


## LETTER

# Novel operational index reveals rapid recovery of genetic connectivity in freshwater fish species after riverine restoration

Jérôme G. Prunier<sup>1</sup>  | Géraldine Loot<sup>2</sup> | Charlotte Veysièrè<sup>2</sup> | Nicolas Poulet<sup>3</sup> | Simon Blanchet<sup>1</sup>

<sup>1</sup>Centre National de la Recherche Scientifique (CNRS), UAR 2029, Station d'Ecologie Théorique et Expérimentale, Moulis, France

<sup>2</sup>CNRS, UPS, UMR 5174 EDB (Laboratoire Évolution & Diversité Biologique), École Nationale de Formation Agronomique (ENFA), Toulouse, France

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

## Correspondence

Jérôme G. Prunier and Simon Blanchet, Centre National de la Recherche Scientifique (CNRS), UAR 2029, Station d'Ecologie Théorique et Expérimentale, 2 route du CNRS, 09200 Moulis, France.  
 Email: [jerome.prunier@gmail.com](mailto:jerome.prunier@gmail.com) and [simon.blanchet@sete.cnrs.fr](mailto:simon.blanchet@sete.cnrs.fr)

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## Abstract

Hyperfragmentation of rivers by anthropogenic barriers is a major threat to biodiversity. Restoration policies are being adopted worldwide to mitigate these impacts, particularly those on fish connectivity. We assessed the utility of a novel genetic index of fragmentation, the  $F_{INDEX}$ , by monitoring real-time responses of two fish genera to restoration operations at 11 weirs in France. The  $F_{INDEX}$  outperformed traditional genetic tools, detecting barriers more efficiently, and thereby improving estimates of recovery of connectivity following restoration. Most weirs had significant impacts on connectivity before restoration, especially the highest and steepest ones. Restoration actions systematically improved genetic connectivity, sometimes completely and in just a few months, with an overall halving of fragmentation levels. Our study demonstrates that current restoration policies are recovering genetic connectivity efficiently, and that practitioners may benefit from the  $F_{INDEX}$  as a new operational tool to assess barrier strength for nonmigratory organisms and to plan and monitor riverine restoration.

## KEYWORDS

dam removal,  $F_{INDEX}$ , fish passes, genetic connectivity, genetic monitoring, low-head dams, potamodromous, restoration, weirs

## 1 | INTRODUCTION

Fragmentation of freshwater habitats by anthropogenic instream barriers such as weirs typically exerts disruptive influences on ecological processes (Pringle, 2003). It does so primarily by altering river flow regimes (Grill et al., 2019), thereby affecting upstream–downstream fluxes of energy, matter, and organisms, and reducing the quality, quantity, and accessibility of habitats that allow individuals to complete their life cycles (Thurow, 2016). With

more than 1 million barriers, European rivers are hyperfragmented (Belletti et al., 2020). Many countries have adopted restrictive legislation to re-establish free-flowing rivers (Thieme et al., 2021) and to restore upstream–downstream connectivity, with a particular focus on fish mobility through barrier removal (Bellmore et al., 2017) or through the creation of fishways (Silva et al., 2018).

Properly planning the restoration of river connectivity should rely on the a priori quantification of the individual and/or cumulative impact of barriers on species

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movements, while a posteriori quantification of the efficiency of implemented measures might allow improving future technical solutions through feedback (Birnie-Gauvin et al., 2017). However, practitioners often stumble over technical challenges that hamper in situ measurement of barrier passability. Direct methods of monitoring movement such as video-counting, capture–mark–recapture, and telemetry often require substantial resources (Cayuela et al., 2018), causing practitioners to mostly rely on less demanding and easy-to-implement assessment methods that provide rough estimates of barrier passability (Appendix S1). However, these estimates are usually conservative and may result in suboptimal restoration planning (Mahlum et al., 2014).

Alternatively, barrier passability may be indirectly quantified through genetic approaches (Deflem et al., 2022; Raeymaekers et al., 2009; see Muha et al. [2021] for a complementary approach based on metabarcoding). These methods, however, are not without practical limits. Assignment methods and parentage analyses require extensive sampling of individuals and moderate to high genetic differentiation between populations (Cayuela et al., 2018). The use of upstream–downstream genetic distances as a proxy for barrier effects is less restrictive, but these measures are not comparable across barriers of different age and/or separating upstream and downstream populations of differing effective sizes (Prunier et al., 2020). These caveats can prevent reliable comparisons across species and barriers. In practice, these molecular tools are therefore rarely used by practitioners (Holderegger et al., 2019), and rarely deployed to assess restoration efficiency. As a result, there is little evidence that restoration strategies deployed worldwide actually improve genetic connectivity (but see Fraik et al., 2021).

Here, we monitored responses of two common fish genera to restoration operations at 11 weirs in France. We used the  $F_{INDEX}$ , a novel genetic index of fragmentation designed to provide *standardized* estimates of the effects of anthropogenic barriers on riverine connectivity by sampling genotypes in the direct upstream and downstream vicinity of barriers (Prunier et al., 2020). The  $F_{INDEX}$  can be computed for any species, provided that separate populations can be identified and sampled on both sides of a barrier, thereby excluding strict migratory fish species such as anadromous salmonids. The method is based on rescaling observed upstream–downstream genetic distances within their theoretical ranges of variation, taking into account the numbers of generations elapsed since barrier creation and the effective sizes of adjacent populations, two confounding parameters when comparing genetic distances. The  $F_{INDEX}$  can be interpreted as the amount of reduction in gene flow, compared to the situation with no barrier. Ranging from 0% (no barrier effect) to 100%

(impassable barrier),  $F_{INDEX}$  estimates are directly comparable across species (whatever their life-history traits), across barriers (whatever their age or typology), and over time (allowing comparisons before and after restoration). These properties should make the  $F_{INDEX}$  a valuable barrier assessment method for practitioners. However, its efficiency remains untested empirically. We performed such a test using the 11 restoration operations, each involving either removing the barrier, or creating or restoring a fishway. If they proved to be consistently associated with a reduction in  $F_{INDEX}$  values, this would simultaneously indicate that (i) the  $F_{INDEX}$  allows proper quantification of riverscape connectivity both before and after restoration and (ii) that longitudinal restoration is an efficient approach to trigger the rapid recovery of genetic connectivity. Additionally, we investigated whether  $F_{INDEX}$  values varied with barrier typology and compared the  $F_{INDEX}$  to classical genetic approaches used to infer interpopulation connectivity.

## 2 | MATERIALS AND METHODS

### 2.1 | Instream barriers

In coordination with practitioners, we identified 11 instream barriers (weirs <4 m high) that were to be dismantled or equipped with a fishway between 2015 and 2019 (Figure 1; Table 1). These restoration actions were carried out opportunistically, preventing us from assessing the cumulative effects of barriers on connectivity. Two of the barriers had already been equipped with fishways but these were judged ineffective by practitioners and were about to be replaced or dismantled. Barriers dated from the 15th to the 20th centuries and were located in three large French river basins, spanning a latitudinal gradient of ~800 km. Our “Typology” procedure classified barriers into four categories (“Low/Gentle,” “Low/Steep,” “High/Gentle,” and “High/Steep”) depending on barrier height (< or  $\geq 2$  m) and slope (< or  $\geq 45^\circ$ ) (Appendix S2).

### 2.2 | Biological models and sampling

We focused on five abundant resident fish species from two genera of cyprinids: minnows (*Phoxinus phoxinus* in the Seine, *Phoxinus fayollarum* in the Loire, and *Phoxinus dragarum* in the Garonne watershed) and gudgeons (*Gobio gobio* in the Loire and the Seine and *Gobio occitaniae* in the Garonne watershed; Denys et al., 2020). Given their maximal body lengths of 140 mm in minnows and 200 mm in gudgeons, these fish should be

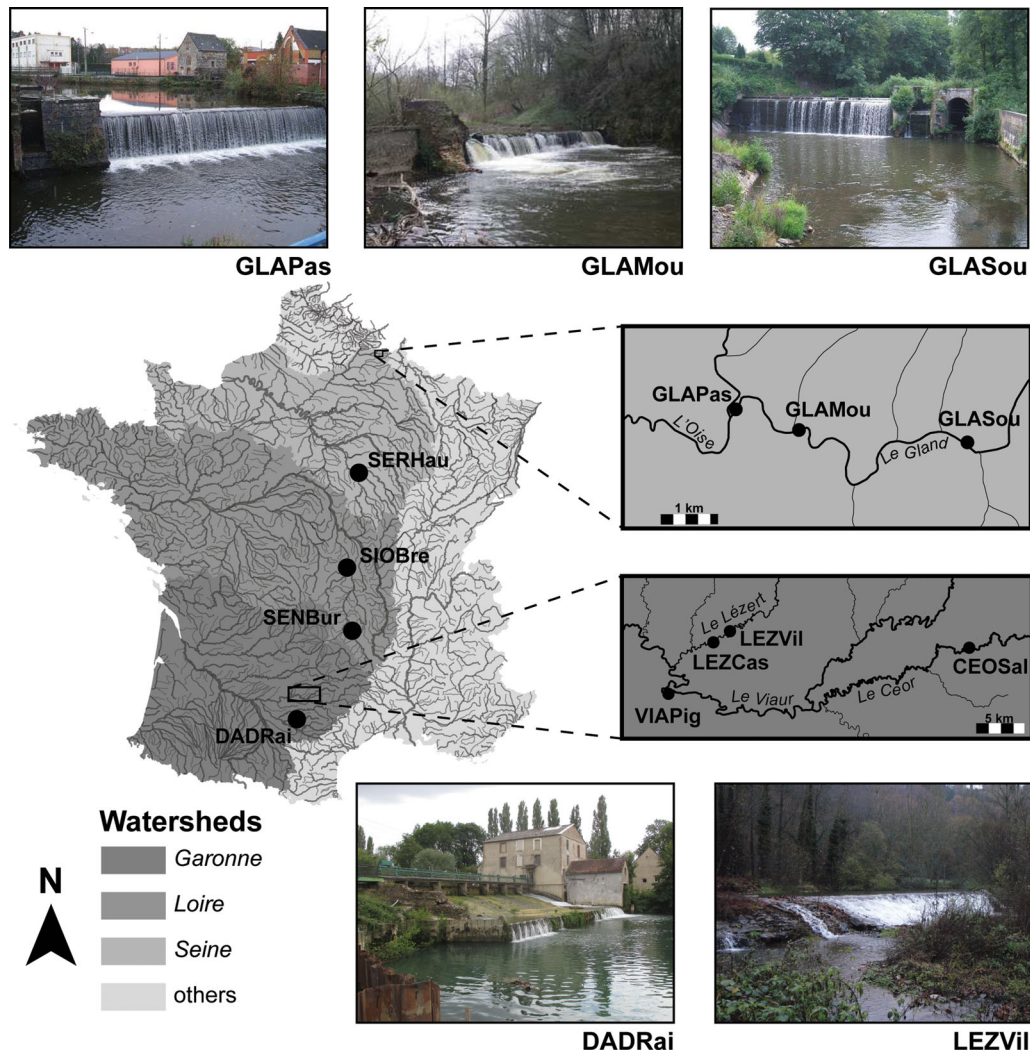


FIGURE 1 Geographic localization of studied instream barriers (black dots) in the main French watersheds

unable to jump over barriers  $>0.2$  m (Baudoin et al., 2014). We thus expected that swimming upstream rather than jumping across barriers was the only option available to them and that the barriers in our low/gentle category might be passable upstreamward (Pflugrath et al., 2019), whereas the steepest and/or the highest barriers would be impassable.

Sampling operations “before” and “after” restoration were performed using electrofishing, which continued until 30 adult individuals of each genus had been captured on each side of each barrier (upstream and downstream), within 200 m of the barrier. A piece of pelvic fin was sampled on each individual and stored in 96% alcohol. All fish were returned alive to their sampling sites. Both genera could be sampled at all sites except in DADRai (gudgeons only) and GLASou (minnows only). The second sampling sessions occurred on average  $9.7 (\pm 3.9 \text{ SD})$  months after restoration (Table 1).

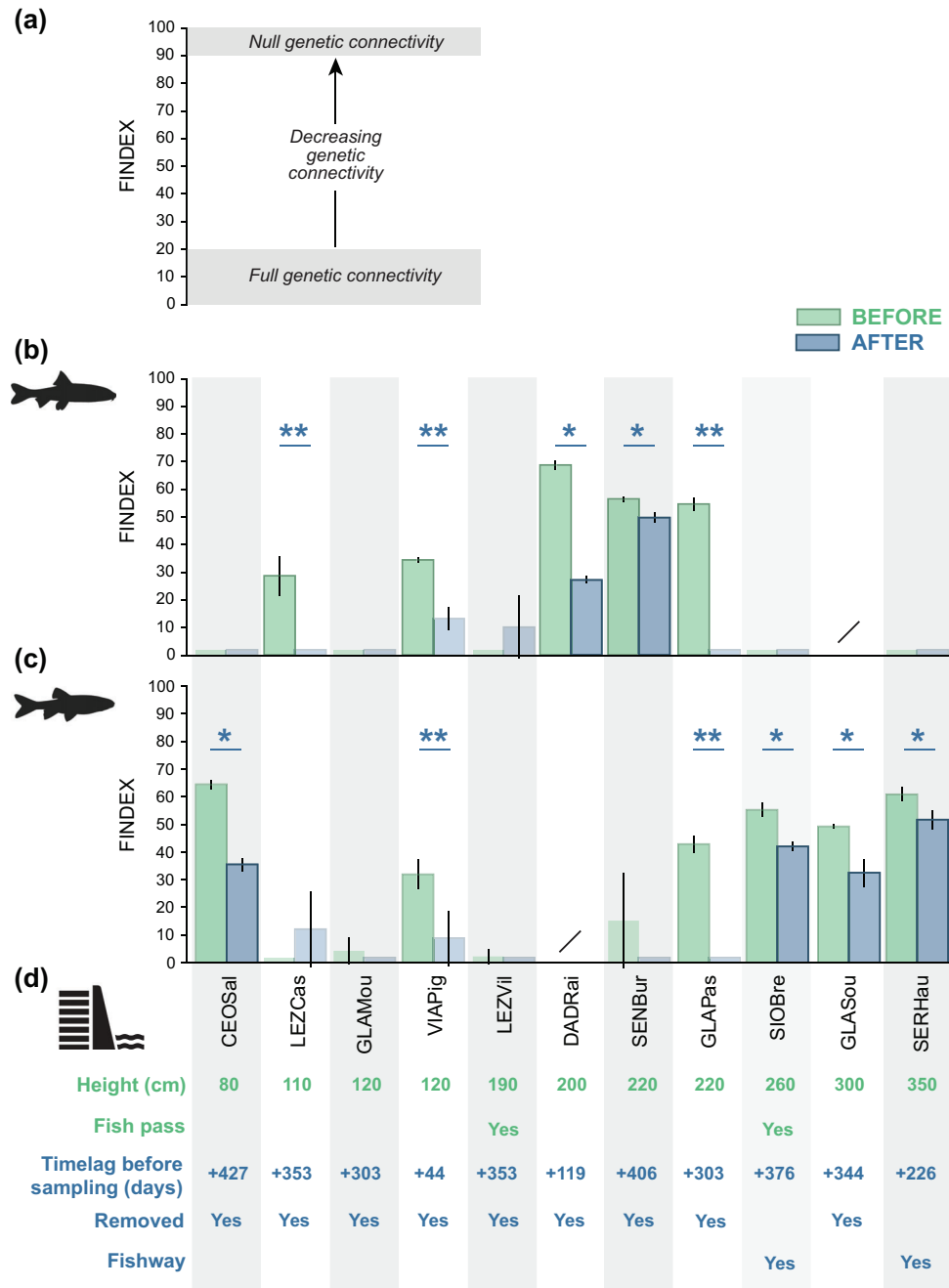
### 2.3 | Genotyping and $F_{INDEX}$ computation

We used 19 microsatellite markers in minnows and 15 in gudgeons. DNA extraction and genotyping followed published procedures (Appendix S3). For each dataset (i.e., each combination of genus and barrier;  $n = 20$ ) and each time period (“Before” and “After”), we computed the  $F_{INDEX}$  and its standard deviation ( $SD_F$ ) as described in Prunier et al. (2020). We used the estimated generation times of 2 years in minnows and 2.5 years in gudgeons (Keith et al., 2011) to calculate numbers of generations elapsed since barrier creation. In practice,  $F_{INDEX}$  values lower than 20% are interpreted as nonsignificant, indicating fully passable structures (Prunier et al., 2020; Figure 2a; Appendix S4).  $F_{INDEX}$  values with a null variance ( $SD_F = 0$ ) are obtained when all upstream–downstream genetic distances used to compute the  $F_{INDEX}$  are null ( $F_{INDEX} = 0$ ), similarly indicating fully passable structures.

**TABLE 1** Main characteristics of barriers (“Lon”: Longitude; “Lat”: Latitude; approximate date of creation; “Height” (in m), “Slope,” and presence of a “fishway”), details about restoration operations (type and date of actions), and time lag (in days) between restoration operations and second sampling sessions

Barrier characteristics				Creation				Restoration operations			
Code	River	Lon	Lat	Year	Height (m)	Slope (°)	Fishway	Action	Date	Time lag (days)	
CEOSal	Ceor	2.57634	44.18213	1800	0.8	55	No	Removal	July 2016	427	
LEZCas	Lézert	2.24649	44.18545	1400	1.1	25	No	Removal	October 2017	353	
GLAMou	Gland	4.09026	49.92292	1800	1.2	90	No	Removal	July 2019	303	
VIAPIg	Viaur	2.18924	44.13735	1400	1.2	60	No	Removal	August 2017	44	
LEZVil	Lézert	2.26765	44.19599	1800	1.9	40	Yes	Removal	October 2017	353	
DADRai	Dadou	2.11982	43.78091	1800	2.0	90	No	Removal	June 2017	119	
SENBur	Senouire	3.41640	45.27123	1500	2.2	40	No	Removal	September 2015	406	
GLAPas	Gland	4.07951	49.92529	1800	2.6	90	No	Removal	July 2019	303	
SIOBre	Sioule	3.29729	46.33353	1500	2.6	30	Yes	Fish pass restoration	October 2015	376	
GLASou	Gland	4.11903	49.92129	1800	3.0	90	No	Removal	November 2016	344	
SERHau	Serein	3.60304	47.92156	1830	3.5	80	No	Fish pass creation	October 2017	226	

Note: Most of the dams were created to supply mills (flour mills, sawmills, etc.), although they have since disappeared. Only two dams currently used for hydroelectric production (SIOBre and SERHau) have not been dismantled.



**FIGURE 2**  $F_{INDEX}$  interpretation (a) and main results of the before–after genetic monitoring (b and c). For each barrier (in columns), bars represent  $F_{INDEX}$  values with  $CI_{95\%}$  as computed before (in green) and after (in blue) restoration in gudgeons (b) and minnows (c). Slashes indicate no data in both panels (b) and (c). Outlined bars represent significant barrier effects ( $F_{INDEX} > 20\%$ ). Blue stars indicate a significant change in  $F_{INDEX}$  values after restoration (nonoverlapping  $CI_{95\%}$ ). Double blue stars indicate the full recovery of connectivity following restoration (see details in Appendix S8). Panel (d) also provides few details about barriers (in green) and restoration (in blue) for direct comparisons with  $F_{INDEX}$  values (see Table 1). Barriers are sorted by increasing height

## 2.4 | $F_{INDEX}$ validity, barrier effects, and restoration efficiency

To assess the validity of the  $F_{INDEX}$ , we tested that  $F_{INDEX}$  values after restoration were consistently and significantly lower than  $F_{INDEX}$  values before restora-

tion. Additionally, we compared the  $F_{INDEX}$  to the outputs of classical genetic approaches used to infer interpopulation connectivity, namely STRUCTURE (Pritchard et al., 2000), GeneClass2 (Piry, 2004), and BAYESASS (Wilson & Rannala, 2003). Details are provided in Appendix S5.

To assess barrier effects and restoration efficiency, we adopted a meta-analytical approach that allows combining effect sizes from several studies into an overall effect size, while taking into account the uncertainty associated with each study: studies with high uncertainty are given less weight in the calculation of the overall effect size than studies with low uncertainty (Borenstein, 2009; Appendix S6). Here, we considered each dataset as an independent study providing two effect sizes: the  $F_{INDEX}$  values ( $\pm SD_F$ , i.e., estimates of uncertainty) before and after restoration. In each dataset, the  $F_{INDEX}$  value before restoration was used as the effect size  $F$  of fragmentation. Similarly, the raw difference between  $F_{INDEX}$  values computed after and before restoration was used as the effect size  $\Delta F$  ( $\pm SD_\Delta$ ) of restoration for each dataset. The raw difference  $\Delta F$  measures the change in the amount of fragmentation following restoration, and constitutes an index of restoration per se. A  $\Delta F$  value lower than 0% indicates a significant reduction in fragmentation following restoration, except when both before and after effect sizes of fragmentation  $F$  are lower than 20%, in which case the effect size of restoration is also deemed nonsignificant (Appendix S4).

We computed the overall effect sizes of fragmentation  $\bar{F}$  and of restoration  $\overline{\Delta F}$  by taking into account different sources of variation: within-datasets ( $SD_F$  or  $SD_\Delta$ , with dataset ID as random factor), between-datasets, and across covariates (Appendix S6). Covariates were “Typology” (four levels: see above), “Genus” (two levels: minnows or gudgeons), and, in the case of the overall effect size of restoration  $\overline{\Delta F}$ , the “Time lag” (in days) between restoration and the second sampling session. For each overall effect size, we performed meta-regressions with all possible interactions among covariates, and identified the best-fit model based on Akaike criterion (Burnham & Anderson, 2002). Covariates identified as significant moderators in the best-fit models were kept as random factors in final models without moderator to get estimates of the overall effect sizes of fragmentation  $\bar{F}$  and of restoration  $\overline{\Delta F}$  along with their 95% confidence interval ( $CI_{95\%}$ ). We similarly computed the overall effect sizes of fragmentation  $\bar{F}'$  and of restoration  $\overline{\Delta F}'$  using only datasets with a significant barrier effect ( $F_{INDEX} > 20\%$ ) before restoration ( $n = 11$ ). The relative amounts  $\bar{G}$  ( $n = 20$ ) and  $\bar{G}'$  ( $n = 11$ ) of genetic recovery were finally computed as the ratio of fragmentation effect sizes after ( $\bar{F} + \overline{\Delta F}$ ) and before ( $\bar{F}$ ) restoration.

### 3 | RESULTS

$F_{INDEX}$  values ranged from 0% to 68.1% before restoration, with a significant barrier effect ( $F_{INDEX} > 20\%$ ) in 11 out of 20 combinations of a genus and a barrier (Appendix

S7). Except when barriers had no effect before restoration ( $F_{INDEX} < 20\%$ ), in which case restoration had no effect either, we observed a systematic and significant decrease in  $F_{INDEX}$  values after restoration (Figure 2), with new  $F_{INDEX}$  values ranging from 0% to 49.1%. This finding held true whatever the nature of restoration actions. Moreover, the  $F_{INDEX}$  outperformed all other genetic tools in quantifying upstream–downstream differences both before and after restoration (Appendix S5).

Before restoration, genera showed contrasted responses to barriers: all barriers but GLAMou and LEZVil had a significant impact on connectivity, but only VIAPig and GLAPas impacted both genera (Figure 2). Nevertheless, “Genus” was not identified as a significant moderator of the overall effect size of fragmentation  $\bar{F}$  (Table 2). The best-fit model for  $\bar{F}$  only included “Typology” (Appendix S8), with steep barriers  $\geq 2$  m showing a significant overall effect size  $\bar{F}$  (45.5%,  $CI_{95\%} > 20\%$ ), a value significantly higher than that of gentle weirs  $< 2$  m (Table 2; Figure 3). Once typology was taken into account, the overall effect size of fragmentation  $\bar{F}$  across all barriers was estimated as 27.7%. It reached a significant value of  $\bar{F} = 48.5\%$  ( $CI_{95\%} > 20\%$ ) when only considering significant barriers (Figure S1).

The observed effect sizes of restoration  $\Delta F$  ranged from  $-55.4\%$  to  $+11.7\%$  (Appendix S7; see Appendix S4 for the interpretation of  $\Delta F > 0$ ). As for  $\bar{F}$ , the best-fit model for the overall effect size of restoration  $\overline{\Delta F}$  only included “Typology” (Appendix S8), with restoration of steep barriers  $\geq 2$  m ( $\overline{\Delta F} = -27.4\%$ ,  $CI_{95\%} < 0\%$ ) significantly more efficient than that of gentle weirs, whatever their height (Table 2; Figure 3).  $\overline{\Delta F}$  was not significant for gentle weirs  $< 2$  m. Neither “Genus” nor “Time lag” were identified as significant moderators of  $\overline{\Delta F}$  (Table 2). Once typology was taken into account, the overall effect size of restoration was  $\overline{\Delta F} = -14.0\%$  ( $CI_{95\%} < 0\%$ ) across all barrier and  $\overline{\Delta F}' = -25.6\%$  ( $CI_{95\%} < 0\%$ ) when only considering significant barriers (Figure S1). The relative amounts of genetic recovery following restoration were  $\bar{G} = 49.4\%$  (all datasets) and  $\bar{G}' = 47.2\%$  (significant barriers only; Figure S1).

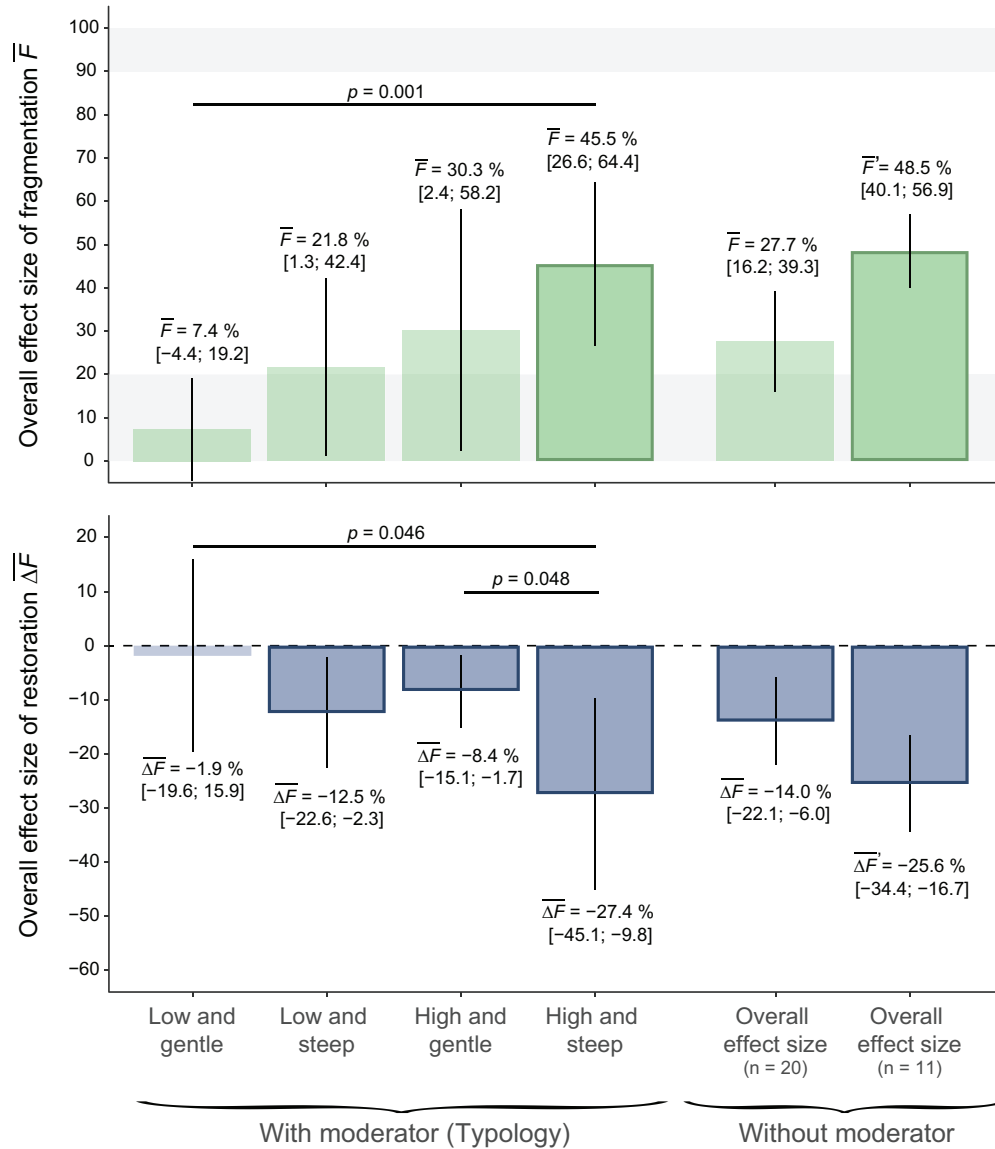
### 4 | DISCUSSION

Quantifying the impact of barriers on river connectivity and the efficiency of restoration operations is a prerequisite for allocating resources toward mitigating the most impactful barriers, for informing trade-offs between ecological and socioeconomic issues and for refining restoration techniques (Hermoso et al., 2012; Silva et al., 2018). This quantification is a difficult task, because operational tools allowing standardized comparisons among species and

TABLE 2 Results of random meta-analyses for the overall effect sizes of fragmentation  $\bar{F}$  and of restoration  $\overline{\Delta F}$  for all datasets ( $n = 20$ )

Overall effect size	Random effects	Moderator	Modalities (INT = Intercept)	$n$	EST	SE	ci.low	ci.high	Zval	pval	
$\bar{F}$	Genus Dataset	Genus	Gudgeons (INT)	10	23.80	8.76	6.62	40.98	2.71	0.007	
			Minnows	10	7.87	11.85	-15.35	31.09	0.665	0.506	
	Typology Dataset	Typology	Low/Gentle (INT)	4	7.39	6.03	-4.42	19.20	1.23	0.220	
			Low/Steep	6	14.44	12.09	-9.26	38.13	1.19	0.232	
			High/Gentle	4	22.90	15.45	-7.37	53.18	1.48	0.138	
	Typology Dataset	Typology	<b>High/Steep</b>	6	<b>38.10</b>	<b>11.38</b>	<b>15.80</b>	<b>60.41</b>	<b>3.35</b>	<b>8.1x10<sup>-4</sup></b>	
			Typology Dataset	20	<b>27.74</b>	<b>5.90</b>	<b>16.16</b>	<b>39.31</b>	<b>4.70</b>	<b>2.6x10<sup>-6</sup></b>	
	$\overline{\Delta F}$	Genus Dataset	Genus	Gudgeons (INT)	11	<b>48.50</b>	<b>4.31</b>	<b>40.06</b>	<b>56.94</b>	<b>11.26</b>	<b>2.1x10<sup>-29</sup></b>
				Minnows	10	-14.02	6.75	-27.24	-0.79	-2.08	0.038
	$\overline{\Delta F}$	Typology Dataset	Typology	Low/Gentle (INT)	10	0.00	8.25	-16.17	16.17	0.00	1.000
Low/Steep				4	-1.88	9.06	-19.64	15.88	-0.21	0.836	
Typology Dataset		Typology	High/Gentle	6	-10.57	10.45	-31.04	9.90	-1.01	0.312	
			<b>High/Steep</b>	4	-6.50	9.69	-25.49	12.50	-0.67	0.503	
Time lag Dataset		Time lag	(INT)	6	<b>-25.55</b>	<b>12.78</b>	<b>-50.60</b>	<b>-0.49</b>	<b>-2.00</b>	<b>0.046</b>	
			Time lag	20	-27.15	11.41	-49.52	-4.78	-2.38	0.017	
Typology Dataset		Typology	Time lag	20	0.04	0.03	-0.03	0.11	1.23	0.220	
			Typology Dataset	11	<b>-25.56</b>	<b>4.53</b>	<b>-34.45</b>	<b>-16.67</b>	<b>-5.64</b>	<b>1.7x10<sup>-8</sup></b>	

Note: In presence of a qualitative moderator, EST is the estimate of the overall effect size for the intercept (INT) and the deviation from the intercept for the alternate modalities. In presence of a quantitative moderator, EST is the estimate of the overall effect size for the intercept (INT) and the estimated slope of the moderator. In absence of moderator, 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). The overall effect sizes of fragmentation  $\bar{F}$  and of restoration  $\overline{\Delta F}$  are also provided for significant barriers only ( $F > 20\%$  before restoration;  $n = 11$ ). The table includes 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). Although not the best fitting models (Appendix S2), results of meta-regressions with "Genus" and "Time lag" as unique moderators are also provided for comparison.



**FIGURE 3** Overall effect sizes of fragmentation  $\bar{F}$  (top panel) and of restoration  $\Delta\bar{F}$  (bottom panel), predicted from intercept-free random-effect meta-regressions with barrier typology as a moderator, and from random-effect meta-regressions without moderator. Also provided are the overall effect sizes  $\bar{F}'$  and  $\Delta\bar{F}'$  computed without moderator for significant barriers only ( $n = 11$ ). Outlined bars are significant effect sizes according to  $CI_{95\%}$  (compared to 20% for  $\bar{F}$  and to 0% for  $\Delta\bar{F}$ ; Appendix S4)

barriers are lacking. Here, we showed that the  $F_{INDEX}$ , a standardized genetic index of fragmentation for nonmigratory organisms that is capable of working across species and barriers, consistently detected recovery of connectivity after barrier removal (Bednarek, 2001), outperforming other traditional genetic tools.

Before restoration, we found a significant barrier effect in 11 out of 20 fish/barrier combinations, with two barriers showing no impact on any genus, two barriers significantly impacting both genera, and five barriers showing large differences between fish genera in response to fragmentation. This great variability in the local response of fish to the presence of barriers (Blanchet et al., 2010;

Deflem et al., 2022) reinforces the need for a reliable and easy-to-implement barrier assessment tool. In absence of fishways, individuals are expected to take advantage of floods to cross barriers (Keller et al., 2012). However, such propitious conditions are not encountered every year and similarly at all locations, which may explain why  $F_{INDEX}$  values differed across datasets and why the effect of fragmentation was genus independent. However,  $\bar{F}$  was significantly influenced by the typology of barriers, with steep barriers  $\geq 2$  m showing an overall effect size 38% higher than gentle barriers  $< 2$  m (Table 2; Figure 3). This 2 m threshold should of course be refined with additional datasets, but it provides a relevant benchmark for



practitioners to adjust restoration planning based on barrier characteristics.

With this effect of typology taken into account, we estimated an overall 27.7% reduction in gene flow on either side of a barrier compared to a situation with no barrier, and 48.5% when only considering significant barriers, suggesting an overall halving of gene flow. Diagnosis based on a standardized tool such as the  $F_{INDEX}$  may help managers easily quantify and compare barrier effects across species and barriers, and thus orientate their restoration efforts toward the most problematic structures. Of course, we acknowledge that other ecological and socioeconomic indicators should be considered in restoration planning (Hermoso et al., 2012). The  $F_{INDEX}$  might also help evaluate the species-specific efficiency of fishways—an important step to drive future technical developments (Foulds & Lucas, 2013). For example, we estimated that the two fishways that were removed or replaced during our study had been beneficial in only three of the four combinations of fish and barrier (Figure 2), illustrating the challenge of designing passes adapted to different species (Birnie-Gauvin et al., 2019; Silva et al., 2018).

We quantified an overall 14% decrease in fragmentation levels following restoration (27.4% in the case of the steepest barriers  $\geq 2$  m) and 25.6% in the case of the most problematic ones. Interestingly, whether considering all barriers or only the most problematic ones, the relative amount of genetic recovery ( $\bar{G}$  or  $\bar{G}'$ ) was always close to 50%, indicating that restoration operations led to an overall halving of fragmentation levels. This systematic gain in connectivity was achieved within only a few months. Barrier removal notably led to the full recovery of genetic connectivity at three localities, ensuring the full mixing of individuals, and thus of allelic frequencies, within a year. The  $F_{INDEX}$  proved sensitive to the direct movements of individuals and allowed quantifying restoration efficiency even in the absence of gene flow *sensu stricto* (i.e., movement followed by reproduction), which constitutes an important operational asset. However, not all restoration actions proved equally efficient, the recovery of connectivity being only partial in several situations. This is the case of the two new fishways that resulted in an 8%–12% recovery in genetic connectivity in minnows. These reductions are highly encouraging, although further temporal monitoring is needed to determine the final gain in connectivity following restoration. Although barrier removal is expected to be more efficient than fishway creation (Birnie-Gauvin et al., 2019), other removal actions only led to a partial recovery of connectivity, sometimes even after a year (e.g., minnows at CEOSal). It is not clear why close-range genetic mixing is sometimes slow despite the absence of any barrier to movement. For instance, weir removal might result in changes in habitat characteristics

(Bednarek, 2001), locally inducing a temporary repelling effect on fish. Nevertheless, we expect the full recovery of genetic connectivity in the coming years.

Despite its strong operational potential, the  $F_{INDEX}$  possesses limitations, some of which could be circumvented by complementary use of other monitoring methods when necessary (e.g., telemetry). First, the  $F_{INDEX}$  assumes similar amounts of upstreamward and downstreamward gene flow (i.e., symmetry; Prunier et al., 2020). We did not detect any sign of asymmetry (Appendix S9), but asymmetry is notoriously difficult to assess from genetic data, especially at such small spatial scales (Sundqvist et al., 2016): passive downstreamward movements might have remained undetected, explaining why  $F_{INDEX}$  values never exceeded 70%, even for the steepest barriers  $\geq 2$  m. Future developments are required to take into account the influence of asymmetry on  $F_{INDEX}$  estimates. Second, the  $F_{INDEX}$  cannot be used to assess barrier effects on migratory fish species or on heavily stocked populations, because individuals sampled on either side of a barrier would not form natural populations *per se*, making pairwise measures of genetic differentiation meaningless. However, a large diversity of freshwater organisms remains qualified for the  $F_{INDEX}$  (nonstocked resident fish species, macroinvertebrates).


There are currently at least 16 million weirs and dams in the world (Lehner et al., 2011). Barrier removal is seen as an economically viable solution to ensuring public safety or restoring the viability of aquatic ecosystems (Bellmore et al., 2017). However, the number of constructions of new dams in developing economies, as well as the number of retrofits of nonpowered dams, particularly in Europe and the United States, is expected to continue to increase in the coming decades, to meet the growing needs for food and hydroelectric production (Hansen et al., 2021; Zarfl et al., 2015). In this context, reconciling the development of dams and the connectivity of rivers appears to be a major challenge that will need to be tackled worldwide (Thieme et al., 2021). Our study provides strong proof-of-concept that river restoration through barrier removal or fishway creation can translate into the rapid recovery of genetic connectivity in different fish species, and we strongly encourage public policies and practitioners to continue in this direction (Blanchet & Tedesco, 2021). To support them in this task, we illustrate the efficiency of the  $F_{INDEX}$ , an operational barrier assessment method providing standardized estimates of fragmentation. We believe that the  $F_{INDEX}$  fills a gap both in diagnosing barrier effects, paving the way for informed restoration planning, and in evaluating the efficiency of implemented measures (either planned or opportunistic), a prerequisite for refining restoration techniques through feedback. The large-scale deployment of this methodology could also make it possible to lift the veil on the

complex links between individual crossing success, life-history traits of organisms, barrier typologies, and cumulative barrier effects, thus bringing new perspectives as to the best means to restore river connectivity.

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## ORCID

Jérôme G. Prunier  <https://orcid.org/0000-0003-4110-2567>

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Additional supporting information can be found online in the Supporting Information section at the end of this article.

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