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ARTICLE

Riverscape genetics in brook lamprey: genetic diversity is less influenced by river fragmentation than by gene flow with the anadromous ecotype

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Abstract

Understanding the effect of human-induced landscape fragmentation on gene flow and evolutionary potential of wild populations has become a major concern. Here, we investigated the effect of riverscape fragmentation on patterns of genetic diversity in the freshwater resident European brook lamprey (*Lampetra planeri*) that has a low ability to pass obstacles to migration. We tested the hypotheses of (i) asymmetric gene flow following water current and (ii) an effect of gene flow with the closely related anadromous river lamprey (*L. fluviatilis)* ecotype on *L. planeri* genetic diversity. We genotyped 2472 individuals, including 225 *L. fluviatilis*, sampled from 81 sites upstream and downstream barriers to migration, in 29 western European rivers. Linear modelling revealed a strong positive relationship between genetic diversity and the distance from the river source, consistent with expected patterns of decreased gene flow into upstream populations. However, the presence of anthropogenic barriers had a moderate effect on spatial genetic structure. Accordingly, we found evidence for downstream-directed gene flow, supporting the hypothesis that barriers do not limit dispersal mediated by water flow. Downstream *L. planeri* populations in sympatry with *L. fluviatilis* displayed consistently higher genetic diversity. We conclude that genetic drift and slight downstream gene flow drive the genetic make-up of upstream *L. planeri* populations whereas gene flow between ecotypes maintains higher levels of genetic diversity in *L. planeri* populations sympatric with *L. fluviatilis*. We discuss the implications of these results for the design of conservation strategies of lamprey, and other freshwater organisms with several ecotypes, in fragmented dendritic river networks.

Introduction

Human activities strongly modify natural ecosystems (Vitousek et al. 1997) and impact evolutionary trajectories of wild species (Palumbi 2001) posing unprecedented threats to the maintenance of biodiversity (Ceballos et al. 2017). In particular, habitat fragmentation is a major threat to wild species (Fahrig 2003; Vitousek et al. 1997). Habitat fragmentation can reduce gene flow among sub-populations, which can ultimately decrease effective population size and genetic diversity (Blanchet et al. 2010; Couvet 2002; DiBattista 2008; Frankham 1998; Whiteley et al. 2013). Habitat fragmentation influences the genetic structure and diversity of various species including birds (Alonso et al. 2009), fishes (Blanchet et al. 2010; Hänfling and Weetman 2006; Raeymaekers et al. 2008; Torterotot et al. 2014) and plants (Young et al. 1996). Small isolated populations are expected to fix weakly deleterious alleles by random drift (Lynch et al. 1995; Wang et al. 1999;

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Glémin 2003) and can suffer higher extinction risks (Saccheri et al. 1998; Carlson et al. 2014; Smith et al. 2014). Maintaining high connectivity and genetic diversity levels is thus fundamental to preserve the evolutionary potential of populations (Frankham et al. 2014; Frankham 2015; Ralls et al. 2018).

Freshwater ecosystems have been particularly affected by fragmentation worldwide (Dynesius and Nilsson 1994; Nilsson et al. 2005) due to the construction of dams, weirs, and to artificial modifications of river channels. Such fragmentation alters the possibility of gene flow between populations of aquatic organisms, so that upstream isolated populations are particularly exposed to genetic drift and its consequences, namely reduced genetic diversity and ultimately increased inbreeding. In addition, river systems are naturally shaped as dendritic networks where migration preferentially occurs following downstream directed water flow, generating patterns of asymmetric gene flow (Hänfling and Weetman 2006; Pollux et al. 2008). As a result, populations are structured following a source–sink model (Hänfling and Weetman 2006; Kawecki and Holt 2002) in which the genetic diversity will be smaller in upstream source populations than in downstream sink populations. Three possible processes may explain this pattern of downstream increase in genetic diversity observed across taxa (Paz-Vinas et al. 2015): (i) downstream biased dispersal generating downstream gene flow (Paz-Vinas et al. 2013); (ii) increase in downstream habitat availability (e.g. Raeymaekers et al. 2008); and iii) upstream founding events with loss of genetic diversity, e.g. following postglacial colonization (Cyr and Angers 2011). However, it remains unclear how human mediated alterations of habitat connectivity in rivers may obscure or exacerbate this pattern.

To date, most studies focused on delineating the effect of barriers to migration in large species targeted by fisheries. This is particularly the case for salmonid fishes that display a strong migratory behaviour and a good ability to pass obstacles (Morita and Yamamoto 2002; Yamamoto et al. 2004). In contrast, few empirical studies have focused on species with modest dispersal abilities or weak capacities to pass obstacles (e.g. Hänfling and Weetman 2006; Raeymaekers et al. 2008; Blanchet et al. 2010), which are expected to be more impacted by the effect of river fragmentation. Even less work has been focussed on the effect of small barriers to migration (e.g. 0.5–5 m) despite the fact that they are more widespread than large dams. For instance, ~58,000 large dams (>15 m) are installed in the world (Mulligan et al. 2020) compared with more than 60,000 obstacles (<5 m) in France alone (sandre.eaufrance.fr). If these small dams affect a species' ability to disperse or migrate then their effect should be widespread across the whole species' range.

In addition, species can display various life history strategies. They may differ in their dispersal capacity and thus be differentially affected by changes in habitat connectivity. For instance, in certain fish species some individuals are freshwater-resident whereas others are anadromous (i.e. reproduce in freshwater and juveniles migrate to sea for growth) (Dodson et al. 2013; Jonsson and Jonsson 1993). Anadromous individuals can either display a homing behaviour as they return back to their natal river to spawn, or disperse into neighbouring rivers, which can enhance gene flow. Consequently, anadromous populations generally display lower levels of population genetic structure than resident populations (Hess et al. 2013; Hohenlohe et al. 2010; Quéméré et al. 2016; Rougemont et al. 2015; 2017; Spice et al. 2012). It has also been shown that anadromous salmonid populations usually display a higher level of genetic diversity than resident populations (e.g. Perrier et al. 2013) but it is not clear whether admixture between both forms may enhance genetic diversity of resident populations when both forms coexist (Tonteri et al. 2007; McPhee et al. 2014).

The European brook lamprey *Lampetra planeri* is a widespread freshwater resident species with a putatively low dispersal ability at the adult stage linked to its small size (15–22 cm) and its particular life cycle (Taverny and Elie 2010). Larvae spend between 4 and 6 years mostly buried in fine sediments, creating large opportunities for long distance passive downstream dispersal at low energetic expense. In contrast, adult upstream migration is limited to return to spawning grounds. However, this spawning migration up to several kilometers might be affected by migratory barriers (Malmqvist 1980; Moser et al. 2015). *L. planeri* is closely related to the river lamprey *L. fluviatilis* that is parasitic and anadromous at the juvenile stage (Eneqvist 1937). The two taxa share many similarities. They were originally recognized as ecotypes of the same species as a consequence of partial reproductive isolation (Eneqvist 1937). They can produce viable hybrids, display a low pre-zygotic and postzygotic isolation (Rougemont et al. 2015) and are best described as partially reproductively isolated ecotypes with patterns of extensive hybridization and genetic admixture in sympatry (Rougemont et al. 2017). Throughout the manuscript we will therefore refer to them as ecotypes, rather than species. *L. fluviatilis* populations from nearby watersheds remain connected and display low genetic differentiation in relation to dispersal abilities through the marine environment and an apparent absence of homing (Bracken et al. 2015; Rougemont et al. 2015). In contrast, *L. planeri* has a highly reduced migratory behaviour: it does not move outside its watershed and generally migrates over short upstream distances within the river for breeding purposes (Malmqvist 1980). Thus, the most isolated brook lamprey

populations located in the upper reaches of rivers can be strongly genetically differentiated from other populations either downstream or in other rivers (Bracken et al. 2015; Mateus et al. 2011; Pereira et al. 2010; Dawson et al. 2015; Rougemont et al. 2015). These isolated populations often display a low genetic diversity at microsatellite loci (Rougemont et al. 2015). The dispersal ability of *Lampetra* larvae is largely unknown but downstream dispersal may be important at this stage. In particular, flood events induce the remobilization of fine sediments where larvae are burrowed and may favour passive drift of larvae. Such downstream dispersal should further enhance the natural tendency of increasing genetic diversity in downstream river networks. Therefore, we hypothesize that in brook lamprey gene flow should clearly be asymmetric. In addition, brook lamprey populations living in sympatry with river lampreys have been found to display a higher level of genetic diversity than populations located in upstream reaches where river lampreys are absent (Rougemont et al. 2015, 2016). Maintaining genetic diversity is key to maintain a species evolutionary potential (reviewed in Frankham 2015). Therefore, gene flow between the two ecotypes may act as a 'reservoir' of genetic diversity, which in turn may contribute to population adaption to an ever changing world (Ceballos et al. 2020). However, to disentangle the effects of gene flow between ecotypes and downstream biased dispersal, the genetic diversity of *L. planeri* populations should be compared between rivers where only *L. planeri* is present and watersheds where both species coexist, which has not yet been tested.

The main aims of this study were to understand: (i) the role of river fragmentation on population genetic diversity and structure of *L. planeri* in various river systems from North western Europe; (ii) the extent of asymmetry in gene flow among *L. planeri* populations from the same river; and (iii) the possible role of *L. fluviatilis* in increasing genetic diversity in sympatric *L. planeri* populations *via* introgression. We performed extensive sampling of *L. planeri* upstream and downstream of small barriers to migrations in 29 rivers from three hydrogeologic regions: Brittany, Normandy and Upper Rhône, in France. Moreover, two watersheds were sampled more extensively to further investigate the combined effects of multiple barriers to migration on patterns of genetic diversity. We were particularly interested in the effect of small obstacles since these are widespread and may therefore have a cumulatively higher impact on fish dispersal. To test the prediction that *L. planeri* populations found in sympatry with river lampreys may display greater levels of genetic diversity than populations where river lampreys are absent, we sampled sympatric and parapatric populations of *L. planeri* in Normandy and populations in Brittany where river lampreys are absent.

Materials and methods

Sampling design

In 2013 and 2014 we sampled with electrofishing 2472 lamprey individuals distributed in 81 sites spread over 29 rivers (Fig. 1). We targeted *L. planeri* located upstream and downstream of a putative barrier to dispersal and, if possible, close to the barrier (<1 km upstream or downstream) to limit the effect of isolation by distance. We considered all kinds of barriers of moderate size (height between 0.50 and 5 m, described in Supplementary Table S1) that may restrict the dispersal of lampreys. The choice of obstacles was made by the description of these obstacles in the ROE data base of the Office Français de la Biodiversité (http://carmen.carmencarto.fr/66/ka_roe_current_metropole.map). None of these obstacles were equipped by fishways or spillover. Our goal was to explicitly test the effect of small migratory barriers, as these are the most widespread sites in France (>60,000; sandre.eaufrance.fr). Under experimental conditions, extremely low efficiency of river lamprey passage through fishways is frequently observed, even for small obstacles (Russon et al. 2011, Kemp et al. 2011). The restriction of passage under natural conditions may be even more pronounced depending on the presence of appropriate fishways and conditions of river flow (Foulds and Lucas 2013; Lucas et al. 2009; Tummers et al. 2018). While no data are available for brook lamprey, they display a smaller adult size. Therefore, we expected a negative relationship between barrier size and the probability of successfully passing the barrier. Although we initially planned to include the age of barriers in our model, such data were rarely available. In some cases, we were unable to capture lampreys immediately downstream or upstream of dams and some sampled points were separated by more than one obstacle. In addition, 12 pairs of sites from eight rivers without barriers to migration were included in the dataset.

A total of 2247 *L. planeri* lampreys were collected from 73 sites in five distinct regions of France (Upper Rhône $n = 575$; Normandy $n = 536$; Atlantic coast $n = 95$;Brittany $n = 969$; and Upper Rhine $n = 36$), as well as two sites in United Kingdom ($n = 83$; from two sites above and below dam) and one in Ireland ($n = 48$; one single site). In Brittany, *L. planeri* were sampled from 32 sites. These were considered as allopatric since no observations of *L. fluviatilis* are currently or historically reported for these coastal rivers (Keith et al. 2011; Germis et al. 2018, Guirec et al. 2018). The same is true in the Upper Rhône and Upper Rhine area where *L. fluviatilis* has been extirpated (Renaud 1997). In Normandy, *L. planeri* were sampled from 17 sites ($n = 536$ individuals) and coexist in sympatry with *L. fluviatilis* at 8 sites ($n = 225$ individuals) or in parapatry for the 9 remaining sites. Here, we defined parapatry to be

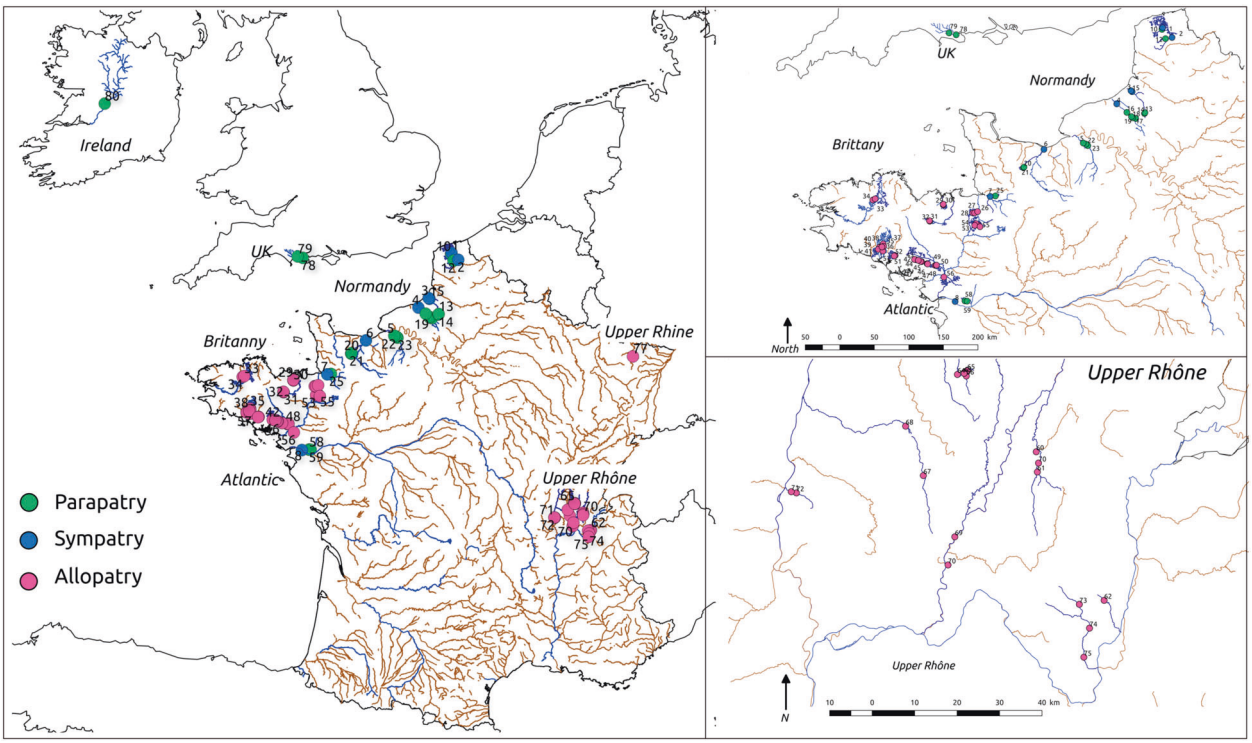

Fig. 1 Sampling map. Each points represents a sample site. Each site is numbered and the corresponding numbers are provided in Table S2 with details of river name and genetic diversity indices.

when populations from the same river were separated by obstacles to migration that are impassable. Sites were classified as sympatric or parapatric based on current expert knowledge (i.e. French Agency for Biodiversity, local angling association, and fisheries managers) and monitoring of these rivers throughout several years. In sympatric sites, populations of the two ecotypes were captured in the same nest, or in close vicinity without being separated by any barriers to gene flow. Among the sites in Brittany, two rivers were subjected to repeated sampling with $n = 8$ and 7 sites, respectively. Our goal was to dissect the joint effect of fragmentation and isolation by distance, independent of any confounding effect (e.g. presence of river lamprey) and in complement to our larger scale analysis. Adults individuals were collected in sympatric and parapatric sites since both ecotypes cannot be distinguished at early larval stage. In allopatric sites (Brittany, Upper Rhône) only brook lamprey larvae were collected during March–May of 2014. Individuals were collected by electric-fishing either on spawning sites of adults or on suitable habitat for larvae. Authorizations were obtained from the prefecture of each department in which a river was sampled.

A fin was clipped on each specimen and preserved in 95% EtOH. For adults, a small piece from the dorsal fin was taken, whereas for larvae we took a caudal fin clip. Explanatory variables of genetic parameters included the number of obstacles, their cumulative height, the geographic distances between each sample point, and the distance from the river source (i.e. distance from the headwaters). Data about obstacle height were gathered from the French "Referentiel des Obstacles à l'Ecoulement" (available at: http://carmen.carmencarto.fr/66/ka_roe_current_metropole.map). Geographic distances were computed using QGis 2.10.1.

Microsatellite genotyping

Genotyping was performed with 13 microsatellite markers specifically developed for *L. planeri* and *L. fluviatilis* after DNA extraction using a Chelex protocol modified from Estoup (1996) and strictly following protocols of Gaigher et al. (2013) and Rougemont et al. (2015).

Broad scale analysis

Genetic diversity within populations

We tested deviations from Hardy–Weinberg equilibrium using GENEPOP 4.1.0 (Rousset 2008) exact tests with Bonferroni corrections (Rice 1989, $\alpha = 0.05$) and computed the inbreeding coefficient (F_{is}) for each population using FSTAT 2.9.3 (Goudet 1995). Genetic diversity indices were

computed and included the number of alleles (A_n), Allelic richness (A_r), observed heterozygosity (H_{obs}), and expected heterozygosity (H_e) using FSTAT 2.9.3 (Goudet 1995) and Genetix 4.05.2 (Belkhir et al. 2004). We also measured relatedness (defined here as the probability that two allleles between two individuals are Identical By Descent) using the Loiselle coefficient (Loiselle et al. 1995). We tested for significant differences in levels of genetic diversity (A_r, H_e, and relatedness) as a function of "geographical connectivity" using Generalized Linear Models in R with Gaussian family. We considered five levels of connectivity: (1) downstream *L. fluviatilis*; (2) sympatry (i.e. the two species occur on the same spawning ground); (3) parapatry (where the two species co-occur on the same watershed but are geographically separated by impassable dams); (4) coastal allopatry (in coastal rivers where *L. fluviatilis* is absent); and (5) terrestrial allopatry (in the Upper Rhône, where *L. fluviatilis* is also absent).

Genetic differentation and structure among populations

To measure genetic differentation among sampling sites, we computed Weir & Cockerham's estimator of F_{ST} (Weir and Cockerham 1984) between all pairs of populations and used permutations tests with Bonferroni corrections to test for significance in FSTAT. We tested for global pairwise differences in F_{ST} between upstream and downstream sites and among the three major regions using permutations tested in FSTAT (10,000–15,000 test permutations in each case) as well as pairwise t.test adjusted for multiple testing using FDR corrections in R (R Development Core Team 2015). However, populations are expected to deviate from migration drift equilibrium and to show a downstream increase in genetic diversity resulting in biased F_{ST} that may reflect this gradient effect rather than true differences. As a result, we also used indices of genetic differentiation that are independent from variations in genetic diversity among populations: the Jost D (Jost 2008) and Hedrick Gst (Hedrick 2005). We illustrated the distribution of pairwise F_{ST} values in R using the heatmap.2 function implemented in the gplots package (Warnes 2015).

To understand how the species were structured at a large geographic scale, a clustering analysis with the whole data was performed using the Bayesian clustering programme STRUCTURE 2.3.3 (Pritchard et al. 2000) and is provided as supplementary material. To evaluate the number of clusters (k), A total of 10 independent replicates per k value were performed. Markov Chain Monte Carlo simulations (MCMC) used 200,000 burn-in and 200,000 iterations under the admixture model with correlated allele frequencies (Falush et al. 2003). We used log likelihood Ln Pr ($X \mid K$) and the ΔK method (Evanno et al. 2005) to determine the most likely number of clusters in STRUCTURE HARVESTER (Earl, vonHoldt 2011). Plots were drawn using DISTRUCT 1.1 (Rosenberg 2003).

Isolation by distance

We were interested in testing if the samples follow isolation by distance (IBD) patterns as previously inferred in a smaller scale data set (Rougemont et al. 2015). To do so, we tested for IBD in each major geographic area separately. Given the complete disconnection between the Rhône drainage (in terms of waterway distance) and the rest of the sampled sites we did not compute IBD over the whole dataset. We computed Mantel tests using the linearized distance $F_{ST}/(1-F_{ST})$ against the waterway geographic distance between all sample sites using the R package Vegan, with the Mantel statistic being based on Spearman's rank correlation rho (Oksanen et al. 2019).

Local scale analysis

Effect of river fragmentation on genetic diversity and differentiation

We aimed to test whether river fragmentation due to barriers to gene flow impacted genetic diversity and genetic differentiation. In particular, we predicted that in the absence of a barrier effect populations sampled upstream and downstream of a given obstacle should not differ in genetic diversity and not be differentiated. Conversely, significant differences should be indicative of a significant effect of river fragmentation. Here, we were interested in this effect on brook lamprey only. Therefore, we removed all river lamprey from the dataset as well as sites where we failed to capture individuals above or below any obstacles.

Effect on genetic diversity

To test this effect, the Allelic richness (*Ar*) *differential* was used as an estimator of difference in genetic diversity between upstream and downstream sites within each river. Independent variables included (i) the cumulative height of obstacles and (ii) geographic waterway distance to the source. We initially also included the number of obstacles as independent variable, but it was highly correlated with the cumulative height ($r = 0.845$) hence we only kept the cumulative height for analyses. All distances were computed manually in Qgis following water flow. Given that more than two upstream/downstream sites were sampled in a number of rivers, the river identity was fit as a random factor. Similarly, given the very different patterns observed in the different geographical areas (presence or absence of lampreys, reduced diversity in the Rhône), we fitted region as a random factor. Models were tested using AIC as

implemented in lme4 (Bates et al. 2015) and car (Fox and Weisberg 2011) packages in the R software (R Development Core Team 2015). The significance of each variable was computed using type III sum-of-squares ANOVA and approximate *F*-tests. Pseudo-R^2 were then calculated using the function r.squaredGLMM implemented in the package MuMIn (Bartoń 2018).

Effect on genetic differentiation

Next, we tested the effect of barriers to gene flow on genetic differentiation. We used the linearized genetic distance $F_{ST}/(1-F_{ST})$ (Rousset 1997) between both sites in each river to test the effect of obstacles on genetic differentiation patterns. The exact same procedure as above with the exact same samples was performed implementing linear mixed models using distance and cumulative height as independent variables, and river as well as region as random variables.

Testing for downstream increase in genetic diversity

A common prediction across river networks or any network where dispersal is constrained (e.g. downstream biased) is that genetic diversity should increased downstream due to biased dispersal (Raeymaekers et al. 2008; Blanchet et al. 2010; Paz-Vinas et al. 2013). More importantly here, we predict that gene flow between ecotypes should further increase genetic diversity in areas of sympatry. To test this hypothesis, we used point estimates of *Ar* in linear mixed models and included all upstream and downstream sites from all rivers. The distance to the source was used as predictor variable (fixed effect) while the river and region were considered as random effects. The two other variables, namely number of obstacles and cumulated height to the source were all highly correlated with the distance to the source ($r = 0.89$ and $r = 0.85$) respectively and between each other ($r = 0.956$) and therefore not included in a single model but only tested separately. We also computed the pseudo-R^2 using the r.squaredGLMM function. As above, we only included brook lamprey in our dataset since river lamprey were only sampled in downstream areas.

Testing for asymmetric gene flow

Another expectation of downstream directed dispersal is that gene flow should be asymetric and follow water currents. Thus, gene flow is expected to be higher from the upstream to downstream direction rather than the reverse. To measure the intensity and symmetry of recent migration between upstream and downstream populations, the software BayesAss 1.3 (Wilson and Rannala 2003) was used. We used a total of 10 millions iterations, discarding the first 1 million as burn-in and sampled the MCMC every 1000 intervals. Following the authors' recommendations, we computed a rough 95% credible interval using the mean ± 1.96 std. We then considered a comparison to be informative only when the credible intervals of downstream and upstream-directed gene flow did not overlap. Then we assessed the symmetry of migration by normalizing the point estimates using $(m_{1\leftarrow 2}-m_{2\leftarrow 1})/\max[m_{1\leftarrow 2},m_{2\leftarrow 1}]$ so that this index varies between −1 and +1. Here $m_{1\leftarrow 2}$ represents the fraction of individuals in population 1 (downstream) that are migrants from population 2 (upstream) each generation and $m_{2\leftarrow 1}$ represents migration in the reverse direction. Therefore, positive values indicate higher downstream directed migration whereas negative values indicate stronger upstream migration.

Effect on local isolation by distance and population genetic structure

Finally, we measured IBD and tested the extent to which it was affected by the presence of obstacles in the Crano and Arz River (Brittany region) where more sites had been sampled (7 and 8 sites, respectively). We used Mantel and partial Mantel tests using the R package Vegan (Oksanen et al. 2019). We constructed matrices of linearized F_{ST}, computed as Fst(1−Fst), A_r and H_e differentials and matrices of pairwise waterway distances, number of obstacles and cumulative height for each river. Next, commonality analysis (Nimon et al. 2008) was applied in order to take into account collinearity between distance and cumulative barrier heights between sampling sites. This method enabled us to better assess the extent to which each predictor variable contributed to the variance in the response variable via a set of unique and shared effects (Prunier et al. 2015). The MBESS R package was used for this analysis (Kelly and Lai 2012).

Next, we evaluated whether population genetic structure increased along these two fragmented networks. We predicted that in the absence of an effect of barriers to gene flow, population admixture should follow a gradient of IBD and decrease as a function of distance separating sites (Meirmans 2012). To test this prediction, the Bayesian clustering programme STRUCTURE 2.3.3 (Pritchard et al. 2000) was used. The extact same settings as for the global analysis were used.

Results

Genetic diversity within populations

F_{is} was significantly >0 (Table S2, $p < 0.01$) in four populations: the downstream site on the Léguer River in Brittany ($F_{is} = 0.259$) and three downstream sites in the Rhône

watershed (Oignin $F_{is} = 0.598$, Calonne $F_{is} = 0.495$, Neyrieux $F_{is} = 0.395$).

Levels of Allelic richness (A_r) based on a minimal sample size of 11 varied from 1.18 (Reyssouze River, Upper Rhône) to 3.79 (Béthune River, Normandy) and from 1.20 to 3.85 for the mean number of alleles per locus (Tables 1, S2, and S3). Levels of H_e averaged over all loci per population also varied substantially, ranging from 0.011 (Reyssouze River) to 0.563 (Aa River, Normandy). Populations of the Upper Rhine displayed similar levels of diversity to those of Brittany (Table 1). On average *L. fluviatilis* populations were significantly more diverse than *L. planeri* populations both in terms of allelic richness (Table 1, $p < 0.0042$, 15,000 permutations) and expected heterozygosity (Table 1, $p < 0.0057$, 15,000 permutations) (see also Fig. 2). *L. fluviatilis* populations in Normandy

Table 1 Summary statistics of genetic diversity of *L. planeri* and *L. fluviatilis* populations for each geographic area.

	N	NbA	A_r	H_e	H_o	F_{ST} [95% IC]
Global	2472	2.73	2.43	0.354	0.344	0.377 [0.334–0.418]
L. fluviatilis (Normandy)	225	3.84	3.39	0.505	0.491	0.003 [0–0.007]
L. planeri	2247	2.65	2.37	0.344	0.334	0.396 [0.353–0.437]
L. planeri (Normandy)	536	3.15	2.88	0.435	0.440	0.139 [0.113–0.170]
Brittany & Normandy	1505	2.94	2.62	0.416	0.411	0.241 [0.207–0.284]
Brittany	969	2.88	2.58	0.406	0.396	0.279 [0.235–0.334]
Upper Rhône	575	1.76	1.52	0.111	0.089	0.249 [0.047–0.317]
UK	83	3.5	2.96	0.476	0.463	NA
Ireland	48	3.31	2.79	0.458	0.453	NA
Upper Rhine	36	3.00	2.58	0.355	0.323	NA

N = number of individuals, NbA = number of alleles (averaged of all loci), A_r = allelic richness, H_e = unbiased expected heterozygosity, H_o = observed heterozygosity. F_{ST} = Weir & Cockerham differentiation index and confidence interval computed in Fstat (Goudet 1995). The different Fst comparisons correspond to: (i) overall Fst, (ii) Fst among population of *L. fluviatilis*, (iii) Fst among *L. planeri* after excluding *L. fluviatilis*; (iv) Fst among *L. planeri* from each of the mentioned region.

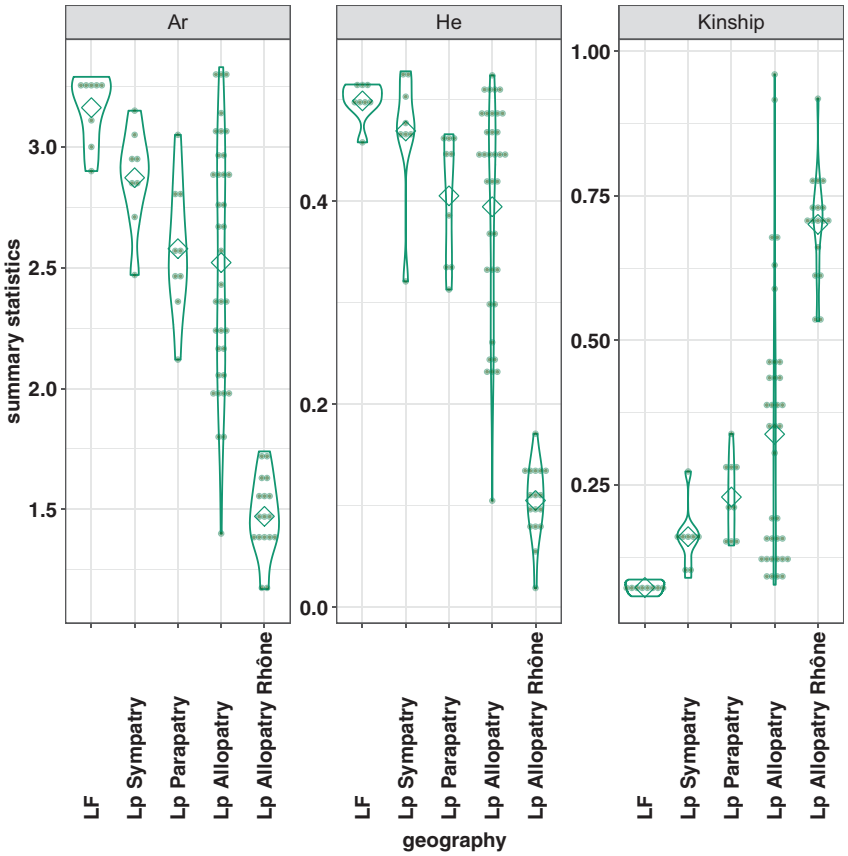

Fig. 2 Violin plots of the distribution of genetic diversity and relatedness as a function of the geographic context and levels of connectivity with *L. fluviatilis*. Diamond display median values. See text for statistical significance.

were not different from downstream *L. planeri* populations in Normandy in terms of expected heterozygosity or allelic richness (GLM, $p > 0.05$) (see Table S4 and Fig. 2). In contrast, the genetic diversity of *L. fluviatilis* populations was systematically higher than that of the upstream *L. planeri* populations of Normandy (i.e. parapatric) and of the neighbouring *L. planeri* populations of Brittany (all $p < 0.05$, see Table S4 and Fig. 2). Levels of genetic diversity of the Frome (UK) and Shannon (Ireland) populations (Table 1) were similar to those observed in Normandy. Comparisons among geographical areas revealed a significantly lower (GLM, $p < 0.05$) genetic diversity of *L. planeri* populations from the upper Rhône compared to Brittany and Normandy (Fig. 2, Table S4 for detailed *p*-values).

Genetic differentiation and structure among populations

Global F_{ST} was 0.377 (95% IC = 0.334–0.418) and reached 0.394 (95% IC = 0.353–0.437) when excluding *L. fluviatilis* (Table 1). Figure S1 illustrates two main groups of populations: the Upper Rhône *vs*. all other populations. *L. fluviatilis* populations were weakly differentiated ($F_{ST} = 0.005$). Populations of *L. planeri* were significantly more differentiated than *L. fluviatilis* populations ($p < 0.00017$, 6000 permutations with Hierfstat after pooling). The highest $F_{ST} = 0.90$, was observed between the Reyssouze (Rhône) and the Moulin du Rocher sites (Brittany). The lowest F_{ST} was 0 as observed in several cases (Fig. S1 and Table S5). Average pairwise F_{ST} between upstream and downstream sites within a river was 0.025 [min = 0−max = 0.095]. The maximal value of 0.095 was observed in the Crano between two sites located near the river source and in the absence of obstacles (Table S5). This difference was likely due to the fact that the uppermost site had the lowest genetic diversity as compared to the rest of the river (e.g. $Ar_{upstream} = 2.13$; $Ar_{all\ \ sites} = 2.4$). Pairwise F_{ST} between upstream and downstream sites were significant in 8 out of 43 pairwise comparisons with upstream–downstream sites from Normandy, with none of the sites where *L. fluviatilis* is present being significantly differentiated. Populations of the Upper Rhine, Frome and Shannon, were moderately differentiated from *L. fluviatilis* (Table S5). The Frome downstream site in particular displayed modest differentiation from *L. fluviatilis* as it was not significantly different from the Hem, Risle and Oir river (F_{ST} below 0.0125). Finally, genetic differentiation between *L. planeri* populations in sympatry with *L. fluviatilis* (i.e. in Normandy) was lower than the average F_{ST} between *L. planeri* population living in allopatry from *L. fluviatilis* (Brittany). Results from analyses using both Hedrick G_{ST} and Jost *D* were largely similar to those based on Weir and Cockerham F_{ST} with correlations of 0.989 and 0.973 between Fst and the two other indices, respectively. Details of population structure over the whole dataset are provided as supplementary materials (Figs. S3 and S4, Table S7). In short, we observed a large degree of admixture between *L. fluviatilis* and *L. planeri* in sympatry, whereas *L. planeri* from Brittany, Upper Rhône, and the Loire formed distinct clusters.

Landscape genetics

Global isolation by distance

Mantel tests, revealed contrasted patterns of isolation by distance, depending on the regions compared. First, we found a significant relationship between distance and linearized genetic differentiation in the upper Rhône area (Mantel $r = 0.469$, $p = 2e^{-4}$). The pattern of IBD was less pronounced in Brittany, but still significant (Mantel $r = 0.188$, $p = 0.016$). In contrast, the pattern of isolation by distance was not significant in the Normandy area (Mantel $r = 0.145$, $p = 0.143$). This absence of relationship was largely driven by the lack of genetic differentiation between the upstream/downstream populations of the Oir River as compared to the remaining *L. planeri* Normandy populations. When this population was removed, the pattern of IBD appeared the strongest among Normandy populations (Mantel $r = 0.55$, $p = 1e^{-4}$). To gain further insights about the evolutionary relationships among populations from coastal areas either connected to *L. fluviatilis* (Normandy) or disconnected (Brittany), we tested the pattern of IBD by keeping only the most downstream site per river. In this case the signal of IBD remained significant in Normandy (Mantel $r = 0.43$, $p = 0.042$) but not in Brittany (Mantel $r = -0.0208$, $p = 0.53$).

Effect of river fragmentation on genetic diversity and differentiation

To test the effect of river fragmentation on diversity and differentiation, a linear model was used, but the strong correlation between distance to the source, cumulative height or number of obstacles (all $r > 0.5$) precluded their joint analysis in a single model. Therefore, each variable was tested separately. The model selection procedure based on AIC indicated that the best model included only the pairwise geographic distance (Table 2) with a highly significant effect on AR differential between downstream and upstream sites. Even though the cumulative height had a significant effect ($p = 0.002$) this model had the highest AIC (Table 2). The amount of variance of the best model explained by fixed factors was $R^2m = 0.21$ whereas the entire model explained a greater part of the variance (pseudo $R^2c = 0.59$). Non-significant results were also observed when using expected heterozygosity differential instead of allelic richness (Table S8).

Table 2 Effect of landscape fragmentation on genetic diversity (A_R differential) and genetic differentiation (F_{ST}) between pairs of sites.

Model	Effect on allelic richness						Effect on genetic differentiation					
	AIC	*F*	*p*	Slope	R^2m	R^2c	AIC	*F*	*p*	Slope	R^2m	R^2c
Model 1	−27.0				0.204	0.604	−230.8				0.043	0.183
Distance		1.30	***<0.001***	0.008				0.993	0.322	0.0026		
Barrier height		14.12	0.25	0.010				0.771	0.383	0.0005		
Model 2	−25.7				0.091	0.505	−245.0				0.032	0.153
Barrier height		10.38	***0.0018***	0.026				2.615	0.110	0.0036		
Model 3	−35.3				0.212	0.586	−241.9				0.036	0.188
Distance		26.45	***<0.001***	0.009				2.432	0.123	0.0008		

Mixed linear models were used with river and region fitted as random factors. Model 1 = distance + barrier size, Model 2 = barrier size, Model 3 = distance. AIC are provided for each model along with *p*-values and slope of the tested variables. R²m corresponds to the marginal R^2 and represents the variance explained by fixed effects. R²c corresponds to the conditioned R^2 and represents the variance explained by both fixed and random effects. Genetic differentiation was linearized using: $Y = F_{ST}/(1 - F_{ST})$.

Significant *p*-values are highlighted in bold.

Regarding genetic differentiation, the linear modelling approach revealed no significant effect of the tested variables (cumulative height, distance) on brook lamprey differentiation within a given watershed (Table 2). Results obtained with the number of obstacles were similar and are provided in Table S9.

Interestingly, sites with no obstacle ($n = 11$ pairwise comparisons) displayed a slightly higher genetic diversity as compared to sites above and below obstacles ($H_e = 0.22$ vs. 0.20, $A_r = 2.67$ vs. 2.49 for sites with no obstacle *versus* sites with obstacles, respectively). Similarly, genetic differentiation between these pairs of sites was lower on average ($Fst = 0.021$) than the differentiation for sites separated by obstacles ($Fst = 0.05$). However, the small amount of pairs of sites with no obstacle prevented a robust comparison (e.g. using linear models) with sites fragmented by barriers to gene flow.

Effect of the distance from the source

As expected, we found a highly significant positive relationship between the distance from the source and the levels of genetic diversity ($p = 1e^{-6}$, $F = 29.7$). Testing each dependant variable separately revealed a significant effect of the barrier count and cumulative height.

Downstream directed gene flow in brook lampreys

We expected that the particular life history of the brook lamprey, buried for several years in the sediments, should favour downstream directed gene-flow (Dawson et al. 2015). Analysis of recent migration rates in BayesAss indicated that confidence intervals do not overlap in 46% of the upstream–downstream comparisons (i.e. 18 out of 39 values were considered further for our analysis below

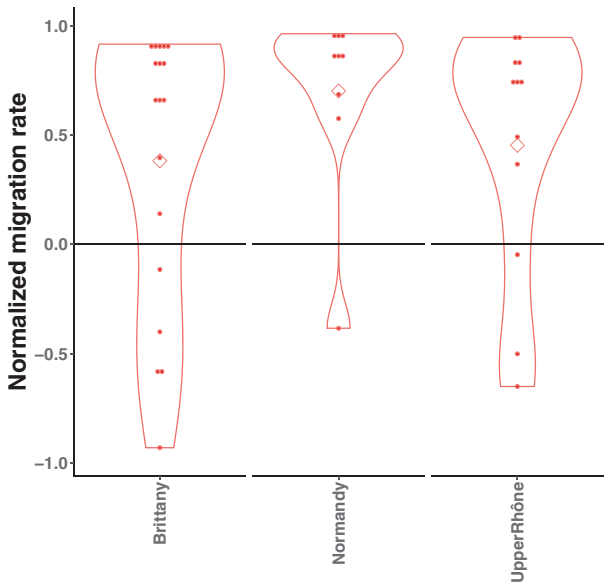

Fig. 3 Evidence for assymetric downstream directed gene flow. Violin plot of normalized estimates of recent migration rate (obtained with BayesAss) accross different geographic areas displaying different levels of connectivity with *L. fluviatilis* (Normandy=-Sympatry+Parapatry, Brittany=Allopatry «coastal» and Upper Rhone=Allopatry).

(Fig. 3, Table S10)). In 100% of these informative cases, we found that migration was predominantly directed from the upstream to the downstream areas with the index of asymmetry reaching a median value of 0.90 (average = 0.88). Furthermore, there was no difference in the intensity of gene flow between *L. planeri* pairs located in sympatry areas (Normandy) and *L. planeri* pairs in allopatry (Brittany and upper Rhône) (Wilcoxon test $W = 50$, $p = 0.117$; GLM $p > 0.1$, Table S11), indicating that the highest genetic diversity observed in Normandy (Fig. 2) was not due to a higher downstream directed gene flow in this area.

Table 3 Results of Mantel tests and partial Mantel tests performed on the Arz (8 sites) and Crano rivers (7 sites).

	$Y = F_{ST}/(1-F_{ST})$				Y = allelic richness differential			
	Arz		Crano		Arz		Crano	
	r	p	r	p	r	p	r	p
Dist	0.163	0.237	0.86	**<0.001**	0.72	**<0.001**	0.645	**0.014**
N.obst	0.018	0.273	0.31	0.152	0.62	**0.002**	0.33	0.133
Height	0.174	0.171	0.222	0.302	0.58	**0.004**	0.59	0.057
Dist (N.obst)	0.338	**0.036**	0.885	**0.006**	0.45	**0.016**	0.513	0.06
Dist (Height)	0.02771	0.389	0.799	**0.024**	0.52	**0.006**	0.379	0.155
N.obst (Dist)	−0.300	0.901	−0.77	0.965	−0.067	0.623	−0.0487	0.581
Height (Dist)	0.066	0.322	−0.592	0.918	−0.102	0.6487	0.222	0.266

Factors in brackets correspond to controlled effects in partial mantel tests. N.obst = number of obstacles, Height = cumulated height (in meters), Dist = distance (in km).

Significant p-values are highlighted in bold.

Table 4 Commonality analyses performed on (a) the Crano River and (b) the Arz River.

(a) Crano River

	F_{ST}							H_e differential						
	R^2 = 0.842	Beta	p	Unique	Common	Total	% Total	R^2 = 0.313	Beta	p	Unique	Common	Total	% Total
Distance		0.027	***<0.001***	0.782	−0.243	0.540	90.42		0.012	***0.029***	0.214	−0.214	0.001	67.51
Number		−0.024	***<0.001***	0.144	−0.095	0.049	16.59		−0.012	0.282	0.047	0.038	0.085	14.73
Height		−0.005	0.29	0.009	0.072	0.081	1.02		−0.012	0.185	0.072	0.029	0.101	22.75

(b) Arz River

	F_{ST}							H_e differential						
	R^2 = 0.096	Beta	p	Unique	Common	Total	% Total	R^2 = 0.313	Beta	p	Unique	Common	Total	% Total
Distance		0.001	0.175	0.066	−0.040	0.026	33.4		0.003	***0.004***	0.291	−0.220	0.072	95.70
Number		−0.006	***0.036***	0.166	−0.166	0	84.7		−0.006	***0.035***	0.144	−0.143	0.001	47.22
Height		0.007	0.137	0.079	−0.050	0.029	40.3		−0.001	0.834	0.001	0.005	0.006	0.43

Unique = predictor unique effect, Common = the sum of effects shared with other predictors, Total = sum of unique and common contributions to the variance in the response variable.

Significant p-values are highlighted in bold.

Effect on local isolation by distance along two linear transects

Mantel tests and partial Mantel tests on the two rivers where more than two sites had been sampled (Arz and Crano) indicated different influences of distance and obstacle-related variables. In the Arz River, all variables significantly influenced allelic richness (Table 3) whereas it was influenced solely by geographic distance in the Crano River. The extent of pairwise differentiation was also influenced by distance in the Crano River whereas this pattern was only revealed in the Arz when the influence of the number of obstacles was controlled for (Table 3). The commonality analysis (Table 4) also indicated a significant influence of the number of obstacles and geographic distance on genetic diversity (measured by heterozygosity) in the Arz with both contributions of unique and common effects, whereas only the number of obstacles influenced the pairwise differentiation. In the Crano River, commonality analysis indicated a strong influence ($p < 0.001$, Table 4) of the number of obstacles and geographic distance on pairwise differentiation whereas most of the variance in expected heterozygosity was explained uniquely by distance (Table 4).

Clustering analyses in the Crano ($n = 7$ sites) and Arz River ($n = 8$ sites) revealed similar patterns of admixture in these two systems. The Crano was composed of two distinct tributaries (Crano and St Sauveur River). The two most upstream sites from each of these tributaries formed distinct clusters with a lower degree of admixture than the downstream populations that displayed increased admixture

values (Fig. S2). In the Arz, the source population formed a slightly distinct cluster with lower admixture than the downstream populations (Fig. S2).

Discussion

The goals of this study were threefold: testing the effect of anthropogenic river fragmentation on patterns of population genetic diversity, testing for asymmetric gene flow and exploring the potential influence of the presence of *L. fluviatilis* on genetic diversity levels in *L. planeri*. We used *L. planeri* as a model to test the effect of fragmentation as this species displays a reduced migratory behaviour (Malmqvist 1980). We found limited evidence for the effect of anthropogenic fragmentation on genetic diversity and differentiation of populations and the distance to the source was a more pertinent variable to explain patterns of genetic diversity within populations. Importantly, this lack of effect of river fragmentation could be explained by the strong downstream dispersal of *L. planeri*, which does not seem limited by obstacles of limited size considered in this study. The comparison of sympatric, parapatric and allopatric populations, located in downstream and isolated areas of different watersheds revealed a key role of *L. fluviatilis* in maintaining genetic diversity of *L. planeri* populations in the lower part of rivers where they co-occur.

Small impact of anthropogenic fragmentation on the distribution of genetic diversity

Several studies have reported strong impacts of barriers to migration on either genetic diversity and/or structure (Blanchet et al. 2010; Faulks et al. 2010; Gouskov et al. 2015; Hänfling and Weetman 2006; Leclerc et al. 2008; Raeymaekers et al. 2008; Torterotot et al. 2014). Here evidence for such effects was low and factors such as the distance to the source or the distance between sites, strong downstream directed gene flow, all contributed to erase genetic differentiation and homogenize diversity levels. Our results are therefore slightly different from those of Bracken et al. (2015) who suggested that barriers increased population differentiation. However, Bracken et al. (2015) analysed the effects of distance and barriers separately, complicating direct comparison with our results. Our results therefore suggest that small dams have only weak effects and that they might not constitue a significant obstacle under appropriate water flow conditions, as observed in the larger *L. fluviatilis* (Tummers et al. 2018) or that they do not restrict downstream dispersal.

Population genetic diversity was mostly affected by distance from the source, as upstream populations showed lower levels of allelic richness and heterozygosity (Fig. 2). This downstream increase in genetic diversity is expected in riverine habitat (Morrissey and de Kerckhove 2009; Paz-Vinas et al. 2015) and is frequently observed in empirical studies (e.g. Hänfling and Weetman 2006; Torterotot et al. 2014; Gouskov et al. 2015). Detailed investigations in the Arz River provided strong evidence for an increased downstream allelic richness and this pattern was also significantly influenced by all other physical variables. In the Crano, an increase in genetic diversity was not influenced by geographic variables other than distance. A recent simulation study investigated the underlying processes that can generate this pattern (Paz-Vinas et al. 2015), namely (i) downstream-biased dispersal, (ii) increase in habitat availability downstream, and (iii) upstream directed colonization. Among the three proposed processes, it appears likely that downstream dispersal plays a key role in *L. planeri* according to our analysis with BayesAss, which indicates higher upstream–downstream dispersal than downstream to upstream dispersal. Such dispersal is expected to occur mainly at the larval stage given the length of this phase that can reach 6 years in *L. planeri* (Hardisty and Potter 1971). Larvae that live mainly buried in the soft sediment may be passively transported downstream during flood events, whereas active downstream dispersal may also occur (Dawson et al. 2015). Accordingly, it has been observed in various lamprey species that older larvae are more frequent in downstream areas, compared to young larvae that are distributed closer to spawning grounds in upper reaches of river systems (Dawson et al. 2015). Admittedly, postglacial colonization history is also expected to shape the present distribution of genetic variation and its role is hard to separate from the above processes (Paz-Vinas et al. 2015). In a closely related lamprey species with similar lifestyle, Spice et al. (2019) found that both long term history and recent connectivity shape the distribution of genetic diversity. Similarly, Paz-Vinas et al. (2015) found that the observed downstream increase in genetic diversity was generally shaped by the interaction of different processes across species. Next, Bayesian clustering analysis (Fig. S2) in the St-Sauveur–Crano river system (the Crano is a small stream flowing into the St Sauveur) revealed another important pattern explaining the increase in downstream genetic diversity via admixture among individuals originating from different upstream sites. The two upstream populations of the St Sauveur and Crano form two genetically distinct clusters ($F_{ST} = 0.265$) and individuals located downstream of the Crano appear admixed, possibly having a shared ancestry stemming from these two source populations (and possibly from other unsampled populations). The second process that may have generated low upstream genetic diversity is the occurrence of bottlenecks through multiple serial founder effects, following usptream river colonization after glacial retreats (Hewitt 1996;

Taberlet et al. 1998). It remains unclear so far whether *L. planeri* populations have recovered from ancestral bottlenecks and disentangling the three hypotheses will require further data. For instance, Spice et al. (2019) suggested that headwater areas tend to have higher stream gradients and less fine sediment than downstream habitat, potentially giving them a lower capacity to support lamprey larvae.

Overall, we hypothesized that our observations of strong downstream directed gene flow may explain our inability to detect the effect of river fragmentation globally. Moreover, even a small amount of upstream directed migration, as inferred here by Bayes Ass may contribute to reduce the effect of obstacles to migration. Alternatively, it is possible that subtle effects will be revealed later in time if most of the studied barriers are still relatively recent (Landguth et al. 2010). For instance, significant effects on genetic differentiation and genetic diversity were found in populations located upstream of a 90-year-old dam (Yamazaki et al. 2011) and of a 45–120 years old series of bigger dams (Coleman et al. 2018). Finer investigations in the Crano and the Arz revealed significant effects of distance and of the number of obstacles (according to the commonality analysis) on differentiation in the Crano River. In contrast, in the Arz River the effect of distance was only revealed when obstacles number was controlled for, in agreement with the commonality analysis. Finally, the impact of river fragmentation may be best revealed by studies focusing on a single catchment and with bigger obstacles to migration (e.g. Raeymaekers et al. 2008; Blanchet et al. 2010; Gouskov et al. 2015). We investigated the impact of obstacles of small to moderate size and it is possible that these obstacles do not influence the downstream passive drift of lamprey larvae, which may be sufficient to homogenize populations and obscure patterns of differentiation (Faubet et al. 2007).

River lamprey as a source of genetic diversity for resident lampreys

Understanding the evolutionary relationships between parasitic and nonparasitic lamprey ecotypes is a long-standing debate (Docker 2009). Recent studies (Bracken et al. 2015; Rougemont et al. 2015) have shown that gene flow is ongoing between the river lamprey and the brook lamprey, locally lowering their level of genetic differentiation. Here, our results also support ongoing introgression between the two ecotypes. Using an extensive SNP data set, Rougemont et al. (2017) inferred the occurrence of locally asymmetric introgression from anadromous to resident sympatric populations following secondary contacts. More specifically, we inferred that between 90 to 95% of the genome was freely introgressing between the two ecotypes, suggestive of partial reproductive isolation. Such results were due to both long term introgression and recent ongoing gene-flow as revealed by demographic analyses and structure analyses. Introgression from a large marine population toward freshwater populations is also known to occur in the stickleback *Gasterosteus aculeatus* (Hohenlohe et al. 2010, 2012). Here, genetic analyses of populations in sympatry (on the same nest), in parapatry (where the two species co-occur on the same watershed but are geographically separated by impassable dams) and in allopatry (in coastal rivers where *L. fluviatilis* is absent) revealed that allopatric *L. planeri* displayed a lower genetic diversity than sympatric and parapatric populations (Fig. 2). These results, with those from previous studies, further suggest that the current genetic makeup of *L. planeri* populations in Normandy is influenced by ongoing gene flow with *L. fluviatilis*. In addition, we found a much stronger pattern of IBD in the connected pairs of *L. planeri* (i.e. populations of downstream areas in Normandy) than in populations from Brittany. In the absence of inter-basin gene flow mediated by *L. fluviatilis*, populations of Brittany evolved independently from each other and do not seem globally at migration-drift equilibrium (which does not imply that subpopulations within rivers deviate from this equilibrium). In addition to this introgression, other factors can contribute to the increased genetic diversity and lower genetic differentiation in populations living in sympatry with *L. fluviatilis*. For instance, larger population sizes in sympatric areas are expected due to more habitat availability (Spice et al. 2019). Moreover, founder events and bottlenecks are expected following the upstream colonization of the rivers, resulting in lower Ne (and lower genetic diversity) upstream. Finally, it is possible that areas of allopatry (Britanny, Upper Rhone) were founded by more ancient colonization events so that *L. planeri* had more time to diverge from *L. fluviatilis*.

Populations from the Upper Rhône area displayed a highly reduced genetic diversity and were strongly differentiated from all other populations, which could be explained by different complementary hypotheses. First, there is evidence for at least three major evolutionary lineages existing in *L. planeri* (Espanhol et al. 2007). It is thus possible that colonization of the Mediterranean area (Upper Rhône region) following postglacial colonization originated from a different lineage than the one having colonized the Atlantic and Channel areas, as observed in various European fish species (Bernatchez and Wilson 1998). Similarly, some tributaries from the Iberian Peninsula have likely been colonized by different populations isolated for a long period of time (Mateus et al. 2011, 2016). In these conditions, it is possible that our microsatellite markers set (originally developed using *L. planeri* and *L. fluviatilis* samples from the Atlantic and Channel areas) is not the most appropriate to perform accurate population

genetic inference of Rhône samples. Second, *L. fluviatilis* no longer colonizes this area and was already reported to be declining during the last century (Bernard 1909; Gensoul 1907). Consequently, it is possible that the history of divergence between Mediterranean and Atlantic populations was initiated a long time ago and that gene flow between neighbouring rivers of the Mediterranean area has been further reduced during the last century. The phylogeography of Iberian populations has been well studied (Espanhol et al. 2007; Mateus et al. 2016), but their relationships with the Upper Rhône and Northern populations still need to be explored. Decreasing costs of low coverage whole genome sequencing should provide insights into these questions.

Conservation implications

Fragmentation of rivers may impact lamprey populations, especially the most upstream populations that do not receive migrants from downstream sites. Whether the most isolated populations from headwaters suffer a mutation load and greater extinctions risks would require further investigation (Frankham 2005, 2015; Higgins and Lynch 2001; Lynch 1991; Spielman et al. 2004). On the other hand, it is not clear if maintaining a possibility for upstream migration by removing obstacles may help preventing the loss of genetic diversity in the most upstream populations of *L. planeri* through gene flow (Frankham 2015). This is of major importance as small isolated populations often undergo less efficient selection and can accumulate more deleterious mutations, threatening their persistence (Lynch 1991). Outbreeding depression is sometimes perceived as a greater risk (Edmands 2007), potentially also reducing local adaptation (Lenormand 2002), but it is rarely observed and meta-analyses revealed that maintaining opportunities for gene-flow between small and isolated populations is key for their maintenance (Ralls et al. 2018; Frankham 2015; Frankham 2016).

For instance, the upstream populations on the Crano, St Sauveur, and Tamoute river displayed increased genetic differentiation despite the absence of migratory barriers. These observations suggest a natural functioning where the most upstream populations are inevitably subject to a loss of genetic diversity (Hänfling and Weetman 2006; Barson et al. 2009; Dehais et al. 2010; Morrissey and de Kerckhove 2009).

Importantly, our study revealed positive impacts of the presence of *L. fluviatilis* on the maintenance of genetic diversity in sympatric populations. However, in Europe, *L. fluviatilis* abundance has strongly declined in some areas, due to habitat alteration and pollution (Maitland et al. 2015) and it is now considered as vulnerable in France on the IUCN red list (UICN Comité français MNHN SFI & AFB 2019). In addition, the low ability of the anadromous ecotype to pass migration barriers often restricts its distribution to downstream areas where *L. planeri* are less abundant. In terms of conservation priorities, it appears fundamental to first ensure that *L. fluviatilis* will have access to upstream reaches of rivers. This will benefit both the river and brook lampreys in sympatric and parapatric areas. In such areas, a joint regional management of the two ecotypes could be envisioned, whereas in allopatric areas, a management at the river scale may be more appropriate.

Conclusion

We have shown here that impacts of anthropogenic barriers to migration were modest on the extent of genetic differentiation, but we provided evidence that headwater populations of *L. planeri* displayed reduced genetic diversity and higher genetic differentiation when compared to downstream sites as a result of isolation by distance and biased downstream gene flow in a river network. Restoring the possibility for upstream active migration from downstream populations could increase genetic diversity and evolutionary potential in the most upstream populations (Brauer et al. 2016; Coleman et al. 2018; Pavlova et al. 2017), but it seems that such restoration practices could hardly counterbalance the strong downstream gene flow probably due to drift of larvae (Dawson et al. 2015).

In addition, our comparative analyses among sympatric, parapatric and allopatric areas support the hypothesis that sympatric populations display higher levels of genetic diversity due to introgression from *L. fluviatilis* (Rougemont et al. 2015, 2016, 2017). Potential strong gene flow or even introgression swamping from anadromous populations to resident populations thus plays a fundamental role in maintaining genetic diversity of *L. planeri* (Rougemont et al. 2017).

Data archiving

Raw genotype data used in this study will be available at the Dryad Digital Repository: https://doi.org/10.5061/dryad.3ffbg79gb.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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