One site contained DNA from either herring or sprat (which are synonymous at the targeted locus) in all three PCR replicates of one sample replicate, albeit at very low concentrations (0.05% of fish reads), which suggests that this marine species was either a lab contaminant or derived from organic fertilisers from nearby farms.

Only three fish species were detected in the upper reaches of the river, Sections 1 and 2 upstream of the Poutes dam (972–1018 m elevation): *Phoxinus phoxinus, Salmo trutta*, and *Cottus gobio*. Other species became progressively more common as one moved downstream (Fig. 3). Eight species only occurred in the lower reaches (between 9 and 531 m elevation): *Ameiurus melas, Silurus glanis, Oncorhynchus* sp., *Esox lucius, Alosa* sp., *Lampetra* sp., *Rhodeus amarus* and *Gymnocephalus cernua*. Three species previously identified with electrofishing sampling were not detected with eDNA (*Anguilla anguilla*, *Lota lota* and *Tinca tinca*) whereas four others were only identified with eDNA but not with electrofishing (*Cyprinus carpio*, *Gymnocephalus cernua*, *Alosa* sp. and *Onchorynchus sp.*) (Supplementary material Fig. S2). The differences in species detection between electrofishing and eDNA were not significant in any of the sectors (T1: McNemar's chi-squared = 0.167, df = 1, $P = 0.683$; T2: McNemar's chi-squared = 1.125, df = 1, $P = 0.289$; T3: McNemar's chi-squared = 0, df = 1, $P = 1$), indicating a good eDNA representation of the distribution of the fish assemblages across the sampling sites.

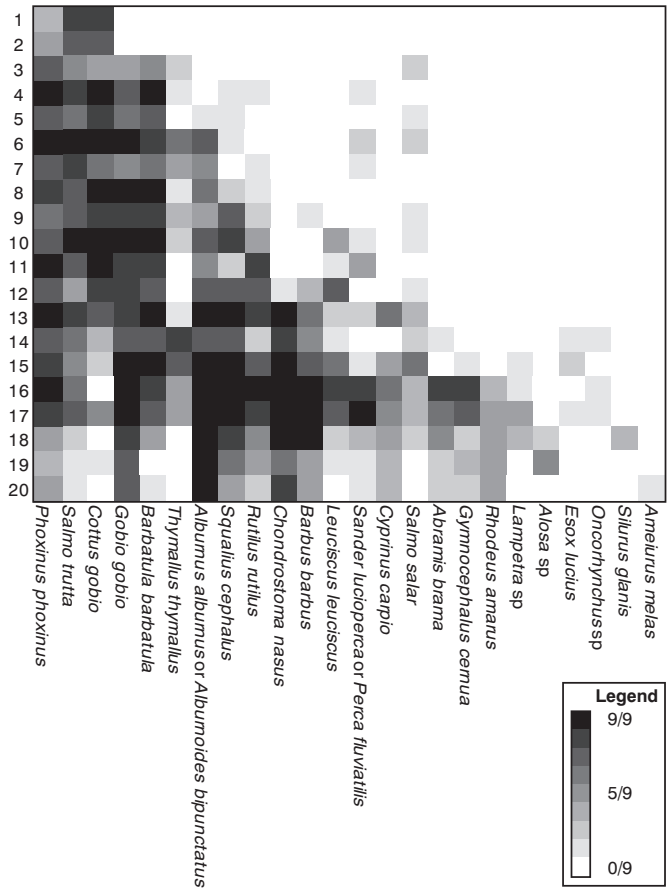

Fig. 3. Heat map of the number of positive PCRs per sample per site for each species of fish detected at the Allier river. Rows are sites with downstream at the figure bottom (site 20). Black squares indicate 9 out of 9 PCRs per site were positive, white indicates all were negative and the gradient corresponds to the fraction of 9 that were positive. Site 11 is immediately downstream of the Poutès and reflects the presence of species flowing from the impounded water and immediately below the dam.

Piecewise linear models were used to determine if break discontinuities would reduce the MSE of species richness as a function of elevation for the response variables: $\text{richness}_{\text{total}}$, $\text{richness}_{\text{rheophilic}}$, $\text{richness}_{\text{non-rheophilic}}$ (Fig. 2b-2e). A single breakpoint (two-piece model) improved the fit of all linear models, with break $\text{richness}_{\text{total}}$ = 413.5 m the ΔAIC = 117.2, with break $\text{richness}_{\text{rheophilic}}$ = 306.9 m the ΔAIC = 106.7 and break $\text{richness}_{\text{non-rheophilic}}$ = 413.5 m, ΔAIC = 82.2. A three-piece linear model (with two breakpoints) also improved the fit, but the change in AIC was considerably lower with ΔAIC for $\text{richness}_{\text{total}}$ = 11.0, only marginally greater than the threshold of 10, whereas the ΔAIC $\text{richness}_{\text{rheophilic}}$ = 7.9 and ΔAIC $\text{richness}_{\text{non-rheophilic}}$ = 7.1. In addition, 95% confidence intervals indicate that the two-piece model is preferable (Fig. 2c, e).

For fish presence/absence (occupancy), the most parsimonious model included all predictors apart from pH. Elevation, the interaction between barrier density and cumulative height, barrier density and average flow were all significant predictors of fish presence/absence (Table 1). In contrast, only two univariate tests were significant, *Rhodeus amarus* was significantly affected by barrier density and *Phoxinus phoxinus* by the average velocity (Supplementary material Table S2). For read count data (i.e., semi-quantitative data) the most parsimonious model included all variables apart from pH and NH4 and elevation, cumulative barrier density and average flow significantly affected read counts (Table 2). Univariate tests indicated that elevation affected the relative abundance (read counts) of all the species apart from *Esox*

Table 1
Analysis of deviance results of the *manyglm* multivariate analyses of fish species presence/absence eDNA data. Elevation represents height above sea level, barrier density is the cumulative number of barriers and cumulative barrier height is the ascending sum of barrier heights.

Variable	Res.Df	Df.diff	Dev	Pr(>Dev)
Elevation	178	1	13,981.3	<0.001
NH4	177	1	12,725.6	0.089
Average velocity	176	1	18,223.2	0.004
Barrier density	175	1	17,995.4	0.007
Cumulative barrier height	174	1	14,019.3	0.284
Barrier density: cum barrier height	173	1	19,137.4	<0.001

lucius, *Gobio gobio*, *Leuciscus leuciscus*, *Oncorhynchus sp.*, *Rutilus rutilus*, *Salmo salar*, *Sander lucioperca* and *Thymallus thymallus*. Cumulative barrier density significantly affected six species (*Barbatula barbatula*, *Barbus barbus*, *Chondrostoma nasus*, *Cyprinus carpio*, *Leuciscus leuciscus*, *Squalius cephalus*) (Supplementary material Table S3).

4. Discussion

Contrary to expectations, the largest discontinuity in fish species richness along the River Allier was not related to the location of the large Poutès hydroelectric dam. Instead, the main two discontinuities in fish richness were identified at 413.5 m altitude for all fish and 306.9 m altitude for rheophilic fish, downstream from the Poutès dam, which is located at an altitude of 651.6 m. Our analyses indicate that the fish assemblage of the Allier is largely determined by river elevation, one of the most common factors in determining fish richness patterns (Van Looy et al., 2014). Together with elevation and water velocity, species presence/absence was also determined by barrier density and its interaction with cumulative barrier height. The relative abundance (read counts) of several fish species decreased near the Poutès dam (Fig. 4) and multivariate models indicated that elevation, velocity and cumulative barrier density were sufficient to explain these changes.

Our work demonstrates how eDNA metabarcoding can be used to examine fish assemblage composition along a large river where other forms of sampling such as electrofishing or netting might be unfeasible. Water samples are easy to collect and can be used to detect taxa across large areas (Civade et al., 2016). With 1 l samples, the volume that we used, fish eDNA has been detected up to 9.1 km downstream from the source (Deiner and Altermatt, 2014), although there is considerable variability in detection distance (Civade et al., 2016; Pont et al., 2018). Abiotic conditions, such as flow rate, water temperature and transport dynamics also influence eDNA distribution in the river and therefore the ability to detect changes (Deiner et al., 2016; Takahara et al., 2012). In this case, this could be the reason for the influence of average water velocity on both presence/absence and read counts. However, although abundance of eDNA in water does not necessarily correlate exactly with abundance (or biomass) of fish in the river (Barnes and Turner, 2015), it represents well the dynamics of relative abundance and can be used to reliably assess changes in fish assemblages (Muha et al., 2021; Ratcliffe et al., 2021).

Table 2
Analysis of deviance results of the *manyglm* multivariate analyses of fish species reads count data. Elevation represents height above sea level, barrier density is the cumulative number of barriers and cumulative barrier height is the ascending sum of barrier heights.

Variable	Res.Df	Df.diff	Dev	Pr(>Dev)
Elevation	178	1	604.0	<0.001
Average velocity	177	1	70.4	0.045
Barrier density	176	1	237.6	0.023
Cumulative barrier height	175	1	56.4	0.108
Barrier density: cum barrier height	174	1	269.6	0.148

We found several species restricted to the lower reaches of the Allier, where there is a relatively high density of small barriers. These included the rheophilic shad (*Alosa sp.*) and lamprey (*Lampetra sp.*), whose distribution tends to be greatly affected by barriers (Lucas et al., 2009),. Conversely, other rheophilic species present upstream, such as *Cottus gobio* and *Barbatula barbatula*, were not detected in the lower reaches. Our data also suggest that cumulative barrier density is affecting the relative abundance of *Barbatula barbatula*. These species are good swimmers and could have drifted downstream, therefore their distribution may suggest that barrier impacts on rheophilic species at low altitude may not be caused simply by blockage of fish passage, but rather by habitat modification (i.e., ponding (Birnie-Gauvin et al., 2017)). Most rheophilic species are therefore good indicators for monitoring river discontinuities resulting from habitat alteration, with the most ubiquitous, such as *Phoxinus phoxinus* (its individual distribution being affected by average velocity) and *S. trutta*, being potentially indicative of extreme fragmentation should they disappear from a river reach. Finally, grayling (*Thymallus thymallus*) and Atlantic salmon (*S. salar*) were present both upstream and downstream of the Poutès, with their abundance declining around the dam. This may reflect strong fragmentation and the recolonization of the upper reaches of the Allier after the dam conversion in the late 1980s (Dauphin and Prévost, 2013). Grayling could be a good indicator species of fragmentation. Although its detection in the lower reaches, below 500 m elevation, might have been affected by effluents from the Conservatoire National du Saumon Sauvage where it is currently cultured (CNSS, 2017), its presence in electrofishing samplings along the whole river suggests it reflects the grayling natural distribution. In the case of Atlantic salmon, its distribution in the Allier is affected by the artificial stocking of juvenile fish over the last six decades with fish from nearby catchments or, more recently, from local hatchery stocks (Dauphin et al., 2016).

The relative abundance of *Barbus barbus*, *Chondrostoma nasus* and *Cyprinus carpio* was affected by cumulative barrier density but their distribution was clearly restricted by the Poutès dam. However, species richness decreased smoothly with increasing elevation over the length of river without barriers downstream of the Poutès dam (between 649 and 680 m of elevation approximately). Thus, while the dam has seriously affected some species like Atlantic salmon, driving its local population to near extinction (Dauphin and Prevost, 2013), and potentially acting as a bottleneck for other species, at the whole fish assemblage level we could not clearly identify a major effect. In contrast, we found that the density of small barriers and its interaction with cumulative height influenced species richness and were associated with the greatest discontinuities in the fish assemblage structure, even if they were fitted with fish passes and were passable for good swimmers like Atlantic salmon. In this sense, our results highlight the benefits of sampling the entire fish assemblage, rather than single charismatic species (Jones et al., 2020b; McLaughlin et al., 2013), across the entire river length to better understand how aquatic ecosystems respond to anthropogenic impacts (Jones et al., 2020a).

An inverse association between barrier density and rheophilic species richness similar to the one we identified had previously been observed in the Loire basin (Van Looy et al., 2014). Small barriers (<5 m) have traditionally been overlooked but are the main cause of river fragmentation because of their abundance and ubiquity in many parts of Europe (Jones et al., 2019), and have the potential to disrupt connectivity and fish passage (Leitão et al., 2018; Perkin and Gido, 2012), altering the structure of fish assemblages (Alexandre and Almeida, 2010). Our study shows that in the Allier, small barriers are also the main cause of discontinuity in fish species richness, most likely because of their cumulative impacts on fish passage (Lucas et al., 2009) and the selective pressures that this entail (Jones et al., 2021; Rahel and McLaughlin, 2018).

Our results also indicate that while adaptive management, lowering of the crest height and retrofitting of the new Poutès dam may facilitate

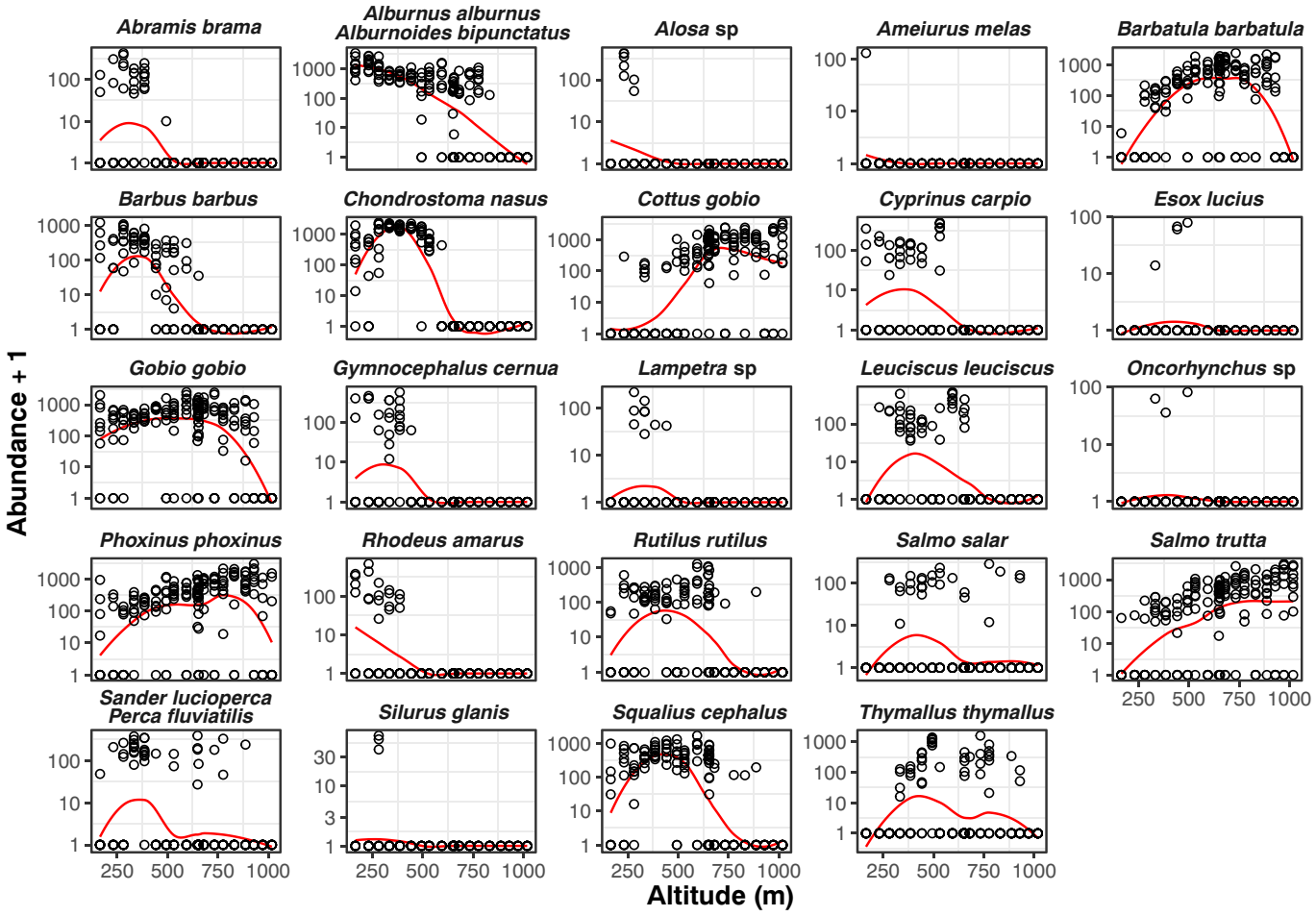

Fig. 4. Change in species abundance estimates as counts of sequence reads for the 24 zOTUs detected in the river Allier. Counts are log transformed. Curve is fitted by loess method.

passage of Atlantic salmon and its recolonization of the headwaters, removing or acting on the smaller barriers in the lower part of the catchment would improve connectivity for more species. Removing small dams can greatly increase fish richness (Ding et al., 2019) and targeting small and obsolete structures, which represent the majority of barriers in Europe, can be a cheaper and more effective strategy for restoring river connectivity than focusing on larger, less abundant structures (Belletti et al., 2020).

5. Conclusion

Our study shows how eDNA metabarcoding can be used to determine the cumulative barrier impacts on the spatial distribution of riverine fish species against the background of altitudinal species richness change predicted by the River Continuum Concept. We observed discontinuities in fish species richness consistent with barrier impacts but, contrary to expectations, these were not associated with the largest dam. Instead, the best model of fish presence indicates that fish occurrence is most likely determined by elevation, barrier density and cumulative barrier height. Although elevation and slope have long been known to affect riverine fish assemblages, our study highlights the role that instream barriers play in shaping fish species richness and relative abundance, as well as the dangers of focusing solely on the impacts of large dams and overlooking small barriers in river management. This study, which precedes a large reconfiguration of the Poutès dam, demonstrates the importance of having baseline data against which the benefits of barrier mitigating actions can be gauged, and the usefulness of eDNA metabarcoding for that purpose, particularly in large rivers that are difficult and costly to sample with more traditional methods.

CRediT authorship contribution statement

Sofia Consuegra: Conceptualization, Methodology, Formal analysis, Writing – original draft, Funding acquisition. **Richard O'Rorke:** Investigation, Formal analysis, Data curation, Writing – original draft. **Deiene Rodriguez-Barreto:** Investigation, Writing – review & editing. **Sara Fernandez:** Investigation, Writing – review & editing. **Joshua Jones:** Investigation, Data curation, Writing-review & editing. **Carlos Garcia de Leaniz:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Sequence reads are available in the European Nucleotide Archive under study accession number PRJNA667064.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.148054.

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