1   **Barrier mitigation measures trigger the rapid recovery of genetic connectivity in**

2   **five freshwater fish species**

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

19   GRAPHICAL ABSTRACT

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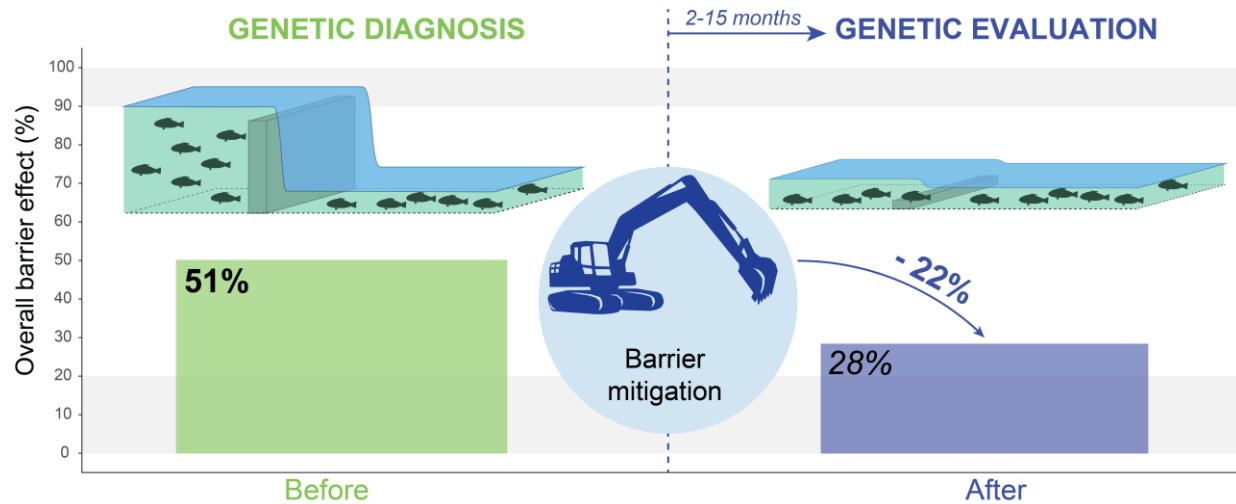
22   ABSTRACT

23   Rivers are heavily fragmented by man-made instream barriers such as dams and weirs. This hyper-

24   fragmentation is a major threat to freshwater biodiversity and restoration policies are now adopted

25   worldwide to mitigate these impacts. However, there is surprisingly little feedback on the efficiency of

26   barrier mitigation measures in restoring riverine connectivity, notably for non-migratory fish species.

27   Here, we implemented a "before-after genetic monitoring" of the restoration of 11 weirs in France using

28   a dedicated genetic index of fragmentation (the F_{INDEX}), with a focus on five fish species from two genera.

29   We found that most obstacles actually had a significant impact on connectivity before restoration,

30   especially the highest and steepest ones, with an overall barrier effect of about 51% of the maximal

31   theoretical impact. Most importantly, we demonstrated for the first time that mitigation measures such

32   as dam removal or fish pass creation significantly and rapidly improved connectivity, with –for some

33   barriers- a complete recovery of the genetic connectivity in less than twelve months. Our study provides

34   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

39   1.   INTRODUCTION

40   Anthropogenic activities exert strong pressures on natural ecosystems, which alter both their

41   physical and biological properties (Crutzen, 2006). This is especially the case for rivers that are highly

42   fragmented by man-made instream barriers such as dams, weirs, water mills, etc. (Grill et al., 2019). In

43   Europe for instance, more than one million obstacles have been reported (Belletti et al., 2020),

44   representing 0,74 barriers per kilometer. Riverscape fragmentation affects the quality, the quantity and

45   the accessibility of natural habitats, and thus prevents organisms to fulfill their life-cycle (Taylor et al.,

46   1993). It is considered one of the most important threat to freshwater biodiversity (Sala, 2000). Given

47   this "hyper-fragmentation" of rivers, the restoration of longitudinal (i.e., upstream-downstream)

48   connectivity to promote fluxes of individuals and genes is hence considered a crucial step to recover the

49   integrity of river ecosystems (Baguette et al., 2013; King & O'Hanley, 2016). Connectivity restoration is

50   moreover the subject of restrictive legislations in many countries, such as in Europe with the Water

51   Framework Directive (2000/60/EC), on which is based the EU's biodiversity strategy for 2030 aiming at

52   restoring at least 25,000 km of rivers to a free-flowing state (COM/2020/380).

53   The restoration of longitudinal connectivity implies barrier mitigation measures: the removal of

54   obstacles, or, when removal is not an option (Blanchet & Tedesco, 2021; Lejon et al., 2009; Magilligan et

55   al., 2017), the equipment of obstacles with natural or artificial fish passes (Seliger & Zeiringer, 2018;

56   Silva et al., 2018). However, the actual efficiency of these barrier mitigation measures to restore genetic

57   and/or demographic connectivity (Lowe & Allendorf, 2010) appear somehow unpredictable, depending

58   on the type of river, the type of obstacle, the chosen type of restoration, as well as the timescale and

59   the species considered (Rodeles et al., 2020). Dam removal has been found beneficial for the rapid

60   recovery of some diadromous fish species (mainly salmonids) whose upstream migratory movements

61   and thus spatial distribution were limited by the presence of barriers (Ding et al., 2019). However other

62   organisms such as potamodromous fish (but also macroinvertebrates, macrophytes, etc.) may not

63   (immediately) benefit from such removal (Brenkman et al., 2019; Gillette et al., 2016). Similarly, fish

64   passes often show uneven levels of permeability across species, depending on pass design and

65   maintenance as well as environmental conditions (Birnie-Gauvin et al., 2019; Harris et al., 2017; Noonan

66   et al., 2012). These effects are yet still poorly documented, notably in the case of low-head structures

67   (weirs, water-mills, sluices...) that do not benefit from the same attractivity as large dams in terms of

68   public interest and research funding (O'Connor et al., 2015). Low-head structures are actually largely

69   understudied when compared to their prevalence and the restoration efforts they represent (Belletti et

70   al., 2020; Ryan Bellmore et al., 2017). Classical methods for the assessment of connectivity restoration

71   efficiency, such as capture-mark-recapture, telemetry and monitoring of spatiotemporal changes in the

72   composition of fish communities (Rodeles et al., 2020; Silva et al., 2018), cannot be systematically

73   deployed at large management scales, especially for practitioners who are generally constrained by time

74   and budget. For low-head barriers, mitigation measures are often undertaken opportunistically (Poff et

75   al., 2003; Tonitto & Riha, 2016), with limited or coarse ecological monitoring of the system before

76   restoration (Barry et al., 2018) and no (or limited) evaluation of the outcome in most cases (Cooke et al.,

77   2019; Rodeles et al., 2017).

78   One challenge for practitioners is notably the lack of rapid and efficient connectivity assessment

79   tools allowing both the *a priori* quantification of the individual impact of instream obstacles and the *a*

80   *posteriori* quantification of the efficiency of implemented measures (removal or equipment; Cooke et

81   al., 2019). If molecular tools are now commonly considered for the *a priori* assessment of barrier

82   effects (Abernethy et al., 2013; Coleman et al., 2018; Dehais et al., 2010; Gouskov et al., 2016; Liu et al.,

83   2020; Meldgaard et al., 2003; Prunier et al., 2018; Raeymaekers et al., 2009), there is still a surprising

84   paucity of genetic studies dedicated to the temporal monitoring of changes in connectivity after

85   restoration (Ding et al., 2019). Only a handful of recent studies could be identified, all focusing on the

86   effect of the creation (Liu et al., 2020; Vega-Retter et al., 2020) or the removal (Fraik et al., 2021) of

87   large dams on gene flow (but see Weigel et al., 2013). As a result, and despite the importance for

88   practitioners and managers to assess (and communicate) the relevance of their actions for natural

89   ecosystems, there are still very few indications that (i) current mitigation measures (barrier removal or

90   fish pass creation) deployed worldwide are improving genetic connectivity (*sensu* Lowe & Allendorf,

91   2010) and (ii) that molecular approaches are efficient and operational tools to prioritize local restoration

92   actions and assess their efficiency.

93   In this study, we implemented a "before-after genetic monitoring" of the restoration of 11 weirs

94   in France using a recently developed genetic index of fragmentation (the F_{INDEX}; Prunier et al., 2020). The

95   F_{INDEX} provides, independently for each obstacle, an absolute and standardized estimate of (species-

96   specific) genetic connectivity, while taking into account two confounding factors: the age of the obstacle

97   and the size of populations on either side of the obstacle. Considering two common potamodromous

98   fish genera, our objectives were (i) to quantify the initial impact of obstacles and thus to determine

99   whether restoration was actually needed in the first place, and (ii) to quantify the gain in connectivity

100   resulting from implemented restoration actions.

101

102 2. MATERIALS AND METHODS

103 2.1. Instream obstacles

104 The study took place at the scale of the French national hydrographic network. In close

105 coordination with the French Office for Biodiversity (OFB) and French Water Agencies, we identified 11

106 obstacles (low-head dams <4m high) whose restoration was scheduled for the coming years (Figure 1;

107 Table 1). These obstacles were located in the three largest French river basins (Garonne, Loire and

108 Seine). On the basis of the consultation of old aerial photos and maps (French Ordnance Survey maps

109 and cadastral plans), completed by surveys of local agencies, we estimated that they were constructed

110 (or reconstructed after destruction or abandonment) between the 15th and the 20th century (Table 1).

111 The obstacles ranged from 0.8 to 3.5m high and showed various slopes (Figure 1). Two of them were

112 already equipped with a fish pass (Table 1). Each obstacle was described according to its height (< or ≥

113 2m high) and slope (< or ≥ 45°) using a unique synthetic factor 'Typology' with four levels ('low and

114 gentle', 'low and steep', 'high and gentle', 'high and steep'). The restoration actions were conducted

115 between 2015 and 2019 and in most cases (9 out of 11) consisted in the dismantlement of the obstacle.

116 Note that we could not statistically assess the specific effects of fish passes on connectivity in the

117 following analyses because there were too few of them, either before or after restoration.

118

119 2.2. Biological models and Before-After sampling sessions

120 We focused on five common potamodromous species from two genera: minnows (*Phoxinus sp.: P.*

121 *phoxinus* in the Seine*, P. fayollarum* in the Loire and *P. dragarum* in the Garonne watershed) and

122 gudgeons (*Gobio sp*: *G. gobio* in the Loire and the Seine and *G. occitaniae* in the Garonne watershed).

123 Within each genus, species are allopatric (Denys et al., 2020) but were here considered to have very

124 similar life history traits and movement behaviors (Keith et al., 2011). These species are small

125 insectivorous cyprinids (maximal body length of 140 and 200mm, in minnows and gudgeons

126   respectively) that show distinct foraging strategies: minnows preferentially feeds in the water column,

127   whereas gudgeons feed on the bottom (Keith et al., 2011). We selected these species because they are

128   widespread and abundant and hence easy to catch for practitioners, which make them ideal models for

129   an operational tool such as the F_{INDEX}.

130   Sampling operations 'Before' and 'After' restoration were performed using electrofishing, until a

131   maximum of 30 adult individuals of each species were captured on either side of obstacles. Fish were

132   captured in the direct downstream and upstream vicinity of each obstacle (Prunier et al., 2020), starting

133   from the downstream site to avoid accidental upstream to downstream movements of individuals. A

134   piece of pelvic fin was sampled on all individuals and stored in 96% alcohol. All fish were returned alive

135   to their sampling site. Electrofishing and fin sampling was performed according to legal authorizations

136   and permits.

137   Both genera could be sampled at all sites except in DADRai (gudgeons only) and GLASou (minnows

138   only). The sampling sessions after the restoration occurred on average 9.7 (± 3.9 SD) months after the

139   end of the restoration (from 44 days in VIAPig to 427 days in CEOSal; Table 1). These after-restoration

140   timelags could not be homogenized better, owing to logistic and administrative difficulties (e.g., high

141   water levels preventing safe fieldwork).

142

143   2.3.   Genotyping and F_{INDEX} computation

144   We considered 19 and 15 microsatellite markers in minnows and gudgeons, respectively. DNA

145   extraction, genotyping and assessment of null alleles and gametic disequilibrium followed previously

146   published procedures (Prunier et al., 2018, 2020; Appendix S1). For each dataset (combination of one

147   obstacle and one genus; n = 20 since only one genus could be sampled at GLASou and DADRai; Table 2)

148   and each passage ('Before' and 'After'), we computed the F_{INDEX} and the standard deviation of the F_{INDEX}

149   as detailed in Prunier et al. (2020) using a dedicated R-based pipeline. Briefly, the F_{INDEX} corresponds to

150   the rescaling of pairwise measures of genetic differentiation within their theoretical range of variation

151   given effective population sizes (approximated from expected heterozygosity) and the number of

152   generations elapsed since barrier creation. The F_{INDEX} is therefore a standardized (across barriers and

153   species) index expressed as a percentage, with values lower than 20% representing fully permeable

154   structures and values higher than 90% representing total barriers to gene flow (see Prunier et al., 2020

155   for details). F_{INDEX} values are computed along with a standard deviation SD_F (or a 95% confidence interval

156    $CI_{95\%}$) that takes into account biological uncertainty stemming from $n_F = 4$ parameters (two mutation

157   rates and two metrics of genetic differentiation; Prunier et al., 2020). We considered a generation time

158   of 2 years in minnows and 2.5 years in gudgeons to compute the number of generations elapsed since

159   barrier creation (Kottelat & Freyhof, 2007).

160

161   2.4.   Barrier effects and restoration efficiency

162   We adopted a meta-analytical approach (Borenstein, 2009), considering each dataset

163   (combination of one obstacle and one genus; n = 20) as an independent study providing two effect sizes

164   (F_{INDEX} values before and after restoration ± SD_F). Six datasets were non-informative ($F_{INDEX} = 0$ and $SD_F =$

165   0 before restoration) and were thus discarded, resulting in 14 datasets, of which three were associated

166   with a non-significant barrier effect (see results). The interpretation of the F_{INDEX} being meaningful

167   (barrier effect expressed as a percentage of maximum fragmentation), the F_{INDEX} value before

168   restoration was directly used as the observed effect size F±SE_F of barrier effect in each dataset, with

169    $SE_F = SD_F/\sqrt{n_F}$. We used the raw difference ΔF(±SE_Δ) between F_{INDEX} values computed after

170   ('treatment') and before ('control') restoration as the observed effect size of restoration for each

171   dataset (Equations 4.2 and 4.6 in Borenstein, 2009). The raw difference ΔF can be directly interpreted as

172   the (positive or negative) change in the amount of fragmentation following restoration and was thus

173   preferred over the standardized mean difference Hedges' g (Borenstein, 2009).

174   We used random-effect meta-regressions with moderators (metafor::rma.mv R-function; Harrer

175   et al., 2021; Viechtbauer, 2010) to compute the overall true effect sizes of the barrier effects $\bar{F}$ and of

176   the restoration of obstacles $\overline{\Delta F}$ (n = 14), while taking into account different possible sources of variation

177   in effect sizes: within-datasets (i.e., SE_F or SE_Δ), between-datasets and, possibly, across different

178   covariate modalities. Considered covariates were 'Genus' (two levels: minnows or gudgeons), 'Typology'

179   (four levels: see above), as well as, in the case of $\overline{\Delta F}$, the 'timelag' (two levels: < 1 year or > 1 year)

180   between the restoration operation and the second sampling session. To account for within-dataset

181   variability (SE_F or SE_Δ), we used dataset ID as an outer random grouping factor. To allow residual

182   heterogeneity to differ across covariate modalities, each covariate was successively defined both as a

183   moderator and an inner random grouping factor. Models were run with a diagonal variance-covariance

184   matrix as a random effect structure. Covariates identified as significant moderators were then kept as

185   inner random grouping factors in final models without moderator to get final estimates of the true

186   effect sizes $\bar{F}$ and $\overline{\Delta F}$ along with their respective 95% confidence interval $CI_{95\%}$.

187   3.   RESULTS

188   Altogether, a total of 1049 and 1038 genotypes could be obtained from the various sampling

189   sessions (through space and time) in minnows and in gudgeons, respectively, with an overall mean of

190   26.1 (± 3.2 SD) genotypes per genus, sampling site and sampling session. The mean number of loci

191   across datasets was 15.7 (± 1.8 SD) and 14.4 (± 0.8 SD) in minnows and in gudgeons, respectively.

192   F_{INDEX} values ranged from 0 to 68.1 % (mean: 27.7 ± 25.8 SD) before restoration, with a significant

193   barrier effect detected in 11 out of 20 datasets (Table 2). Genera showed highly contrasted responses to

194   obstacles: all obstacles but GLAMou and LEZVil had a significant impact on connectivity before

195   restoration, but only VIAPig and GLAPas did impact both genera simultaneously (Table 2; Figure 2).

196   Accordingly, 'Genus' was not identified as a significant moderator of the overall effect size $\bar{F}$ (Table 3).

197   Only 'Typology' did significantly influence $\bar{F}$, with steep obstacles higher than 2 m showing an overall

198   effect size $\bar{F}$ 40 % ($CI_{95\%}$ = [14.2; 65.8]) higher than gentle weirs lower than 2 m (Table 3; Figure 3). Once

199   all possible sources of variation in effect sizes were taken into account (both within-datasets and

200   between modalities of typology), the final true effect size of fragmentation $\bar{F}$ was of 51.4 %, a value

201   significantly different from 0 and higher than the F_{INDEX} significance threshold of 20% ($CI_{95\%}$ = [45.1;

202   57.7]).

203   Except when obstacles had no effect before restoration (F_{INDEX} < 20%), in which case restoration

204   had no effect either (F_{INDEX} < 20% after restoration; 5/10 obstacles in gudgeons and 4/10 obstacles in

205   minnows), restoration systematically led to a significant decrease in F_{INDEX} values (as indicated by non-

206   overlapping confidence intervals; Figure 2), with F_{INDEX} values after restoration ranging from 0 to 49.1 %

207   (mean: 13.7 ± 17.8 SD). This decrease led to the full recovery of connectivity (F_{INDEX} < 20% after

208   restoration) in 3/5 obstacles in gudgeons and 2/6 obstacles in minnows. The observed effect sizes of

209   restoration ΔF ranged from -55.4% to +11.7% (mean: -14 ± 18 SD; Table 2). Neither 'Genus', 'Typology'

210   or 'Timelag' were identified as significant moderators of the overall effect size of restoration $\overline{\overline{\Delta F}}$ (Table

211   3, Appendix S2). The final overall effect size of restoration $\overline{\overline{\Delta F}}$ was of -21.7%, a value significantly

212   different from 0 ($CI_{95\%}$ = [-30.2; -13.2]). Overall, the effect of restoration was thus the same across

213   genera, did not depend on the typology of the obstacle and, interestingly, was independent from the

214   timelag between the restoration and the second sampling session. In other words, we found evidence

215   that genetic connectivity could be recovered (entirely in some cases, partly in most cases) in just a few

216   months after restoration.

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218

219   4.   DISCUSSION

220   Quantifying the impact of instream barriers on potamodromous fish species as well as the

221   efficiency of mitigation measures is primordial in the context of restoration planning, so as to properly

222   allocate limited resources towards the most impactful obstacles, inform trade-offs between ecological

223   and socio-economic issues, and refine restoration techniques (Hermoso et al., 2012; Rodeles et al.,

224   2020; Silva et al., 2018). Quantification is yet a difficult task, notably because of technical and financial

225   constraints preventing the parallel monitoring of multiple obstacles and because of the relative lack of

226   operational tools allowing valid comparisons across both contexts and species (Cayuela et al., 2018). The

227   response of freshwater organisms to connectivity restoration has often been studied at the community-

228   or at the population-levels (Brenkman et al., 2019; Frey, 2021; Magilligan et al., 2021; Muha et al., 2021;

229   Stanley et al., 2002; Sun et al., 2021), rarely at the genetic level (Fraik et al., 2021), and our study is the

230   first to document the systematic and rapid recovery of gene flow over a series of independent

231   restoration actions. In this study, we used a standardized genetic index of fragmentation to quantify

232   both the impact of 11 low-head dams on gene flow in five freshwater fish species, and the efficiency of

233   mitigation actions in restoring genetic connectivity.

234   Before restoration, we found a significant barrier effect in 11 out of 20 datasets, with two

235   obstacles showing no impact on any genus and two obstacles significantly impacting both genera.

236   Surprisingly, out of nine obstacles with genetic data in both genera, five showed large discrepancies in

237   genus response to fragmentation, with either only gudgeons or only minnows significantly impacted

238   (Figure 2). These discrepancies illustrate how barrier effects can be highly species- or genus-dependent

239   (Amaral et al., 2021; Blanchet et al., 2010; Prunier et al., 2018), and thus hardly predictable given our

240   limited knowledge about fish movement behavior and capacities (Baudoin et al., 2014; Thurow, 2016).

241   In absence of a dedicated fish pass, individuals are supposed to take advantage of drowned conditions,

242   that is, of periods where water level rises above the height of the dam, to cross the obstacle (Keller et

243   al., 2012). However, such propitious conditions of obstacle drowning might not be encountered every

244   year, at all localities, and equally across all species/individuals, depending on their swimming behavior

245   and capabilities in various environmental conditions and to the timing of submersion compared with the

246   timing of individual movements (Carpenter-Bundhoo et al., 2020; Holthe et al., 2005; Keller et al., 2012).

247   This may explain why F_{INDEX} values differed so much across datasets, and why the overall effect size of

248   fragmentation $\bar{F}$ was unrelated to the considered genus. However, $\bar{F}$ was significantly influenced by the

249   typology of obstacles, with high and steep obstacles showing an overall effect size $\bar{F}$ 40% higher than

250   low and gentle obstacles (Table 3, Figure 3). This finding is in line with classical expectations about the

251   impact of dam typology (e.g., Januchowski-Hartley et al., 2019) and conclusions from other studies (e.g.,

252   Amaral et al., 2019; Keller et al., 2012; Zigler et al., 2004), as well as with the obstacle drowning

253   hypothesis: the highest dams (≥ 2 m) might rarely be drowned (leading to the increase in $\bar{F}$), but, in

254   presence of a gentle slope (< 45°), they might become partly crossable by some individuals, at least at

255   intermediate drowning conditions, so that only the highest and steepest obstacles have an overall

256   significant effect size ($\bar{F}$ = 54.6%; Figure 3). This result should of course be confirmed and refined with

257   additional datasets, but it provides a relevant and meaningful benchmark for practitioners to adjust

258   restoration planning even in the absence of any individual quantification of barrier effects.

259   With this effect of typology taken into account, we estimated the true effect size of fragmentation

260   as of $\bar{F}$ = 51.4 %: in other words, we might expect a 51 % decrease in gene flow in presence of a low-

261   head dam (irrespective of the species or the context). Given these different findings, it appears that a

262   preliminary diagnosis based on a standardized genetic tool such as the F_{INDEX} may actually help managers

263   quantify and compare barrier effects across species and obstacles, and thus orientate their restoration

264   efforts towards the most problematic structures (Prunier et al., 2020), keeping in mind that the most

265   impactful obstacles might be the highest and steepest ones, but that more seemingly anodyne obstacles

266   might also be particularly impacting for some species. Of course, we willingly acknowledge that other

267   ecological and socio-economic indicators should be considered in restoration planning as well (Hermoso

268   et al., 2012). It is also noteworthy that the F_{INDEX} might help evaluate the species-specific efficiency of

269   fish passes, an important step to drive future technical developments (Foulds & Lucas, 2013): the two

270   considered fish passes before restoration appeared beneficial in all situations except for minnows at

271   SIOBre (Figure 2), illustrating the challenge of locally designing passes adapted to different fish species

272   with distinct life-history traits and requirements (Birnie-Gauvin et al., 2019; Silva et al., 2018).

273   Our most striking result concerns the efficiency of mitigation measures. All restoration actions led

274   to a significant reduction of barrier effects, provided there was an actual barrier effect in the first place.

275   We quantified an overall 22% decrease in fragmentation levels following restoration. This means that

276   mitigation measures may allow the full recovery of genetic connectivity for any initial fragmentation

277   level of up to 42% (i.e., 20% < F_{INDEX} ≤ 42%), a value to be compared to the overall effect size of

278   fragmentation $\bar{F}$ = 51% before restoration. Most interestingly, this systematic gain in connectivity was

279   achieved within a few months after restoration only. For instance, weir removal led to the full recovery

280   of genetic connectivity at three localities (LEZCas, VIAPig and GLAPas), indicating that it ensured the full

281   mixing of individuals, and thus of allelic frequencies, within a year, and even within two months only at

282   VIAPig. However, not all restoration actions proved equally efficient within the same timeframe, the

283   recovery of connectivity being only partial in several situations. This is the case of the two (new or

284   improved) fish passes at SERHau and SIOBre that resulted in an 8 to 12% recovery in genetic connectivity

285   in minnows within a year. These reductions are highly encouraging, but it is still unknown whether

286   recovery is still ongoing: further temporal monitoring is needed to detect migration-drift equilibrium

287   and determine the final gain in connectivity following restoration. Furthermore, and although weir

288   removal is expected to be more efficient than fish pass creation as a restoration option (Birnie-Gauvin et

289   al., 2019; Sun et al., 2021), other removal actions only led to a partial recovery of connectivity,

290   sometimes even after a year (e.g., at CEOSal in minnows). The migration behavior of fish being still

291   poorly documented and likely to deeply differ across species and contexts, it probably explains why the

292   true effect size of restoration was unrelated to the considered genus, the typology of the obstacle or the

293   timelag between restoration and sampling. We can only speculate on why close-range genetic mixing

294   would sometimes take so long despite the absence of any obstacle to movement. For instance, weir

295   removal might result in profound changes in upstream and downstream habitat characteristics

296   (Bednarek, 2001; Doyle et al., 2005), locally inducing a temporary repelling effect on fish. It has also

297   been suggested that, in some conditions, fragmentation might lead individuals to adjust their life-history

298   strategies towards residency (Branco et al., 2017), which might further delay genetic connectivity after

299   removal. Nevertheless, we expect the full recovery of genetic connectivity in the coming years, which

300   will however require further genetic monitoring of these situations.

301

302   5.   CONCLUSION

303   Our study provides a strong proof-of-concept that barrier removal and, probably to a lesser extent, fish

304   pass creation, are efficient mitigation strategies to restore riverine genetic connectivity in just a few

305   months. We also illustrated how before-after genetic monitoring based on a standardized tool such as

306   the F_{INDEX} constitute a promising support for practitioners in the planning and the monitoring of

307   restoration. We believe that the large-scale deployment of this methodology in the future, with a

308   growing number of case studies, will make it possible to lift the veil on the complex links between

309   individual crossing success, life history traits of organisms and barrier typologies.

310

311   ACKNOWLEDGEMENTS

317

318   AUTHORS'CONTRIBUTIONS

319   JGP, SB and NP conceived the ideas and designed methodology; JGP and SB collected the samples in the

320   field; CV and GL produced molecular data; JGP analyzed the data; JGP and SB led the writing of the

321   manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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535 FIGURES AND TABLES

536 Table 1: Main characteristics of obstacles ('Lon': longitude; 'Lat': latitude; 'Height' (in m), 'Slope' and

537 presence of a 'fish pass'), details about mitigation measures (type and date of actions) and timelag (in

538 days) between mitigation measures and second sampling sessions.

539

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
DADRai	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

540

541

542

543   Table 2: For each dataset (that is, a combination of one obstacle and one genus), observed effect sizes

544   of barrier effects (F±SD$_F$) both before and after restoration and observed effect sizes of restoration (ΔF±

545   SE$_Δ$), respectively. Note that SD$_F$ = 2 x SE$_F$. Significant effect sizes (see Figure 2) are in bold and indicated

546   with a star. The last column 'Recovery' indicates whether the restoration action (Removal or Fish pass

547   creation) led to the partial or the full recovery of connectivity, when applicable.

548

Obstacle code	Genus	Barrier effect					Restoration efficiency			
		Before restoration		After restoration						
		F	SD$_F$		F	SD$_F$		Action	ΔF	SE$_Δ$		Recovery
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		/
DADRai	**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**

549

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551

552 Table 3: Results of random meta-analyses for the overall effect sizes of fragmentation $\bar{F}$ and of

553 restoration $\overline{\Delta F}$. In presence of a moderator, EST is the estimate of the overall effect size for the

554 intercept (INT) and the deviation from the intercept for the alternate modalities, with the moderator as

555 a random effect. In absence of moderator (indicated with a slash), EST is the final estimate of the overall

556 effect size taking into account all sources of variation (including the previously identified significant

557 moderators as random effects, when applicable). Also provided are the number n of datasets in each

558 modality, the standard error (SE) and CI$_{95\%}$ (ci.low and ci.high) around EST, the Wald-Type Z statistic

559 (Zval) and the associated p-value (pval), and the QM test of moderator effect (QM statistic and

560 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**		
$\overline{\Delta F}$	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**		

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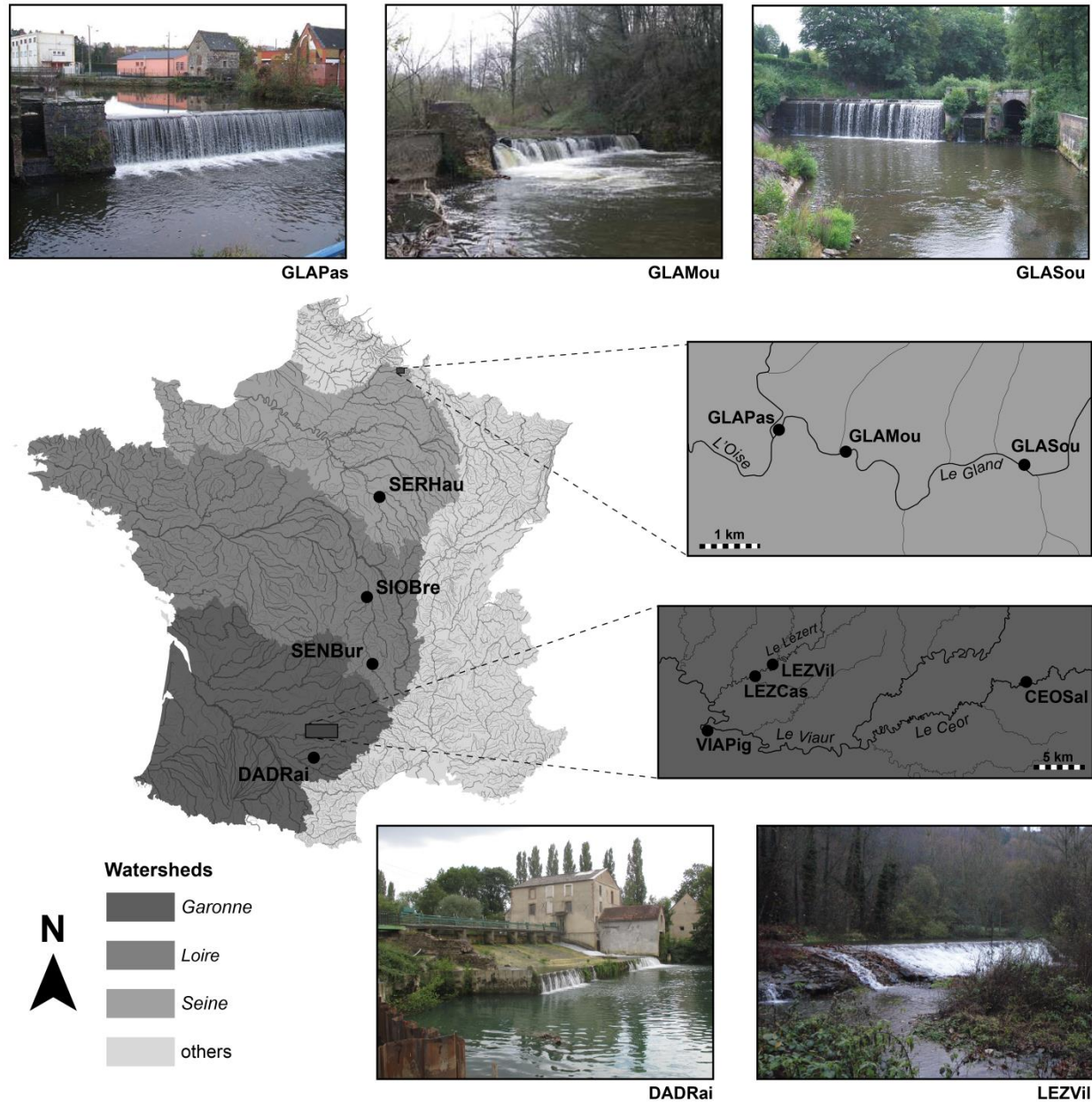
563 watersheds.

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566   Figure 2: Main results of the before-after genetic monitoring. For each obstacle (in columns), bars

567   represent F_{INDEX} values with 95% confidence intervals as computed before (in green) and after (in purple)

568   restoration in gudgeons (panel A) and minnows (panel B). Slashes indicate no data in both A and B.

569   Outlined bars represent significant barrier effects ($F_{INDEX} > 20\%$). Green stars indicate a significant change

570   in F_{INDEX} values after restoration (non-overlapping confidence intervals). Double purple stars indicate the

571   full recovery of connectivity following restoration (see details in Table 2). Panel C also provides few

572   details about obstacles (in green) and restoration (in purple) for direct comparisons with F_{INDEX} values

573   (see Table 1). Obstacles are sorted by their increasing height. Note that F_{INDEX} values actually increased

574   after restoration in two datasets (LEZVil in gudgeons and LEZCas in minnows), but that new values were

575   lower than 20 % and non-significantly different from 0 (and thus from F_{INDEX} values before restoration)

576   according to $CI_{95\%}$, a phenomenon owing to the stochasticity of genetic approaches

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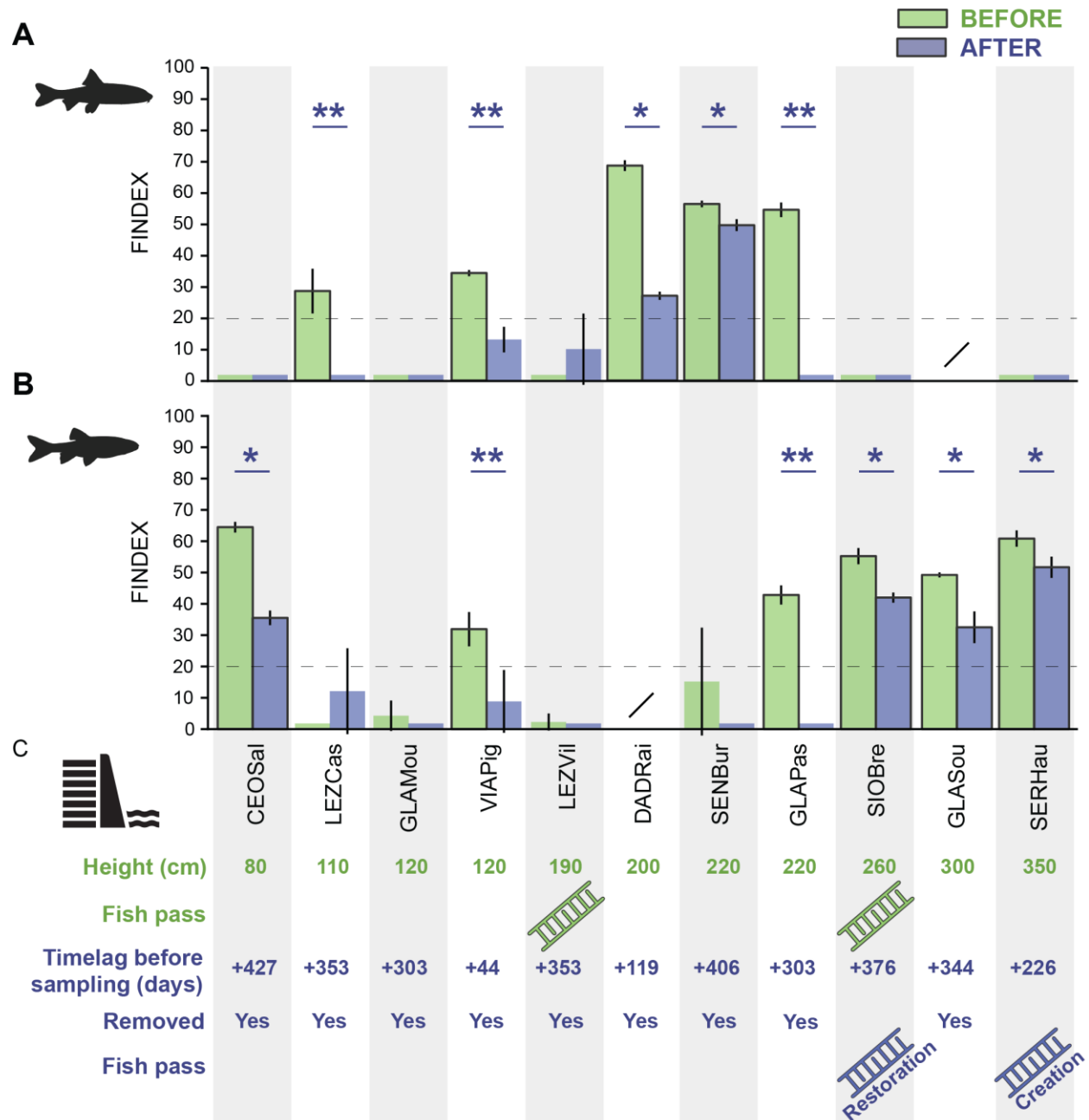
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590   Figure 3: Overall effect sizes $\bar{F}$ of fragmentation for each modality of typology and overall true effect size

591    $\bar{F}$ taking all significant sources of variation (within datasets and across modalities) into account. Washed

592   out bars indicate that the effect size of fragmentation is not significant ($\bar{F} > 20\%$ or $CI_{95\%}$ including 20%;

593   Prunier et al., 2020).

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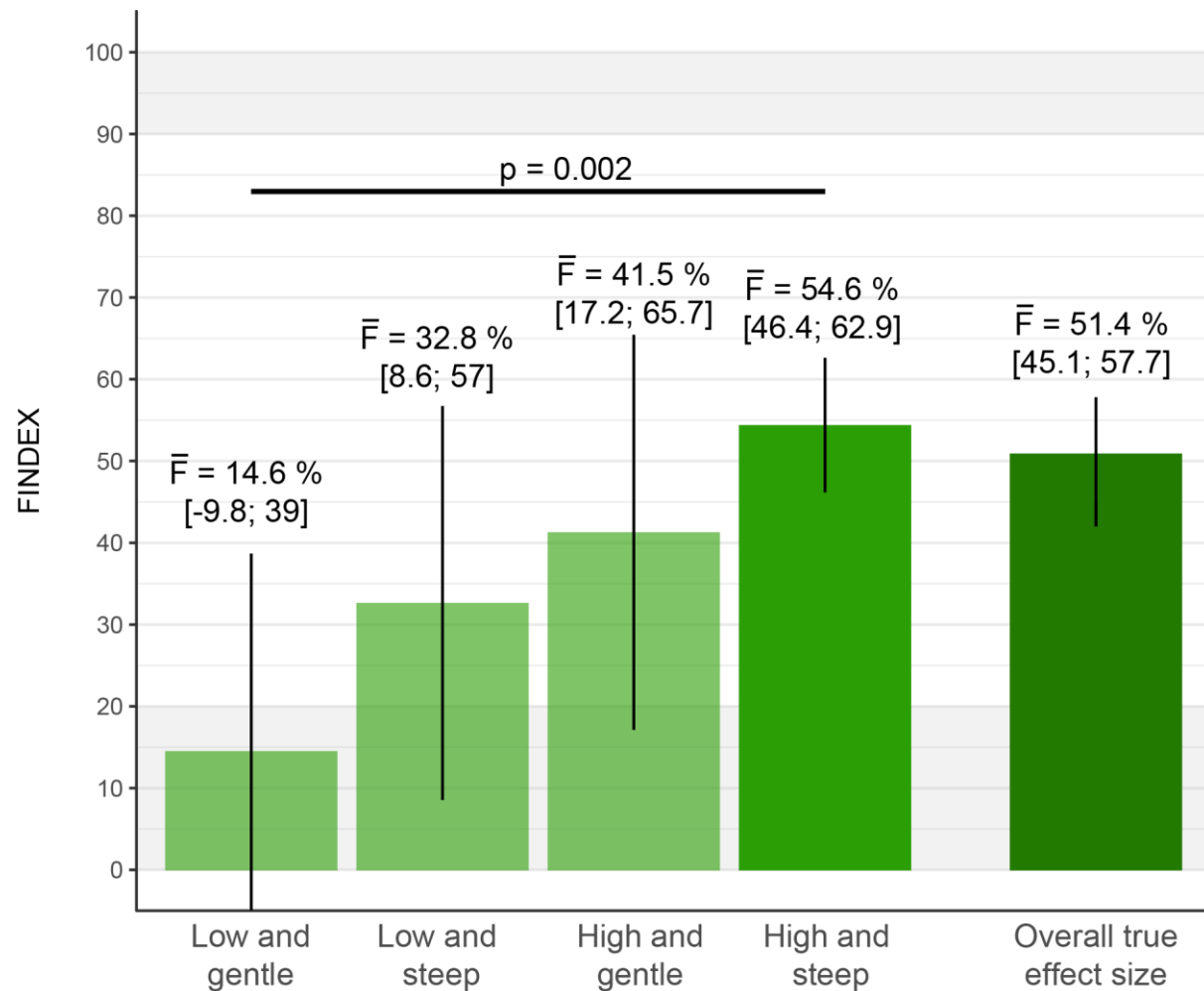
599   APPENDIX S1: DNA EXTRACTION AND GENOTYPING

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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, Rvla21177,

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).

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

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

616   timelag between restoration and sampling.

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