

Barrier mitigation measures trigger the rapid recovery of genetic connectivity in five freshwater fish species

Jérôme G. PRUNIER ^{1*}, Géraldine LOOT ², Charlotte VEYSSIERE ², Nicolas POULET ³, Simon BLANCHET ^{1,2*}

¹ Centre National de la Recherche Scientifique (CNRS), Université Paul Sabatier (UPS), UPR 2001, Station d'Ecologie Théorique et Expérimentale, Moulis, France

² CNRS, UPS, UMR 5174 EDB (Laboratoire Évolution & Diversité Biologique), École Nationale de Formation Agronomique (ENFA), Toulouse Cedex 4, France

³ DRAS, Pôle R&D écohydraulique OFBIMFT-PPRIME, Office Français de la Biodiversité, Toulouse, France

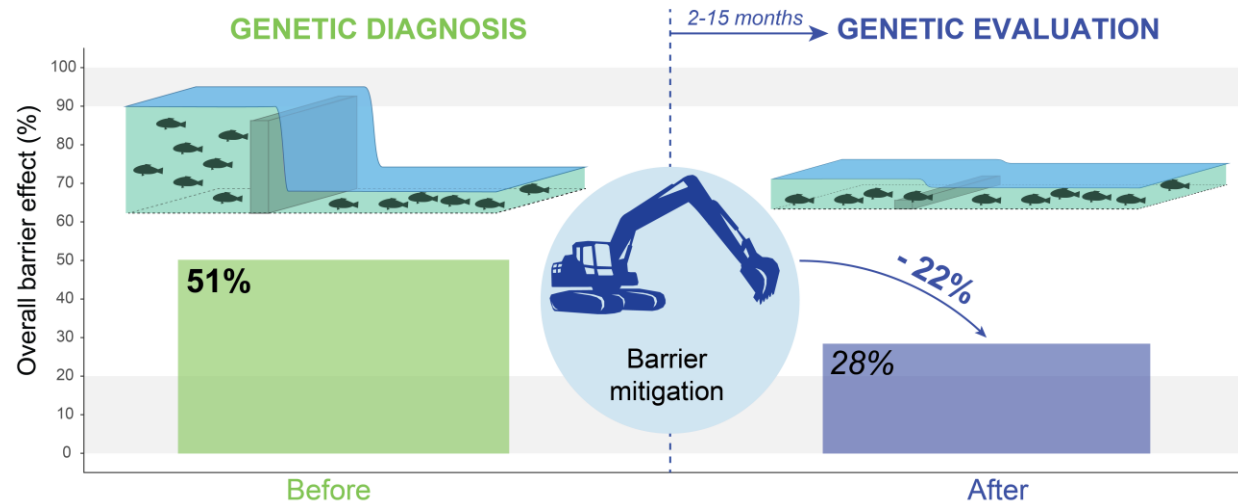
* Corresponding authors:

Jérôme G. Prunier, Centre National de la Recherche Scientifique (CNRS), UPR 2001, Station d'Ecologie Théorique et Expérimentale, 2 route du CNRS, 09200 Moulis, France ; Email : jerome.prunier@gmail.com; Tel : +33671640804

Simon Blanchet, Centre National de la Recherche Scientifique (CNRS), UPR 2001, Station d'Ecologie Théorique et Expérimentale, 2 route du CNRS, 09200 Moulis, France ; Email : simon.blanchet@sete.cnrs.fr

Running title: **Rapid recovery of genetic connectivity**

GRAPHICAL ABSTRACT



ABSTRACT

Rivers are heavily fragmented by man-made instream barriers such as dams and weirs. This hyper-fragmentation is a major threat to freshwater biodiversity and restoration policies are now adopted worldwide to mitigate these impacts. However, there is surprisingly little feedback on the efficiency of barrier mitigation measures in restoring riverine connectivity, notably for non-migratory fish species. Here, we implemented a “before-after genetic monitoring” of the restoration of 11 weirs in France using a dedicated genetic index of fragmentation (the F_{INDEX}), with a focus on five fish species from two genera. We found that most obstacles actually had a significant impact on connectivity before restoration, especially the highest and steepest ones, with an overall barrier effect of about 51% of the maximal theoretical impact. Most importantly, we demonstrated for the first time that mitigation measures such as dam removal or fish pass creation significantly and rapidly improved connectivity, with –for some barriers- a complete recovery of the genetic connectivity in less than twelve months. Our study provides a unique and strong proof-of-concept that barrier removal is an efficient strategy to restore riverine

35 connectivity and that molecular tools can provide accurate measures of restoration efficiency within a
36 few months.

37 Keywords: Genetic monitoring; Restoration; Weirs; Low-head dams; FINDEX; Fish passes; Dam removal;
38 Genetic connectivity; Potamodromous

1. INTRODUCTION

Anthropogenic activities exert strong pressures on natural ecosystems, which alter both their physical and biological properties (Crutzen, 2006). This is especially the case for rivers that are highly fragmented by man-made instream barriers such as dams, weirs, water mills, etc. (Grill et al., 2019). In Europe for instance, more than one million obstacles have been reported (Belletti et al., 2020), representing 0,74 barriers per kilometer. Riverscape fragmentation affects the quality, the quantity and the accessibility of natural habitats, and thus prevents organisms to fulfill their life-cycle (Taylor et al., 1993). It is considered one of the most important threat to freshwater biodiversity (Sala, 2000). Given this “hyper-fragmentation” of rivers, the restoration of longitudinal (i.e., upstream-downstream) connectivity to promote fluxes of individuals and genes is hence considered a crucial step to recover the integrity of river ecosystems (Baguette et al., 2013; King & O’Hanley, 2016). Connectivity restoration is moreover the subject of restrictive legislations in many countries, such as in Europe with the Water Framework Directive (2000/60/EC), on which is based the EU’s biodiversity strategy for 2030 aiming at restoring at least 25,000 km of rivers to a free-flowing state (COM/2020/380).

The restoration of longitudinal connectivity implies barrier mitigation measures: the removal of obstacles, or, when removal is not an option (Blanchet & Tedesco, 2021; Lejon et al., 2009; Magilligan et al., 2017), the equipment of obstacles with natural or artificial fish passes (Seliger & Zeiringer, 2018; Silva et al., 2018). However, the actual efficiency of these barrier mitigation measures to restore genetic and/or demographic connectivity (Lowe & Allendorf, 2010) appear somehow unpredictable, depending on the type of river, the type of obstacle, the chosen type of restoration, as well as the timescale and the species considered (Rodeles et al., 2020). Dam removal has been found beneficial for the rapid recovery of some diadromous fish species (mainly salmonids) whose upstream migratory movements and thus spatial distribution were limited by the presence of barriers (Ding et al., 2019). However other

organisms such as potamodromous fish (but also macroinvertebrates, macrophytes, etc.) may not (immediately) benefit from such removal (Brenkman et al., 2019; Gillette et al., 2016). Similarly, fish passes often show uneven levels of permeability across species, depending on pass design and maintenance as well as environmental conditions (Birnie-Gauvin et al., 2019; Harris et al., 2017; Noonan et al., 2012). These effects are yet still poorly documented, notably in the case of low-head structures (weirs, water-mills, sluices...) that do not benefit from the same attractivity as large dams in terms of public interest and research funding (O'Connor et al., 2015). Low-head structures are actually largely understudied when compared to their prevalence and the restoration efforts they represent (Belletti et al., 2020; Ryan Bellmore et al., 2017). Classical methods for the assessment of connectivity restoration efficiency, such as capture-mark-recapture, telemetry and monitoring of spatiotemporal changes in the composition of fish communities (Rodeles et al., 2020; Silva et al., 2018), cannot be systematically deployed at large management scales, especially for practitioners who are generally constrained by time and budget. For low-head barriers, mitigation measures are often undertaken opportunistically (Poff et al., 2003; Tonitto & Riha, 2016), with limited or coarse ecological monitoring of the system before restoration (Barry et al., 2018) and no (or limited) evaluation of the outcome in most cases (Cooke et al., 2019; Rodeles et al., 2017).

One challenge for practitioners is notably the lack of rapid and efficient connectivity assessment tools allowing both the *a priori* quantification of the individual impact of instream obstacles and the *a posteriori* quantification of the efficiency of implemented measures (removal or equipment; Cooke et al., 2019). If molecular tools are now commonly considered for the *a priori* assessment of barrier effects (Abernethy et al., 2013; Coleman et al., 2018; Dehais et al., 2010; Gouskov et al., 2016; Liu et al., 2020; Meldgaard et al., 2003; Prunier et al., 2018; Raeymaekers et al., 2009), there is still a surprising paucity of genetic studies dedicated to the temporal monitoring of changes in connectivity after restoration (Ding et al., 2019). Only a handful of recent studies could be identified, all focusing on the

effect of the creation (Liu et al., 2020; Vega-Retter et al., 2020) or the removal (Fraik et al., 2021) of large dams on gene flow (but see Weigel et al., 2013). As a result, and despite the importance for practitioners and managers to assess (and communicate) the relevance of their actions for natural ecosystems, there are still very few indications that (i) current mitigation measures (barrier removal or fish pass creation) deployed worldwide are improving genetic connectivity (*sensu* Lowe & Allendorf, 2010) and (ii) that molecular approaches are efficient and operational tools to prioritize local restoration actions and assess their efficiency.

In this study, we implemented a “before-after genetic monitoring” of the restoration of 11 weirs in France using a recently developed genetic index of fragmentation (the F_{INDEX} ; Prunier et al., 2020). The F_{INDEX} provides, independently for each obstacle, an absolute and standardized estimate of (species-specific) genetic connectivity, while taking into account two confounding factors: the age of the obstacle and the size of populations on either side of the obstacle. Considering two common potamodromous fish genera, our objectives were (i) to quantify the initial impact of obstacles and thus to determine whether restoration was actually needed in the first place, and (ii) to quantify the gain in connectivity resulting from implemented restoration actions.

2. MATERIALS AND METHODS

2.1. Instream obstacles

The study took place at the scale of the French national hydrographic network. In close coordination with the French Office for Biodiversity (OFB) and French Water Agencies, we identified 11 obstacles (low-head dams <4m high) whose restoration was scheduled for the coming years (Figure 1; Table 1). These obstacles were located in the three largest French river basins (Garonne, Loire and Seine). On the basis of the consultation of old aerial photos and maps (French Ordnance Survey maps and cadastral plans), completed by surveys of local agencies, we estimated that they were constructed (or reconstructed after destruction or abandonment) between the 15th and the 20th century (Table 1). The obstacles ranged from 0.8 to 3.5m high and showed various slopes (Figure 1). Two of them were already equipped with a fish pass (Table 1). Each obstacle was described according to its height (< or ≥ 2m high) and slope (< or ≥ 45°) using a unique synthetic factor ‘Typology’ with four levels (‘low and gentle’, ‘low and steep’, ‘high and gentle’, ‘high and steep’). The restoration actions were conducted between 2015 and 2019 and in most cases (9 out of 11) consisted in the dismantlement of the obstacle. Note that we could not statistically assess the specific effects of fish passes on connectivity in the following analyses because there were too few of them, either before or after restoration.

2.2. Biological models and Before-After sampling sessions

We focused on five common potamodromous species from two genera: minnows (*Phoxinus sp.*: *P. phoxinus* in the Seine, *P. fayollarum* in the Loire and *P. dragarum* in the Garonne watershed) and gudgeons (*Gobio sp.*: *G. gobio* in the Loire and the Seine and *G. occitaniae* in the Garonne watershed). Within each genus, species are allopatric (Denys et al., 2020) but were here considered to have very similar life history traits and movement behaviors (Keith et al., 2011). These species are small insectivorous cyprinids (maximal body length of 140 and 200mm, in minnows and gudgeons

respectively) that show distinct foraging strategies: minnows preferentially feeds in the water column, whereas gudgeons feed on the bottom (Keith et al., 2011). We selected these species because they are widespread and abundant and hence easy to catch for practitioners, which make them ideal models for an operational tool such as the F_{INDEX} .

Sampling operations ‘Before’ and ‘After’ restoration were performed using electrofishing, until a maximum of 30 adult individuals of each species were captured on either side of obstacles. Fish were captured in the direct downstream and upstream vicinity of each obstacle (Prunier et al., 2020), starting from the downstream site to avoid accidental upstream to downstream movements of individuals. A piece of pelvic fin was sampled on all individuals and stored in 96% alcohol. All fish were returned alive to their sampling site. Electrofishing and fin sampling was performed according to legal authorizations and permits.

Both genera could be sampled at all sites except in DADRai (gudgeons only) and GLASou (minnows only). The sampling sessions after the restoration occurred on average $9.7 (\pm 3.9 \text{ SD})$ months after the end of the restoration (from 44 days in VIAPig to 427 days in CEOSal; Table 1). These after-restoration timelags could not be homogenized better, owing to logistic and administrative difficulties (e.g., high water levels preventing safe fieldwork).

2.3. Genotyping and F_{INDEX} computation

We considered 19 and 15 microsatellite markers in minnows and gudgeons, respectively. DNA extraction, genotyping and assessment of null alleles and gametic disequilibrium followed previously published procedures (Prunier et al., 2018, 2020; Appendix S1). For each dataset (combination of one obstacle and one genus; $n = 20$ since only one genus could be sampled at GLASou and DADRai; Table 2) and each passage (‘Before’ and ‘After’), we computed the F_{INDEX} and the standard deviation of the F_{INDEX} as detailed in Prunier et al. (2020) using a dedicated R-based pipeline. Briefly, the F_{INDEX} corresponds to

the rescaling of pairwise measures of genetic differentiation within their theoretical range of variation given effective population sizes (approximated from expected heterozygosity) and the number of generations elapsed since barrier creation. The F_{INDEX} is therefore a standardized (across barriers and species) index expressed as a percentage, with values lower than 20% representing fully permeable structures and values higher than 90% representing total barriers to gene flow (see Prunier et al., 2020 for details). F_{INDEX} values are computed along with a standard deviation SD_F (or a 95% confidence interval $CI_{95\%}$) that takes into account biological uncertainty stemming from $n_F = 4$ parameters (two mutation rates and two metrics of genetic differentiation; Prunier et al., 2020). We considered a generation time of 2 years in minnows and 2.5 years in gudgeons to compute the number of generations elapsed since barrier creation (Kottelat & Freyhof, 2007).

2.4. Barrier effects and restoration efficiency

We adopted a meta-analytical approach (Borenstein, 2009), considering each dataset (combination of one obstacle and one genus; $n = 20$) as an independent study providing two effect sizes (F_{INDEX} values before and after restoration $\pm SD_F$). Six datasets were non-informative ($F_{\text{INDEX}} = 0$ and $SD_F = 0$ before restoration) and were thus discarded, resulting in 14 datasets, of which three were associated with a non-significant barrier effect (see results). The interpretation of the F_{INDEX} being meaningful (barrier effect expressed as a percentage of maximum fragmentation), the F_{INDEX} value before restoration was directly used as the observed effect size $F \pm SE_F$ of barrier effect in each dataset, with $SE_F = SD_F / \sqrt{n_F}$. We used the raw difference $\Delta F (\pm SE_{\Delta})$ between F_{INDEX} values computed after ('treatment') and before ('control') restoration as the observed effect size of restoration for each dataset (Equations 4.2 and 4.6 in Borenstein, 2009). The raw difference ΔF can be directly interpreted as

the (positive or negative) change in the amount of fragmentation following restoration and was thus preferred over the standardized mean difference Hedges' g (Borenstein, 2009).

We used random-effect meta-regressions with moderators (metafor::rma.mv R-function; Harrer et al., 2021; Viechtbauer, 2010) to compute the overall true effect sizes of the barrier effects \bar{F} and of the restoration of obstacles $\overline{\Delta F}$ ($n = 14$), while taking into account different possible sources of variation in effect sizes: within-datasets (i.e., SE_F or SE_{Δ}), between-datasets and, possibly, across different covariate modalities. Considered covariates were 'Genus' (two levels: minnows or gudgeons), 'Typology' (four levels: see above), as well as, in the case of $\overline{\Delta F}$, the 'timelag' (two levels: < 1 year or > 1 year) between the restoration operation and the second sampling session. To account for within-dataset variability (SE_F or SE_{Δ}), we used dataset ID as an outer random grouping factor. To allow residual heterogeneity to differ across covariate modalities, each covariate was successively defined both as a moderator and an inner random grouping factor. Models were run with a diagonal variance-covariance matrix as a random effect structure. Covariates identified as significant moderators were then kept as inner random grouping factors in final models without moderator to get final estimates of the true effect sizes \bar{F} and $\overline{\Delta F}$ along with their respective 95% confidence interval $CI_{95\%}$.

3. RESULTS

Altogether, a total of 1049 and 1038 genotypes could be obtained from the various sampling sessions (through space and time) in minnows and in gudgeons, respectively, with an overall mean of 26.1 (\pm 3.2 SD) genotypes per genus, sampling site and sampling session. The mean number of loci across datasets was 15.7 (\pm 1.8 SD) and 14.4 (\pm 0.8 SD) in minnows and in gudgeons, respectively.

F_{INDEX} values ranged from 0 to 68.1 % (mean: 27.7 \pm 25.8 SD) before restoration, with a significant barrier effect detected in 11 out of 20 datasets (Table 2). Genera showed highly contrasted responses to obstacles: all obstacles but GLAMou and LEZVil had a significant impact on connectivity before restoration, but only VIAPig and GLAPas did impact both genera simultaneously (Table 2; Figure 2). Accordingly, 'Genus' was not identified as a significant moderator of the overall effect size \bar{F} (Table 3). Only 'Typology' did significantly influence \bar{F} , with steep obstacles higher than 2 m showing an overall effect size \bar{F} 40 % ($CI_{95\%} = [14.2; 65.8]$) higher than gentle weirs lower than 2 m (Table 3; Figure 3). Once all possible sources of variation in effect sizes were taken into account (both within-datasets and between modalities of typology), the final true effect size of fragmentation \bar{F} was of 51.4 %, a value significantly different from 0 and higher than the F_{INDEX} significance threshold of 20% ($CI_{95\%} = [45.1; 57.7]$).

Except when obstacles had no effect before restoration ($F_{\text{INDEX}} < 20\%$), in which case restoration had no effect either ($F_{\text{INDEX}} < 20\%$ after restoration; 5/10 obstacles in gudgeons and 4/10 obstacles in minnows), restoration systematically led to a significant decrease in F_{INDEX} values (as indicated by non-overlapping confidence intervals; Figure 2), with F_{INDEX} values after restoration ranging from 0 to 49.1 % (mean: 13.7 \pm 17.8 SD). This decrease led to the full recovery of connectivity ($F_{\text{INDEX}} < 20\%$ after restoration) in 3/5 obstacles in gudgeons and 2/6 obstacles in minnows. The observed effect sizes of restoration ΔF ranged from -55.4% to +11.7% (mean: -14 \pm 18 SD; Table 2). Neither 'Genus', 'Typology'

or ‘Timelag’ were identified as significant moderators of the overall effect size of restoration $\overline{\Delta F}$ (Table 3, Appendix S2). The final overall effect size of restoration $\overline{\Delta F}$ was of -21.7%, a value significantly different from 0 ($CI_{95\%} = [-30.2; -13.2]$). Overall, the effect of restoration was thus the same across genera, did not depend on the typology of the obstacle and, interestingly, was independent from the timelag between the restoration and the second sampling session. In other words, we found evidence that genetic connectivity could be recovered (entirely in some cases, partly in most cases) in just a few months after restoration.

4. DISCUSSION

Quantifying the impact of instream barriers on potamodromous fish species as well as the efficiency of mitigation measures is primordial in the context of restoration planning, so as to properly allocate limited resources towards the most impactful obstacles, inform trade-offs between ecological and socio-economic issues, and refine restoration techniques (Hermoso et al., 2012; Rodeles et al., 2020; Silva et al., 2018). Quantification is yet a difficult task, notably because of technical and financial constraints preventing the parallel monitoring of multiple obstacles and because of the relative lack of operational tools allowing valid comparisons across both contexts and species (Cayuela et al., 2018). The response of freshwater organisms to connectivity restoration has often been studied at the community- or at the population-levels (Brenkman et al., 2019; Frey, 2021; Magilligan et al., 2021; Muha et al., 2021; Stanley et al., 2002; Sun et al., 2021), rarely at the genetic level (Fraik et al., 2021), and our study is the first to document the systematic and rapid recovery of gene flow over a series of independent restoration actions. In this study, we used a standardized genetic index of fragmentation to quantify both the impact of 11 low-head dams on gene flow in five freshwater fish species, and the efficiency of mitigation actions in restoring genetic connectivity.

Before restoration, we found a significant barrier effect in 11 out of 20 datasets, with two obstacles showing no impact on any genus and two obstacles significantly impacting both genera. Surprisingly, out of nine obstacles with genetic data in both genera, five showed large discrepancies in genus response to fragmentation, with either only gudgeons or only minnows significantly impacted (Figure 2). These discrepancies illustrate how barrier effects can be highly species- or genus-dependent (Amaral et al., 2021; Blanchet et al., 2010; Prunier et al., 2018), and thus hardly predictable given our limited knowledge about fish movement behavior and capacities (Baudoin et al., 2014; Thurow, 2016). In absence of a dedicated fish pass, individuals are supposed to take advantage of drowned conditions,

that is, of periods where water level rises above the height of the dam, to cross the obstacle (Keller et al., 2012). However, such propitious conditions of obstacle drowning might not be encountered every year, at all localities, and equally across all species/individuals, depending on their swimming behavior and capabilities in various environmental conditions and to the timing of submersion compared with the timing of individual movements (Carpenter-Bundhoo et al., 2020; Holthe et al., 2005; Keller et al., 2012). This may explain why F_{INDEX} values differed so much across datasets, and why the overall effect size of fragmentation \bar{F} was unrelated to the considered genus. However, \bar{F} was significantly influenced by the typology of obstacles, with high and steep obstacles showing an overall effect size \bar{F} 40% higher than low and gentle obstacles (Table 3, Figure 3). This finding is in line with classical expectations about the impact of dam typology (e.g., Januchowski-Hartley et al., 2019) and conclusions from other studies (e.g., Amaral et al., 2019; Keller et al., 2012; Zigler et al., 2004), as well as with the obstacle drowning hypothesis: the highest dams (≥ 2 m) might rarely be drowned (leading to the increase in \bar{F}), but, in presence of a gentle slope ($< 45^\circ$), they might become partly crossable by some individuals, at least at intermediate drowning conditions, so that only the highest and steepest obstacles have an overall significant effect size ($\bar{F} = 54.6\%$; Figure 3). This result should of course be confirmed and refined with additional datasets, but it provides a relevant and meaningful benchmark for practitioners to adjust restoration planning even in the absence of any individual quantification of barrier effects.

With this effect of typology taken into account, we estimated the true effect size of fragmentation as of $\bar{F} = 51.4\%$: in other words, we might expect a 51 % decrease in gene flow in presence of a low-head dam (irrespective of the species or the context). Given these different findings, it appears that a preliminary diagnosis based on a standardized genetic tool such as the F_{INDEX} may actually help managers quantify and compare barrier effects across species and obstacles, and thus orientate their restoration efforts towards the most problematic structures (Prunier et al., 2020), keeping in mind that the most impactful obstacles might be the highest and steepest ones, but that more seemingly anodyne obstacles

might also be particularly impacting for some species. Of course, we willingly acknowledge that other ecological and socio-economic indicators should be considered in restoration planning as well (Hermoso et al., 2012). It is also noteworthy that the F_{INDEX} might help evaluate the species-specific efficiency of fish passes, an important step to drive future technical developments (Foulds & Lucas, 2013): the two considered fish passes before restoration appeared beneficial in all situations except for minnows at SIOBre (Figure 2), illustrating the challenge of locally designing passes adapted to different fish species with distinct life-history traits and requirements (Birnie-Gauvin et al., 2019; Silva et al., 2018).

Our most striking result concerns the efficiency of mitigation measures. All restoration actions led to a significant reduction of barrier effects, provided there was an actual barrier effect in the first place. We quantified an overall 22% decrease in fragmentation levels following restoration. This means that mitigation measures may allow the full recovery of genetic connectivity for any initial fragmentation level of up to 42% (i.e., $20\% < F_{INDEX} \leq 42\%$), a value to be compared to the overall effect size of fragmentation $\bar{F} = 51\%$ before restoration. Most interestingly, this systematic gain in connectivity was achieved within a few months after restoration only. For instance, weir removal led to the full recovery of genetic connectivity at three localities (LEZCas, VIAPig and GLAPas), indicating that it ensured the full mixing of individuals, and thus of allelic frequencies, within a year, and even within two months only at VIAPig. However, not all restoration actions proved equally efficient within the same timeframe, the recovery of connectivity being only partial in several situations. This is the case of the two (new or improved) fish passes at SERHau and SIOBre that resulted in an 8 to 12% recovery in genetic connectivity in minnows within a year. These reductions are highly encouraging, but it is still unknown whether recovery is still ongoing: further temporal monitoring is needed to detect migration-drift equilibrium and determine the final gain in connectivity following restoration. Furthermore, and although weir removal is expected to be more efficient than fish pass creation as a restoration option (Birnie-Gauvin et al., 2019; Sun et al., 2021), other removal actions only led to a partial recovery of connectivity,

sometimes even after a year (e.g., at CEOSal in minnows). The migration behavior of fish being still poorly documented and likely to deeply differ across species and contexts, it probably explains why the true effect size of restoration was unrelated to the considered genus, the typology of the obstacle or the timelag between restoration and sampling. We can only speculate on why close-range genetic mixing would sometimes take so long despite the absence of any obstacle to movement. For instance, weir removal might result in profound changes in upstream and downstream habitat characteristics (Bednarek, 2001; Doyle et al., 2005), locally inducing a temporary repelling effect on fish. It has also been suggested that, in some conditions, fragmentation might lead individuals to adjust their life-history strategies towards residency (Branco et al., 2017), which might further delay genetic connectivity after removal. Nevertheless, we expect the full recovery of genetic connectivity in the coming years, which will however require further genetic monitoring of these situations.

5. CONCLUSION

Our study provides a strong proof-of-concept that barrier removal and, probably to a lesser extent, fish pass creation, are efficient mitigation strategies to restore riverine genetic connectivity in just a few months. We also illustrated how before-after genetic monitoring based on a standardized tool such as the F_{INDEX} constitute a promising support for practitioners in the planning and the monitoring of restoration. We believe that the large-scale deployment of this methodology in the future, with a growing number of case studies, will make it possible to lift the veil on the complex links between individual crossing success, life history traits of organisms and barrier typologies.

ACKNOWLEDGEMENTS

This study was financially supported by the Office Français pour la Biodiversité and the Région Occitanie. We warmly thank all the members of local agencies (Office Français pour la Biodiversité, Fédérations de Pêche, Syndicats Mixtes, Agences de l'Eau) that have been involved in this project and directly helped us fulfill our objectives, either by performing the sampling or by providing valuable information about local restoration actions.

AUTHORS' CONTRIBUTIONS

JGP, SB and NP conceived the ideas and designed methodology; JGP and SB collected the samples in the field; CV and GL produced molecular data; JGP analyzed the data; JGP and SB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

REFERENCES

323 Abernethy, E., McCombs, E., Siefferman, L., & Gangloff, M. (2013). Effect of Small Dams on Freshwater
324 Mussel Population Genetics in Two Southeastern Usa Streams. *Freshwater Mollusk Biology and*
325 *Conservation*, 16(1), 21. <https://doi.org/10.31931/fmbc.v16i1.2013.21-28>

326 Adamack, A. T., & Gruber, B. (2014). POPGENREPORT: Simplifying basic population genetic analyses in R.
327 *Methods in Ecology and Evolution*, 5(4), 384–387. <https://doi.org/10.1111/2041-210X.12158>

328 Aljanabi, S. M., & Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for
329 PCR-based techniques. *Nucleic Acids Research*, 25(22), 4692–4693.

330 Amaral, S. D., Branco, P., Katopodis, C., Ferreira, M. T., Pinheiro, A. N., & Santos, J. M. (2019). Passage
331 Performance of Potamodromous Cyprinids over an Experimental Low-Head Ramped Weir: The
332 Effect of Ramp Length and Slope. *Sustainability*, 11(5), 1456.
333 <https://doi.org/10.3390/su11051456>

334 Amaral, S. D., Branco, P., Romão, F., Ferreira, M. T., Pinheiro, A. N., & Santos, J. M. (2021). Evaluation of
335 Low-Head Ramped Weirs for a Potamodromous Cyprinid: Effects of Substrate Addition and
336 Discharge on Fish Passage Performance, Stress and Fatigue. *Water*, 13(6), 765.
337 <https://doi.org/10.3390/w13060765>

338 Baguette, M., Blanchet, S., Legrand, D., Stevens, V. M., & Turlure, C. (2013). Individual dispersal,
339 landscape connectivity and ecological networks: Dispersal, connectivity and networks. *Biological*
340 *Reviews*, 88(2), 310–326. <https://doi.org/10.1111/brv.12000>

341 Barry, J., Coghlan, B., Cullagh, A., Kerr, J. R., & King, J. J. (2018). Comparison of coarse-resolution rapid
342 methods for assessing fish passage at riverine barriers: ICE and SNIFFER protocols: Protocol
343 comparison of fish passability at barriers. *River Research and Applications*, 34(9), 1168–1178.
344 <https://doi.org/10.1002/rra.3358>

Baudoin, J.-M., Burgun, V., Chanseau, M., Larinier, M., Ovidio, M., Sremski, W., Steinbach, P., & Voegtle, B. (2014). *The ICE protocol for ecological continuity, Assessing the passage of obstacles by fish: Concepts, design and application*. Onema.

Bednarek, A. T. (2001). Undamming Rivers: A Review of the Ecological Impacts of Dam Removal. *Environmental Management*, 27(6), 803–814. <https://doi.org/10.1007/s002670010189>

Belletti, B., Garcia de Leaniz, C., Jones, J., Bizzi, S., Börger, L., Segura, G., Castelletti, A., van de Bund, W., Aarestrup, K., Barry, J., Belka, K., Berkhuisen, A., Birnie-Gauvin, K., Bussettini, M., Carolli, M., Consuegra, S., Dopico, E., Feierfeil, T., Fernández, S., ... Zalewski, M. (2020). More than one million barriers fragment Europe’s rivers. *Nature*, 588(7838), 436–441. <https://doi.org/10.1038/s41586-020-3005-2>

Birnie-Gauvin, K., Franklin, P., Wilkes, M., & Aarestrup, K. (2019). Moving beyond fitting fish into equations: Progressing the fish passage debate in the Anthropocene. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 29(7), 1095–1105. <https://doi.org/10.1002/aqc.2946>

Blanchet, S., Rey, O., Etienne, R., Lek, S., & Loot, G. (2010). Species-specific responses to landscape fragmentation: Implications for management strategies. *Evolutionary Applications*, 3(3), 291–304. <https://doi.org/10.1111/j.1752-4571.2009.00110.x>

Blanchet, S., & Tedesco, P. A. (2021). French vote for river barriers defies biodiversity strategy. *Nature*, 594(7861), 26–26. <https://doi.org/10.1038/d41586-021-01467-0>

Borenstein, M. (Ed.). (2009). *Introduction to meta-analysis*. John Wiley & Sons.

Branco, P., Amaral, S. D., Ferreira, M. T., & Santos, J. M. (2017). Do small barriers affect the movement of freshwater fish by increasing residency? *Science of The Total Environment*, 581–582, 486–494. <https://doi.org/10.1016/j.scitotenv.2016.12.156>

Brenkman, S. J., Peters, R. J., Tabor, R. A., Geffre, J. J., & Sutton, K. T. (2019). Rapid Recolonization and Life History Responses of Bull Trout Following Dam Removal in Washington’s Elwha River. *North*

American Journal of Fisheries Management, 39(3), 560–573.

<https://doi.org/10.1002/nafm.10291>

Carpenter-Bundhoo, L., Butler, G. L., Bond, N. R., Bunn, S. E., Reinfelds, I. V., & Kennard, M. J. (2020).

Effects of a low-head weir on multi-scaled movement and behavior of three riverine fish species.

Scientific Reports, 10(1), 6817. <https://doi.org/10.1038/s41598-020-63005-8>

Cayuela, H., Rougemont, Q., Prunier, J. G., Moore, J.-S., Clobert, J., Besnard, A., & Bernatchez, L. (2018).

Demographic and genetic approaches to study dispersal in wild animal populations: A

methodological review. *Molecular Ecology*, 27(20), 3976–4010.

<https://doi.org/10.1111/mec.14848>

Coleman, R. A., Gauffre, B., Pavlova, A., Beheregaray, L. B., Kearns, J., Lyon, J., Sasaki, M., Leblois, R.,

Sgro, C., & Sunnucks, P. (2018). Artificial barriers prevent genetic recovery of small isolated

populations of a low-mobility freshwater fish. *Heredity*. <https://doi.org/10.1038/s41437-017->

0008-3

Cooke, S. J., Bennett, J. R., & Jones, H. P. (2019). We have a long way to go if we want to realize the

promise of the “Decade on Ecosystem Restoration.” *Conservation Science and Practice*, 1(12).

<https://doi.org/10.1111/csp2.129>

Crutzen, P. J. (2006). The “Anthropocene.” In E. Ehlers & T. Krafft (Eds.), *Earth System Science in the*

Anthropocene (pp. 13–18). Springer-Verlag. https://doi.org/10.1007/3-540-26590-2_3

Dehais, C., Eudeline, R., Berrebi, P., & Argillier, C. (2010). Microgeographic genetic isolation in chub

(Cyprinidae: *Squalius cephalus*) population of the Durance River: estimating fragmentation by

dams: Genetic isolation in chub. *Ecology of Freshwater Fish*, 19(2), 267–278.

<https://doi.org/10.1111/j.1600-0633.2010.00411.x>

- Denys, G. P. J., Dettai, A., Persat, H., Daszkiewicz, P., Hauteceœur, M., & Keith, P. (2020). *Revision of Phoxinus in France with the description of two new species (Teleostei, Leuciscidae)*. <https://doi.org/10.26028/CYBIUM/2020-443-003>
- Ding, L., Chen, L., Ding, C., & Tao, J. (2019). Global Trends in Dam Removal and Related Research: A Systematic Review Based on Associated Datasets and Bibliometric Analysis. *Chinese Geographical Science*, 29(1), 1–12. <https://doi.org/10.1007/s11769-018-1009-8>
- Doyle, M. W., Stanley, E. H., Orr, C. H., Selle, A. R., Sethi, S. A., & Harbor, J. M. (2005). Stream ecosystem response to small dam removal: Lessons from the Heartland. *Geomorphology*, 71(1–2), 227–244. <https://doi.org/10.1016/j.geomorph.2004.04.011>
- Foulds, W. L., & Lucas, M. C. (2013). Extreme inefficiency of two conventional, technical fishways used by European river lamprey (*Lampetra fluviatilis*). *Ecological Engineering*, 58, 423–433. <https://doi.org/10.1016/j.ecoleng.2013.06.038>
- Fraik, A. K., McMillan, J. R., Liermann, M., Bennett, T., McHenry, M. L., McKinney, G. J., Wells, A. H., Winans, G., Kelley, J. L., Pess, G. R., & Nichols, K. M. (2021). The Impacts of Dam Construction and Removal on the Genetics of Recovering Steelhead (*Oncorhynchus mykiss*) Populations across the Elwha River Watershed. *Genes*, 12(1), 89. <https://doi.org/10.3390/genes12010089>
- Frey, R. (2021). Immediate response of black basses to dam removal is determined by habitat preference. *Masters Theses*, 4862. <https://thekeep.eiu.edu/theses/4862>
- Gillette, D. P., Daniel, K., & Redd, C. (2016). Fish and Benthic Macroinvertebrate Assemblage Response to Removal of a Partially Breached Lowhead Dam: Assemblage Response to Lowhead Dam Removal. *River Research and Applications*, 32(8), 1776–1789. <https://doi.org/10.1002/rra.3017>
- Gouskov, A., Reyes, M., Wirthner-Bitterlin, L., & Vorburger, C. (2016). Fish population genetic structure shaped by hydroelectric power plants in the upper Rhine catchment. *Evolutionary Applications*, 9(2), 394–408. <https://doi.org/10.1111/eva.12339>

415 Grenier, R., Costedoat, C., Chappaz, R., & Dubut, V. (2013). Two multiplexed sets of 21 and 18
416 microsatellites for *Phoxinus phoxinus* (L.) and *Gobio gobio* (L.) developed by cross-species
417 amplification. *European Journal of Wildlife Research*, 59(2), 291–297.
418 <https://doi.org/10.1007/s10344-013-0693-z>

419 Grill, G., Lehner, B., Thieme, M., Geenen, B., Tickner, D., Antonelli, F., Babu, S., Borrelli, P., Cheng, L.,
420 Crochetiere, H., Ehalt Macedo, H., Filgueiras, R., Goichot, M., Higgins, J., Hogan, Z., Lip, B.,
421 McClain, M. E., Meng, J., Mulligan, M., ... Zarfl, C. (2019). Mapping the world’s free-flowing
422 rivers. *Nature*, 569(7755), 215–221. <https://doi.org/10.1038/s41586-019-1111-9>

423 Harrer, M., Cuijpers, P., Furukawa, T. A., & Ebert, D. D. (2021). *Doing meta-analysis with R: A hands-on*
424 *guide* (First edition). CRC Press.

425 Harris, J. H., Kingsford, R. T., Peirson, W., & Baumgartner, L. J. (2017). Mitigating the effects of barriers to
426 freshwater fish migrations: The Australian experience. *Marine and Freshwater Research*, 68(4),
427 614. <https://doi.org/10.1071/MF15284>

428 Hermoso, V., Pantus, F., Olley, J., Linke, S., Mugodo, J., & Lea, P. (2012). Systematic planning for river
429 rehabilitation: Integrating multiple ecological and economic objectives in complex decisions.
430 *Freshwater Biology*, 57(1), 1–9. <https://doi.org/10.1111/j.1365-2427.2011.02693.x>

431 Holthe, E., Lund, E., Finstad, B., Thorstad, E. B., & McKinley, R. S. (2005). A fish selective obstacle to
432 prevent dispersion of an unwanted fish species, based on leaping capabilities. *Fisheries*
433 *Management and Ecology*, 12(2), 143–147.

434 Januchowski-Hartley, S. R., Jézéquel, C., & Tedesco, P. A. (2019). Modelling built infrastructure heights to
435 evaluate common assumptions in aquatic conservation. *Journal of Environmental Management*,
436 232, 131–137. <https://doi.org/10.1016/j.jenvman.2018.11.040>

437 Keith, P., Persat, H., Feunteun, E., Adam, B., & Geniez, M. (2011). *Les Poissons d’eau douce de France*
438 (Muséum National d’Histoire Naturelle and Publications Biotope).

- Keller, R. J., Peterken, C. J., & Berghuis, A. P. (2012). Design and assessment of weirs for fish passage under drowned conditions. *Ecological Engineering*, 48, 61–69.
<https://doi.org/10.1016/j.ecoleng.2011.06.037>
- Kemp, P. S., & O’Hanley, J. R. (2010). Procedures for evaluating and prioritising the removal of fish passage barriers: A synthesis. *Fisheries Management and Ecology*, no-no.
<https://doi.org/10.1111/j.1365-2400.2010.00751.x>
- King, S., & O’Hanley, J. R. (2016). Optimal Fish Passage Barrier Removal—Revisited. *River Research and Applications*, 32(3), 418–428. <https://doi.org/10.1002/rra.2859>
- Kottelat, M., & Freyhof, J. (2007). *Handbook of european freshwater fishes*. Publications Kottelat.
- Lejon, A. G. C., Malm Renöfält, B., & Nilsson, C. (2009). Conflicts Associated with Dam Removal in Sweden. *Ecology and Society*, 14(2), art4. <https://doi.org/10.5751/ES-02931-140204>
- Liu, D., Li, X., & Song, Z. (2020). No decline of genetic diversity in elongate loach (*Leptobotia elongata*) with a tendency to form population structure in the upper Yangtze River. *Global Ecology and Conservation*, 23, e01072. <https://doi.org/10.1016/j.gecco.2020.e01072>
- Lowe, W. H., & Allendorf, F. W. (2010). What can genetics tell us about population connectivity? *Molecular Ecology*, 19(15), 3038–3051. <https://doi.org/10.1111/j.1365-294X.2010.04688.x>
- Magilligan, F. J., Nislow, K. H., Dietrich, J. T., Doyle, H., & Kynard, B. (2021). Transient versus sustained biophysical responses to dam removal. *Geomorphology*, 389, 107836.
<https://doi.org/10.1016/j.geomorph.2021.107836>
- Magilligan, F. J., Sneddon, C. S., & Fox, C. A. (2017). The Social, Historical, and Institutional Contingencies of Dam Removal. *Environmental Management*, 59(6), 982–994. <https://doi.org/10.1007/s00267-017-0835-2>
- Meldgaard, T., Nielsen, E. E., & Loeschcke, V. (2003). Fragmentation by weirs in a riverine system: A study of genetic variation in time and space among populations of European grayling (*Thymallus*

thymallus) in a Danish river system. *Conservation Genetics*, 4(6), 735–747.

<https://doi.org/10.1023/B:COGE.00000006115.14106.de>

Muha, T. P., Rodriguez-Barreto, D., O'Rourke, R., Garcia de Leaniz, C., & Consuegra, S. (2021). Using eDNA Metabarcoding to Monitor Changes in Fish Community Composition After Barrier Removal. *Frontiers in Ecology and Evolution*, 9, 629217. <https://doi.org/10.3389/fevo.2021.629217>

Noonan, M. J., Grant, J. W. A., & Jackson, C. D. (2012). A quantitative assessment of fish passage efficiency: Effectiveness of fish passage facilities. *Fish and Fisheries*, 13(4), 450–464. <https://doi.org/10.1111/j.1467-2979.2011.00445.x>

O'Connor, J. E., Duda, J. J., & Grant, G. E. (2015). 1000 dams down and counting. *Science*, 348(6234), 496–497. <https://doi.org/10.1126/science.aaa9204>

Poff, N. L., Allan, J. D., Palmer, M. A., Hart, D. D., Richter, B. D., Arthington, A. H., Rogers, K. H., Meyer, J. L., & Stanford, J. A. (2003). River flows and water wars: Emerging science for environmental decision making. *Frontiers in Ecology and the Environment*, 1(6), 298–306.

Prunier, J. G., Dubut, V., Loot, G., Tudesque, L., & Blanchet, S. (2018). The relative contribution of river network structure and anthropogenic stressors to spatial patterns of genetic diversity in two freshwater fishes: A multiple-stressors approach. *Freshwater Biology*, 63(1), 6–21. <https://doi.org/10.1111/fwb.13034>

Prunier, J. G., Poesy, C., Dubut, V., Veyssi re, C., Loot, G., Poulet, N., & Blanchet, S. (2020). Quantifying the individual impact of artificial barriers in freshwaters: A standardized and absolute genetic index of fragmentation. *Evolutionary Applications*, eua.13044. <https://doi.org/10.1111/eva.13044>

Raeymaekers, J. A. M., Raeymaekers, D., Koizumi, I., Geldof, S., & Volckaert, F. A. M. (2009). Guidelines for restoring connectivity around water mills: A population genetic approach to the

management of riverine fish. *Journal of Applied Ecology*, 46(3), 562–571.

<https://doi.org/10.1111/j.1365-2664.2009.01652.x>

Rodeles, A. A., Galicia, D., & Miranda, R. (2017). Recommendations for monitoring freshwater fishes in river restoration plans: A wasted opportunity for assessing impact. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 27(4), 880–885. <https://doi.org/10.1002/aqc.2753>

Rodeles, A. A., Miranda, R., & Galicia, D. (2020). Barriers to longitudinal river connectivity: Review of impacts, study methods and management for Iberian fish conservation. *Limnetica*, 39(2), 1. <https://doi.org/10.23818/limn.39.39>

Rousset, F. (2008). GENEPOP '007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, 8(1), 103–106.

Ryan Bellmore, J., Duda, J. J., Craig, L. S., Greene, S. L., Torgersen, C. E., Collins, M. J., & Vittum, K. (2017). Status and trends of dam removal research in the United States. *Wiley Interdisciplinary Reviews: Water*, 4(2), e1164. <https://doi.org/10.1002/wat2.1164>

Sala, O. E. (2000). Global Biodiversity Scenarios for the Year 2100 *Science*, 287(5459), 1770–1774. <https://doi.org/10.1126/science.287.5459.1770>

Seliger, C., & Zeiringer, B. (2018). River Connectivity, Habitat Fragmentation and Related Restoration Measures. In S. Schmutz & J. Sendzimir (Eds.), *Riverine Ecosystem Management* (pp. 171–186). Springer International Publishing. https://doi.org/10.1007/978-3-319-73250-3_9

Silva, A. T., Lucas, M. C., Castro-Santos, T., Katopodis, C., Baumgartner, L. J., Thiem, J. D., Aarestrup, K., Pompeu, P. S., O'Brien, G. C., Braun, D. C., Burnett, N. J., Zhu, D. Z., Fjeldstad, H.-P., Forseth, T., Rajaratnam, N., Williams, J. G., & Cooke, S. J. (2018). The future of fish passage science, engineering, and practice. *Fish and Fisheries*, 19(2), 340–362. <https://doi.org/10.1111/faf.12258>

508 Stanley, E. H., Luebke, M. A., Doyle, M. W., & Marshall, D. W. (2002). Short-Term Changes in Channel
509 Form and Macroinvertebrate Communities Following Low-Head Dam Removal. *Journal of the*
510 *North American Benthological Society*, 21(1), 172–187. <https://doi.org/10.2307/1468307>

511 Sun, J., Galib, S. M., & Lucas, M. C. (2021). Rapid response of fish and aquatic habitat to removal of a
512 tidal barrier. *Aquatic Conservation: Marine and Freshwater Ecosystems*, aqc.3576.
513 <https://doi.org/10.1002/aqc.3576>

514 Taylor, P. D., Fahrig, L., Henein, K., & Merriam, G. (1993). Connectivity is a vital element of landscape
515 structure. *Oikos*, 571–573.

516 Thurow, R. F. (2016). Life Histories of Potamodromous Fishes. In P. Morais & F. Daverat (Eds.), *An*
517 *Introduction to Fish Migration* (p. 26). CRC Press.

518 Tonitto, C., & Riha, S. J. (2016). Planning and implementing small dam removals: Lessons learned from
519 dam removals across the eastern United States. *Sustainable Water Resources Management*,
520 2(4), 489–507. <https://doi.org/10.1007/s40899-016-0062-7>

521 Vega-Retter, C., Muñoz-Rojas, P., Rojas-Hernández, N., Copaja, S., Flores-Prado, L., & Véliz, D. (2020).
522 Dammed river: Short- and long-term consequences for fish species inhabiting a river in a
523 Mediterranean climate in central Chile. *Aquatic Conservation: Marine and Freshwater*
524 *Ecosystems*, aqc.3425. <https://doi.org/10.1002/aqc.3425>

525 Viechtbauer, W. (2010). Conducting Meta-Analyses in R with the **metafor** Package. *Journal of Statistical*
526 *Software*, 36(3). <https://doi.org/10.18637/jss.v036.i03>

527 Weigel, D. E., Connolly, P. J., Martens, K. D., & Powell, M. S. (2013). Colonization of Steelhead in a Natal
528 Stream after Barrier Removal. *Transactions of the American Fisheries Society*, 142(4), 920–930.
529 <https://doi.org/10.1080/00028487.2013.788560>

530 Zigler, S. J., Dewey, M. R., Knights, B. C., Runstrom, A. L., & Steingraeber, M. T. (2004). Hydrologic and
531 Hydraulic Factors Affecting Passage of Paddlefish through Dams in the Upper Mississippi River.

532 *Transactions of the American Fisheries Society*, 133(1), 160–172. <https://doi.org/10.1577/T02->
533 161
534

FIGURES AND TABLES

Table 1: Main characteristics of obstacles ('Lon': longitude; 'Lat': latitude; 'Height' (in m), 'Slope' and presence of a 'fish pass'), details about mitigation measures (type and date of actions) and timelag (in days) between mitigation measures and second sampling sessions.

Obstacle characteristics					Mitigation measures					
Code	River	Lon	Lat	Creation	Height (m)	Slope (°)	Fish pass	Action	Date	Timelag (days)
CEOSal	Ceor	2.57634	44.18213	1800	0.8	> 45°	No	Removal	Jul-2016	427
LEZCas	Lézert	2.24649	44.18545	1400	1.1	< 45°	No	Removal	Oct-2017	353
GLAMou	Gland	4.09026	49.92292	1800	1.2	> 45°	No	Removal	Jul-2019	303
VIAPig	Viaur	2.18924	44.13735	1400	1.2	> 45°	No	Removal	Aug-2017	44
LEZVil	Lézert	2.26765	44.19599	1800	1.9	< 45°	Yes	Removal	Oct-2017	353
DAD Rai	Dadou	2.11982	43.78091	1800	2.0	> 45°	No	Removal	Jun-2017	119
SENBur	Senouire	3.41640	45.27123	1500	2.2	< 45°	No	Removal	Sept-2015	406
GLAPas	Gland	4.07951	49.92529	1800	2.6	> 45°	No	Removal	Jul-2019	303
SIOBre	Sioule	3.29729	46.33353	1500	2.6	< 45°	Yes	Fish pass restoration	Oct-2015	376
GLASou	Gland	4.11903	49.92129	1800	3.0	> 45°	No	Removal	Nov-2016	344
SERHau	Serein	3.60304	47.92156	1830	3.5	> 45°	No	Fish pass creation	Oct-2017	226

Table 2: For each dataset (that is, a combination of one obstacle and one genus), observed effect sizes of barrier effects ($F \pm SD_F$) both before and after restoration and observed effect sizes of restoration ($\Delta F \pm SE_\Delta$), respectively. Note that $SD_F = 2 \times SE_F$. Significant effect sizes (see Figure 2) are in bold and indicated with a star. The last column ‘Recovery’ indicates whether the restoration action (Removal or Fish pass creation) led to the partial or the full recovery of connectivity, when applicable.

Obstacle code	Genus	Barrier effect				Restoration efficiency			
		Before restoration		After restoration		Action	ΔF	SE_Δ	Recovery
		F	SD_F	F	SD_F				
CEOSal	Gudgeons	0.00	0.00	0.00	0.00	Removal	0.00	0.00	/
	Minnows	64.42	1.59	* 35.52	2.22	* Removal	-28.91	1.37	* Partial
LEZCas	Gudgeons	27.25	6.26	* 0.00	0.00	Removal	-27.25	3.13	* Full
	Minnows	0.00	0.00	11.66	12.47	Removal	11.66	6.23	/
GLAMou	Gudgeons	0.00	0.00	0.00	0.00	Removal	0.00	0.00	/
	Minnows	4.27	4.57	0.00	0.00	Removal	-4.27	2.28	/
VIAPig	Gudgeons	32.48	0.99	* 12.41	3.70	Removal	-20.07	1.91	* Full
	Minnows	29.80	4.59	* 8.35	8.93	Removal	-21.45	5.02	* Full
LEZVil	Gudgeons	0.00	0.00	10.39	11.14	Removal	10.39	5.57	/
	Minnows	2.32	2.63	0.00	0.00	Removal	-2.32	1.31	/
DAD Rai	Gudgeons	68.09	1.58	* 26.82	1.20	* Removal	-41.27	0.99	* Partial
SENBur	Gudgeons	54.75	1.02	* 48.22	1.87	* Removal	-6.53	1.07	* Partial
	Minnows	14.59	15.60	0.00	0.00	Removal	-14.59	7.80	* /
GLAPas	Gudgeons	55.42	2.24	* 0.00	0.00	Removal	-55.42	1.12	* Full
	Minnows	42.88	2.87	* 0.00	0.00	Removal	-42.88	1.43	* Full
SIOBre	Gudgeons	0.00	0.00	0.00	0.00	Fish pass	0.00	0.00	/
	Minnows	51.84	2.57	* 39.46	1.61	* Fish pass	-12.38	1.52	* Partial
GLASou	Minnows	49.26	0.76	* 32.51	4.77	* Removal	-16.75	2.42	* Partial
SERHau	Gudgeons	0.00	0.00	0.00	0.00	Fish pass	0.00	0.00	/
	Minnows	57.33	1.31	* 49.10	2.05	* Fish pass	-8.23	1.22	* Partial

Table 3: Results of random meta-analyses for the overall effect sizes of fragmentation \bar{F} and of restoration $\bar{\Delta F}$. In presence of a moderator, EST is the estimate of the overall effect size for the intercept (INT) and the deviation from the intercept for the alternate modalities, with the moderator as a random effect. In absence of moderator (indicated with a slash), EST is the final estimate of the overall effect size taking into account all sources of variation (including the previously identified significant moderators as random effects, when applicable). Also provided are the number n of datasets in each modality, the standard error (SE) and CI_{95%} (ci.low and ci.high) around EST, the Wald-Type Z statistic (Zval) and the associated p-value (pval), and the QM test of moderator effect (QM statistic and associated p-value).

Overall effect size	Random effects	Moderator	Modalities (INT = Intercept)	n	EST	SE	ci.low	ci.high	Zval	pval	Test of moderator	
											QM	pval
\bar{F}	Species Dataset	Genus	Gudgeons (INT)	5	47.72	7.63	32.77	62.67	6.25	<.0001		
			Minnows	9	-12.26	10.91	-33.65	9.13	-1.12	0.261	1.26	0.261
	Typology Dataset	Typology	Low and gentle (INT)	2	14.62	12.46	-9.80	39.05	1.17	0.241		
			Low and steep	4	18.18	17.53	-16.18	52.53	1.04	0.300		
			High and gentle	3	26.84	17.55	-7.56	61.25	1.53	0.126		
			High and steep	5	40.00	13.15	14.22	65.78	3.04	0.002	11.45	0.010
	Typology Dataset			14	51.42	3.22	45.12	57.72	16.00	<.0001		
	$\bar{\Delta F}$	Species Dataset	Genus	Gudgeons (INT)	5	-30.14	8.50	-46.80	-13.47	-3.54	0.000	
Minnows				9	13.24	9.60	-5.57	32.06	1.38	0.168	1.90	0.168
Typology Dataset		Typology	Low and Gentle (INT)	2	-14.62	12.46	-39.05	9.80	-1.17	0.241		
			Low and Steep	4	-4.03	13.57	-30.62	22.56	-0.30	0.766		
			High and Gentle	3	4.81	12.73	-20.13	29.75	0.38	0.705		
			High and Steep	5	-18.32	15.25	-48.22	11.57	-1.20	0.230	7.79	0.051
Timelag Dataset		Timelag	<1year (INT)	10	-24.04	5.67	-35.16	-12.92	-4.24	<.0001		
			>1year	4	8.35	7.74	-6.81	23.51	1.08	0.281	1.16	0.281
	1 Dataset			14	-21.71	4.35	-30.24	-13.18	-4.99	<.0001		

Figure 1: Geographic localization of studied instream barriers (black dots) in the main French watersheds.

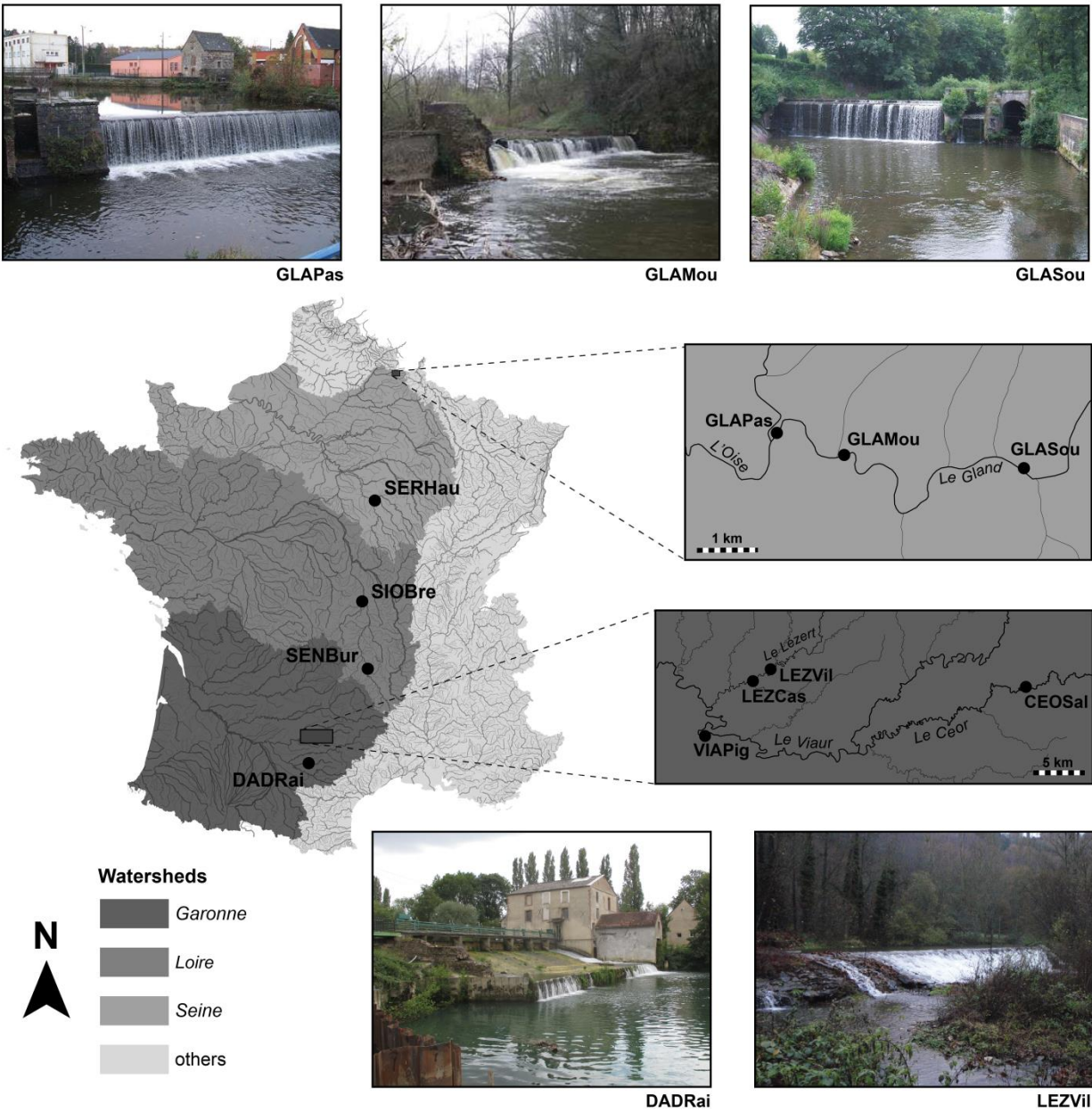


Figure 2: Main results of the before-after genetic monitoring. For each obstacle (in columns), bars represent F_{INDEX} values with 95% confidence intervals as computed before (in green) and after (in purple) restoration in gudgeons (panel A) and minnows (panel B). Slashes indicate no data in both A and B. Outlined bars represent significant barrier effects ($F_{INDEX} > 20\%$). Green stars indicate a significant change in F_{INDEX} values after restoration (non-overlapping confidence intervals). Double purple stars indicate the full recovery of connectivity following restoration (see details in Table 2). Panel C also provides few details about obstacles (in green) and restoration (in purple) for direct comparisons with F_{INDEX} values (see Table 1). Obstacles are sorted by their increasing height. Note that F_{INDEX} values actually increased after restoration in two datasets (LEZVil in gudgeons and LEZCas in minnows), but that new values were lower than 20 % and non-significantly different from 0 (and thus from F_{INDEX} values before restoration) according to $CI_{95\%}$, a phenomenon owing to the stochasticity of genetic approaches

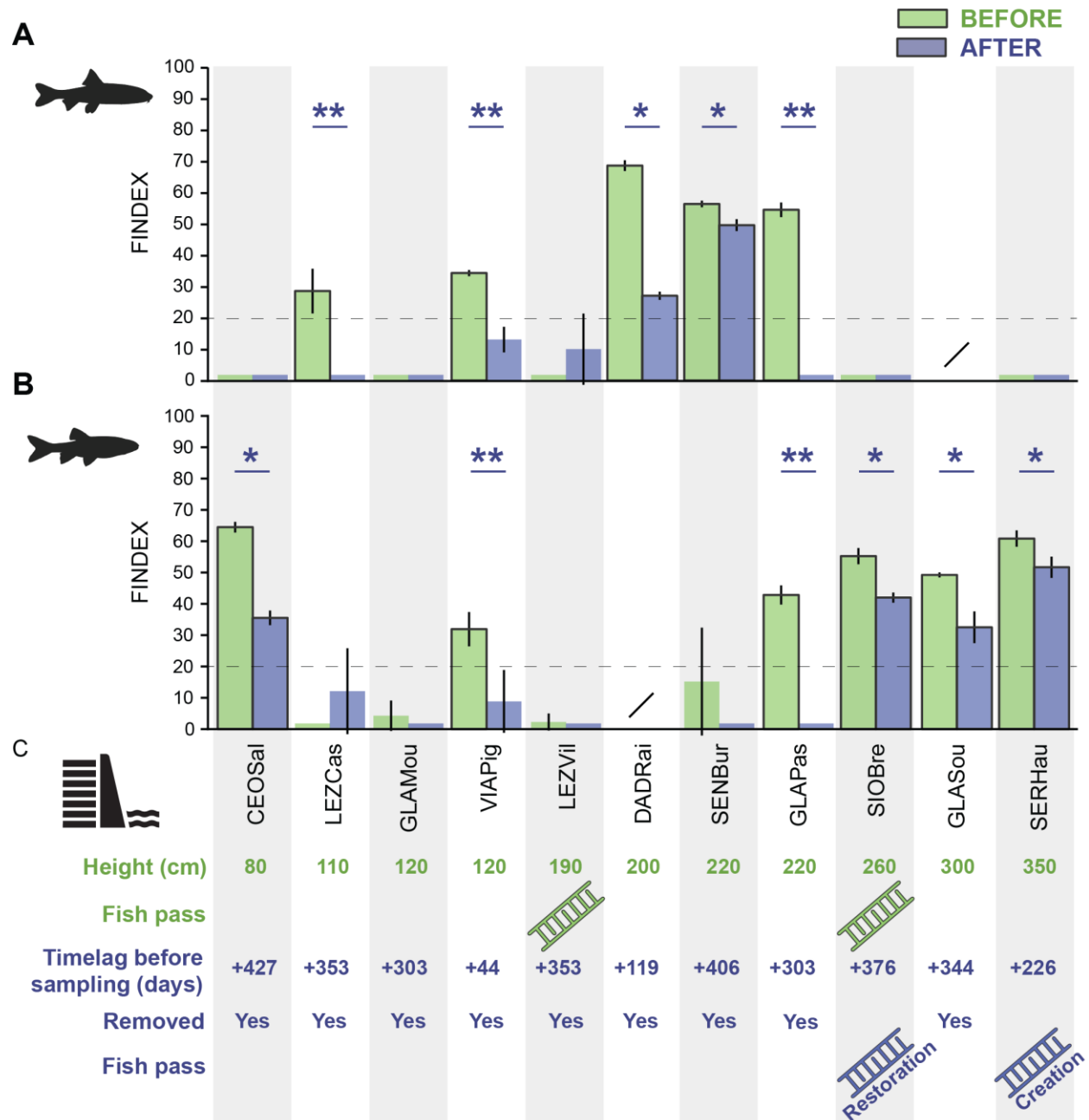
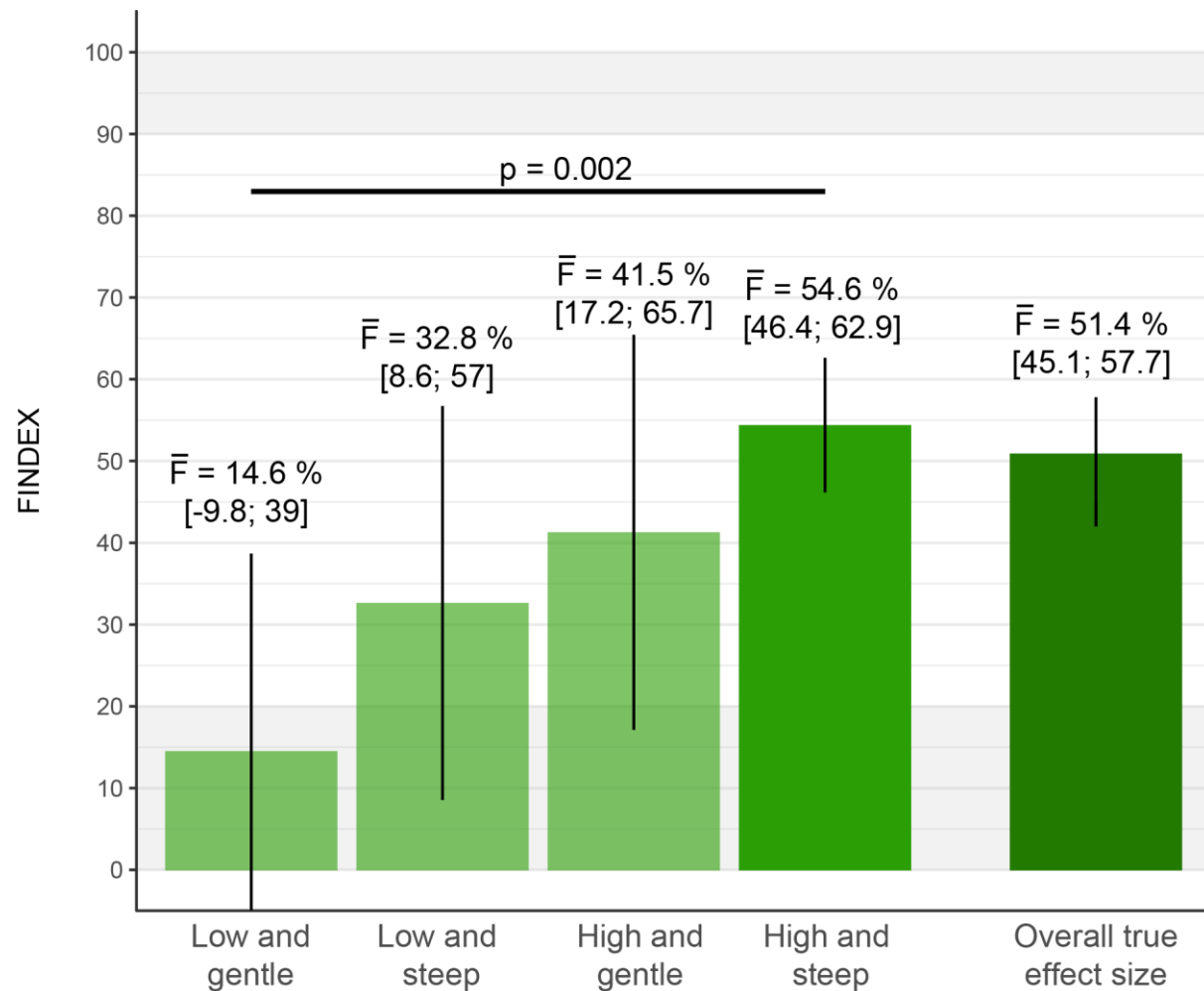


Figure 3: Overall effect sizes \bar{F} of fragmentation for each modality of typology and overall true effect size \bar{F} taking all significant sources of variation (within datasets and across modalities) into account. Washed out bars indicate that the effect size of fragmentation is not significant ($\bar{F} > 20\%$ or $CI_{95\%}$ including 20%; Prunier et al., 2020).



599 APPENDIX S1: DNA EXTRACTION AND GENOTYPING

600

601 Genomic DNA was extracted using a salt-extraction protocol (Aljanabi & Martinez, 1997). A subset of 15
602 and 19 autosomal microsatellite loci from Grenier et al. (2013) were amplified and genotyped in gudgeons
603 (BL1-153, Gob15, Gob16, Gob22, LC293, Lco4, MFW1, Ca1, CypG24, Gob12, Gob28, Lsou5, Rv1a21177,
604 Smv03 and Lro12) and minnows (BL1-153, Ca3, CtoA-247, CtoG-075, CypG9, LSou8, LleA-071, LleB-072,
605 Ppro-132, Rru4, BL1-44, BL1-84, BL1-98, LC27, LceC1, LleC-090, Lsou5, MFW1 and Rhca20), respectively,
606 following PCR conditions described in Grenier et al. (2013).

607 Each combination of a genus, an obstacle and a sampling session ($n = 40$) was considered a unique
608 genotypic dataset, with corresponding genotypes coded in the *genepop* format (Rousset, 2008). For each
609 dataset, we assessed the presence of null alleles and checked for gametic disequilibrium using the *null.all*
610 function (R-package *PopGenReport*; Adamack & Gruber, 2014) and the *test_LD* function (R-package
611 *genepop*; Rousset, 2008), respectively. Any locus showing significant gametic disequilibrium and/or
612 evidence of null alleles was discarded before F_{INDEX} calculation (see Table 2 in main text for outputs).

613

APPENDIX S2: THE EFFECT OF TIMELAG ON THE OBSERVED EFFECT SIZES OF RESTORATION ΔF

The observed effect sizes of restoration ΔF ($\pm CI_{95\%}$) did not show any significant trend when increasing the timelag between restoration and sampling.

