

Guidelines for restoring connectivity around water mills: a population genetic approach to the management of riverine fish

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Summary

1. While freshwater systems provide important goods and services for society, they are threatened by human activity. Fragmentation is one of the most serious ecological concerns in the riverine environment.

2. Historical and cultural values may conflict with nature restoration. Here we use the Zwalm sub-basin (Scheldt basin, Belgium) as a case study for reconciling the restoration of the native fish fauna with the preservation of historical water mills (320–1000 years old).

3. We assessed the genetic structure of a barrier-sensitive species, the three-spined stickleback *Gasterosteus aculeatus*, to estimate the impact of fragmentation on a local to catchment scale. We show how population genetic approaches may be used to generate guidelines for restoration and management, and advance the science of river restoration.

4. Dispersal was lower in above- than in below-mill populations, and water mills provoked an average loss of almost 4% of the genetic variation. This loss accumulated to 40% over the entire system (~23 km, 13 barriers). The impact of individual mills strongly increased with upstream distance and water mill height. One mill provoked significant genetic differentiation, despite the presence of a fish passage.

5. This detailed picture of the genetic connectivity in stickleback is indicative for the basin's depauperate fauna. Many species share the same migratory pathways and barriers to dispersal. The physical properties of the water mills are likely to have similar effects on species with a similar genetic structure to stickleback.

6. *Synthesis and applications.* Population genetic studies may be particularly useful during the planning of river restoration and associated ecological studies. In the case of the Zwalm sub-basin, we propose a number of management actions, such as building new fish passages and translocating individuals to above-mill populations. These will counter the negative impact of the water mills on the genetic variation in aquatic fauna, whilst retaining their cultural–economical value and limiting the restoration costs. Simulations suggest that reassessment of stickleback genetic structure after a decade should reveal whether or not restoration actions have been effective.

Key words: animal translocation, conservation genetics, dispersal, ecologically effective, fragmentation, migration, restoration ecology, river connectivity, river restoration

Introduction

While freshwater systems provide important and unique goods and services for society, they are threatened by several human activities, including engineering, pollution and

overexploitation of natural resources (Vrijenhoek 1998). Ecologists and managers of aquatic habitats face the challenge of reconciling important human services with vital ecological services of freshwater systems. In the riverine environment, one of the most serious ecological concerns is fragmentation. Human activities, such as flood alleviation and canalization, water regulation and irrigation, modify the hydrological cycle

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(Giller 2005). Many of these activities obstruct rivers, modify the habitat, prevent aquatic animals from reaching upstream habitats, and isolate upstream populations. Populations that are physically and genetically isolated suffer from decreasing population sizes and inbreeding, which may increase the risk of extinction (Shaffer 1981; Brook *et al.* 2002; Morita *et al.* 2009).

The conflict between vital ecological services and human activities has a spatial dimension (Fausch *et al.* 2002). Streams are regulated at the local level to catchment scale, whereas local and basin-wide connectivity is vital for the reproductive success and survival of various organisms (Vrijenhoek 1998). One goal of river restoration ecology is to collect information and understand functionality that might contribute to sound river management. However, the scales of river management often exceed the scales at which ecological information is collected (Wiens 2002). The spatial and temporal resolution of ecological studies is often limited and constrained by financial resources.

Through analysis of molecular markers, population genetics provides insights into population divergence and dispersal at local to catchment scales. This allows us to draw inference about population connectivity and evolutionary viability, and has important applications in conservation management (conservation genetics; e.g. Frankham 1996; Latta 2008). In the context of river restoration, population connectivity of a target species may be indicative of the majority of the species in the basin, because they are all subject to the same barriers to dispersal and share the same migratory pathways. This does not hold true for most terrestrial animals in which dispersal occurs in many directions. Arguably, genetic parameters may be too imprecise to serve specific conservation targets, and are unlikely to include important ecological processes such as seasonal migration. However, a great advantage in the context of global ecosystem health and river restoration is that population genetic estimates can be compared over large geographical scales, providing a local as well as basin-wide overview of the relative connectivity in the system and the associated human impact (Raeymaekers *et al.* 2008).

We demonstrate this approach in the river Zwalm, a mid-catchment sub-basin of the Scheldt River (Flanders, Belgium), which drains into the North Sea. A set of historical water mills (see Supporting Information, Fig. S1) enhances the cultural value of the Zwalm region. These mills date back to the Middle Ages and the Early Modern era [320–1000 years before present (BP)], and have considerable historical, cultural, economical and recreational value, mainly through tourism. The preservation and maintenance of the mills is therefore of importance, but conflicts with the high priority accorded to the restoration of the river Zwalm (Monden *et al.* 2004). The mills not only prevent upstream migration of fish by blocking the river channel, but also alter stream conditions. Historical pollution and limited accessibility to spawning grounds contributed to the extinction of migratory fishes such as Atlantic salmon *Salmo salar* L. and twaite shad *Alosa fallax fallax*, and to the decline of the river lamprey *Lampetra fluviatilis* (Maes *et al.* 2008). In the lower sections of the Zwalm, limited connectivity is threatening typical stream

fishes such as chub *Leuciscus cephalus* and common dace *L. leuciscus* (Buysse *et al.* 2007). Two other fragile species, the bullhead *Cottus gobio* and the brook lamprey *Lampetra planeri*, persist in the upstream sections of the basin.

Our target species for assessing the impact of the water mills on population connectivity is the three-spined stickleback *Gasterosteus aculeatus* L. Its populations colonized the Flemish uplands 10 000–16 000 BP following the end of the Last Glacial Maximum (Raeymaekers *et al.* 2005). A small size and short generation time make the stickleback a barrier-sensitive species from a physical and genetic perspective (Raeymaekers *et al.* 2008). Its non-commercial value meant there was no bias caused by angling or restocking. We used the relative genetic connectivity among the populations in the Zwalm basin as a guideline for river restoration and water mill preservation. We first evaluated the functioning of the system's oldest fish passage. Secondly, we assessed the impact of four mills targeted by a restoration project, and three mills not considered for the construction of a fish passage. Thirdly, we investigated which factors determine water mill impact. Finally, we investigated the potential of population genetic approaches for evaluating the ecological effectiveness of river restoration (Giller 2005).

Material and methods

SAMPLE COLLECTION

Sticklebacks were sampled at 20 sites in the Zwalm sub-basin of the Scheldt River (Flanders, Belgium; Fig. 1; Table 1). Local sticklebacks belong to the low-plated upland freshwater ecotype (Raeymaekers *et al.* 2007). Sixteen sites (coded Z) were selected immediately upstream (above mill; index a) and downstream (below mill; index b) of eight historical water mills on the main channel and on principal tributaries. Additional sites were chosen on the main channel (Z10), and at upstream positions on principal tributaries (Z6, Z14, Z13). All sites were visited on a single day in spring 2004, after the construction of a fish passage for water mill Z9 (°1995), and before the construction of fish passages for water mills Z2, Z3, Z4 and Z7 (°2005). Fifty adults per site were caught with a dip net, anaesthetized with MS222 and frozen on dry ice. In the laboratory, fin clips were taken and stored in 100% ethanol.

DNA EXTRACTION AND GENOTYPING

Genomic DNA was extracted from fin clips using a silica-based purification method (Elphinstone *et al.* 2003). Allelic variation was assessed at six microsatellite loci (Gac5196, Gac2111, Gac4170, Gac1097, Gac7033, Gac1125; Largiadèr *et al.* 1999). All loci were amplified simultaneously with the QIAGEN® Multiplex PCR Kit (QIAGEN, Venlo, The Netherlands). The 12.5 µL polymerase chain reaction (PCR) cocktail contained 1–100 ng genomic DNA, 0.050 µM (Gac5196, Gac2111, Gac7033, Gac1125), 0.75 µM (Gac4170), 1 µM (Gac1097) forward and reverse primer, 1 × QIAGEN Multiplex PCR Master Mix (3 mM MgCl₂) and RNase-free water. The reaction consisted of an initial activation step of 15 min at 95 °C, followed by 30 cycles of 30 s at 94 °C, 90 s at 55 °C and 1 min at 72 °C. A final elongation step of 10 min at 72 °C was performed. PCR products were visualized on an ABI3130 Avant Genetic analyser (Applied

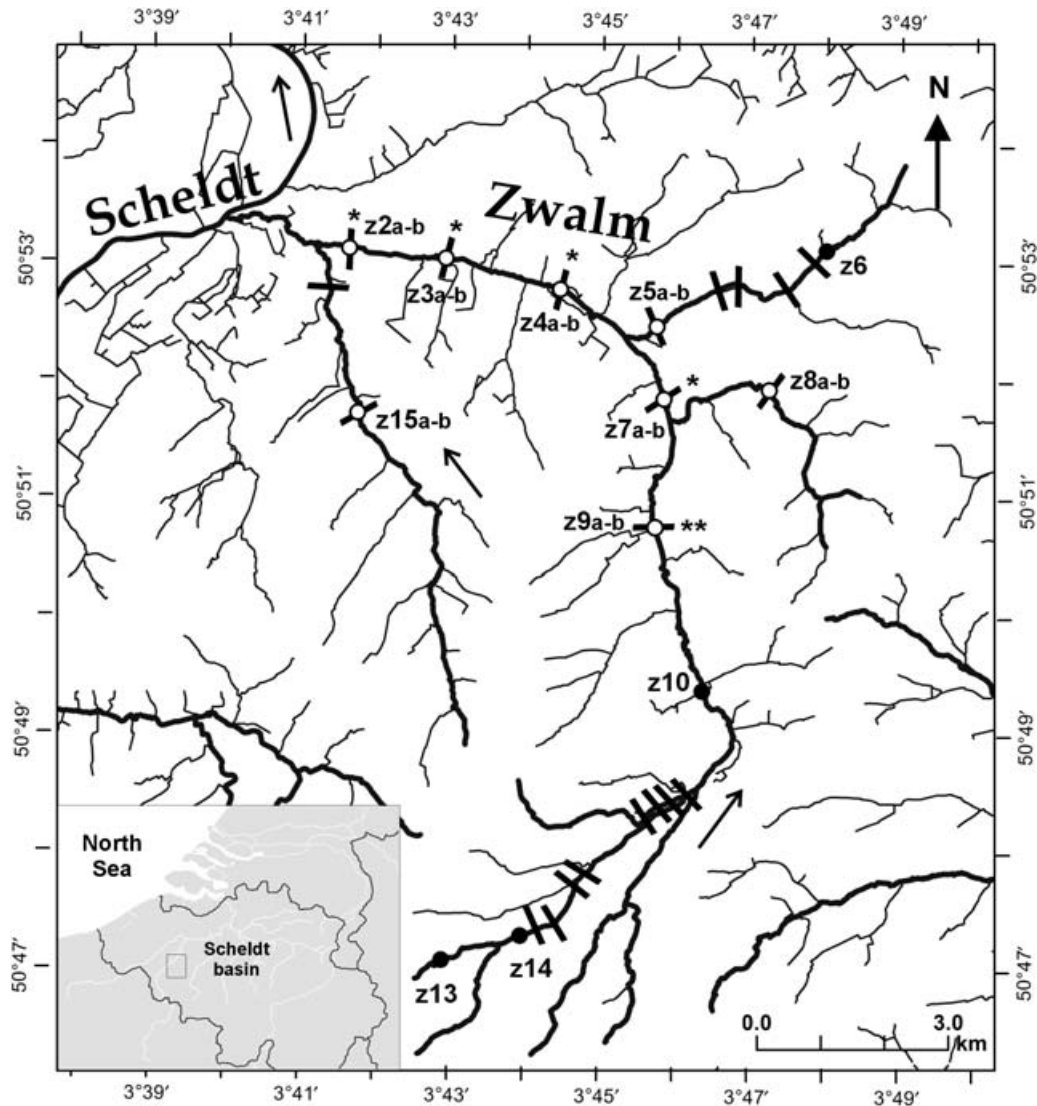


Fig. 1. Locations of 20 three-spined stickleback *Gasterosteus aculeatus* populations from the Zwalm basin (Scheldt River, Belgium; see inset) sampled in 2004. Bars indicate the position of artificial barriers, including eight historical water mills (marked with white dots). Sixteen populations were sampled immediately upstream (index a) or downstream (index b) from these mills. Black dots mark the location of four additional populations. Water mills marked with a star have been by-passed by a fish passage since 2005 (*) or 1995 (**). Arrows indicate the flow direction. Population codes are as in Table 1.

Biosystems, Foster City, CA). Allele sizes were determined by means of an internal GeneScan 500-LIZ size standard and genotypes were obtained using GeneMapper 3.7 (Applied Biosystems). Genotypes were checked for scoring errors attributable to stutter-products, large allele dropout or null-alleles, using Micro-Checker 2.3 (Van Oosterhout *et al.* 2004).

GENETIC DATA ANALYSIS

Genetic variation was evaluated based on observed and expected heterozygosities (H_O and H_E) using Genetix 4.04 (Belkhir *et al.* 2002). Allelic richness was quantified in FSTAT 2.9.3.2 (Goudet 1995) and averaged over loci. Standard errors were obtained by jackknifing over loci. Tests for linkage disequilibrium (LD) were performed according to a permutation method in Genetix. Departures from Hardy–Weinberg equilibrium (HWE) were calculated in FSTAT.

Population differentiation was quantified in Genetix using the standardized allelic variance F_{ST} , estimated as θ (Weir & Cockerham 1984). Overall and pairwise F_{ST} values were tested for significance against 10^4 random permutations of the data in Genetix. Pairwise F_{ST} values were visualized by non-metric multidimensional scaling (NMDS) plots with the function ISOMDS in the R-library VEGAN (Oksanen *et al.* 2007).

In order to estimate contemporary rates of dispersal, we assigned individual multilocus genotypes to their most likely population of origin with GeneClass2 (Piry *et al.* 2004). For each individual, the likelihood of site membership was calculated based on Rannala & Mountain's (1997) Bayesian criterion. Probability of assignment was based on Monte Carlo resampling with 10^4 simulated individuals (Paetkau *et al.* 2004). Individuals were identified as immigrants based on likelihood ratio tests (L_{HOME}/L_{MAX}) evaluated at a significance level of 5%. For each population, dispersal rate was estimated as the

Table 1. Genetic and geographical characteristics of 20 three-spined stickleback populations from the Zwalm basin (Belgium). Sixteen populations were sampled upstream (index a) or downstream (index b) from ancient water mills. The year indicates the mill's first historical record as reported by Vandeputte (1974). Standard errors (SE) are obtained by jackknifing over loci for allelic richness, and from a bootstrap distribution (1000 iterations) for dispersal

Code	Water mill (year of first record)	Upstream distance (km)	Water mill height (m)	Allelic richness (SE)	Dispersal (SE)
Z2b	Z2 – Ter Biestmolen (1063)	2.40	2.5	8.73 (1.22)	0.106 (0.044)
Z2a				9.45 (1.18)	0.102 (0.045)
Z3b	Z3 – Ijzerkotmolen (1571)	4.30	2.1	9.65 (1.18)	0.087 (0.040)
Z3a				9.52 (1.13)	0.102 (0.043)
Z4b	Z4 – Zwalmolen (1040)	6.30	2.3	9.14 (1.10)	0.102 (0.044)
Z4a				9.40 (1.14)	0.125 (0.049)
Z5b	Z5 – Temsche molen (1690)	8.40	1	9.56 (0.97)	0.140 (0.048)
Z5a				8.69 (0.72)	0.130 (0.052)
Z6	–	11.86	–	5.74 (0.83)	0.031 (0.031)
Z7b	Z7 – Bostmolen (1571)	9.35	2.5	8.14 (0.95)	0.128 (0.048)
Z7a				7.44 (1.09)	0.063 (0.043)
Z8b	Z8 – Van den Borre's molen (1571)	11.39	5	6.79 (1.10)	0.102 (0.043)
Z8a				5.89 (1.19)	0.042 (0.029)
Z9b	Z9 – Boembekemolen (1544)	11.42	4	7.18 (0.64)	0.14 (0.048)
Z9a				6.64 (0.55)	0.082 (0.039)
Z10	–	14.56	–	6.82 (0.47)	0.109 (0.045)
Z14	–	21.13	–	5.36 (0.75)	0.070 (0.039)
Z13	–	22.35	–	4.15 (0.43)	0.14 (0.047)
Z15b	Z15 – Moldergemolen (1229)	5.70	4	7.90 (0.97)	0.149 (0.052)
Z15a				7.74 (1.19)	0.023 (0.023)

proportion of immigrants. Upstream and downstream dispersers were pooled, following recommendations in Crispo *et al.* (2006). Standard errors were calculated from a bootstrap procedure with 10^3 iterations. Dispersal estimates typically have limited precision (e.g. Hänfling & Weetman 2006; McCairns & Bernatchez 2008), but this does not preclude comparison on a relative scale.

WATER MILL CHARACTERISTICS AND GEOGRAPHICAL INFORMATION

The age of the water mills was estimated based on first historical records as documented in Vandeputte (1974) (Table 1). Water mill Z5 has been destroyed, but a relief drop of 1 m still hinders fish migration. Geographical information was obtained from a digital map of the river system (AMINAL Section Water 2000), and from a digital map containing most of the migration barriers, including the water mills (Monden *et al.* 2004; Fig. 1). We carried out complementary field surveys with a Global Positioning System (Etrex, Garmin) to locate additional barriers. We measured the vertical height of the mills between upper and lower water level as a measure of potential impact. As the Zwalm basin is a shallow watershed, there are no natural rapids influencing dispersal or gene flow.

All geographical information was combined in a Geographical Information System (GeoMedia Professional 5.2, Intergraph Co., Huntsville, AL, USA) and rasterized in GeoMedia Grid 5.2 (Intergraph Co.). Using the standard cost analysis tool in GeoMedia Grid, we calculated the upstream distance from the Zwalm's river mouth into the Scheldt River to all sampling sites. We also calculated upstream size, defined as the total river distance (main channel and all tributaries) upstream from each mill. Upstream size measures the ecological benefit in terms of the habitat that becomes accessible if a bypass or fish passage is constructed. An automated command developed for GeoMedia Grid (Raeymaekers *et al.* 2008) revealed that sampling sites were separated by up to 24 km of river, 15 barriers, and 24 m of drop in relief.

WATER MILL IMPACT

Our sampling design included eight population pairs separated by a water mill (without geographical separation), and eight pairs spanning a stretch of on average 2130 m (range: 1200–2900 m) barrier-free river. Inference is particularly strong here, because the effects of barriers and geography on genetic parameters can be analysed independently (Koizumi *et al.* 2006; Raeymaekers *et al.* 2008). First, we compared the *average* impact of the eight mills on allelic richness and dispersal with the average effect of the eight uninterrupted river stretches. For both genetic properties, we expected a reduction in above-mill compared to below-mill populations, and contrasting patterns along uninterrupted river stretches. Neighbouring sites were paired in a repeated measures ANOVA, including geographical separation (water mill or river stretch) as a main effect, and the relative upstream or downstream position of each population as the *within* effect. Secondly, we assessed the impact of each of the mills *individually*. We used three different scores: (i) reduction of the allelic richness in the above-mill versus the neighbouring below-mill populations (tested with Wilcoxon matched pairs tests replicating over loci); (ii) reduction in dispersal rate (calculated and compared as outlined above); (iii) genetic differentiation between the above- and below-mill populations (pairwise F_{ST} ; tested as outlined above). Finally, we investigated which water mill characteristics determine water mill impact. Water mill impact was either quantified as F_{ST} (high values suggesting high impact), or as the ratio of above-mill vs. below-mill allelic richness or dispersal (high values suggesting low impact). These scores were then correlated with the year of the water mill's first historical record, its vertical height, and upstream distance. All analyses were executed in STATISTICA 6.0 (StatSoft, Tulsa, OK, USA).

ECOLOGICALLY EFFECTIVE RIVER RESTORATION

We performed simulations of population genetic dynamics with the software EASYPPOP 1.8 (Balloux 2001) to estimate when the functioning

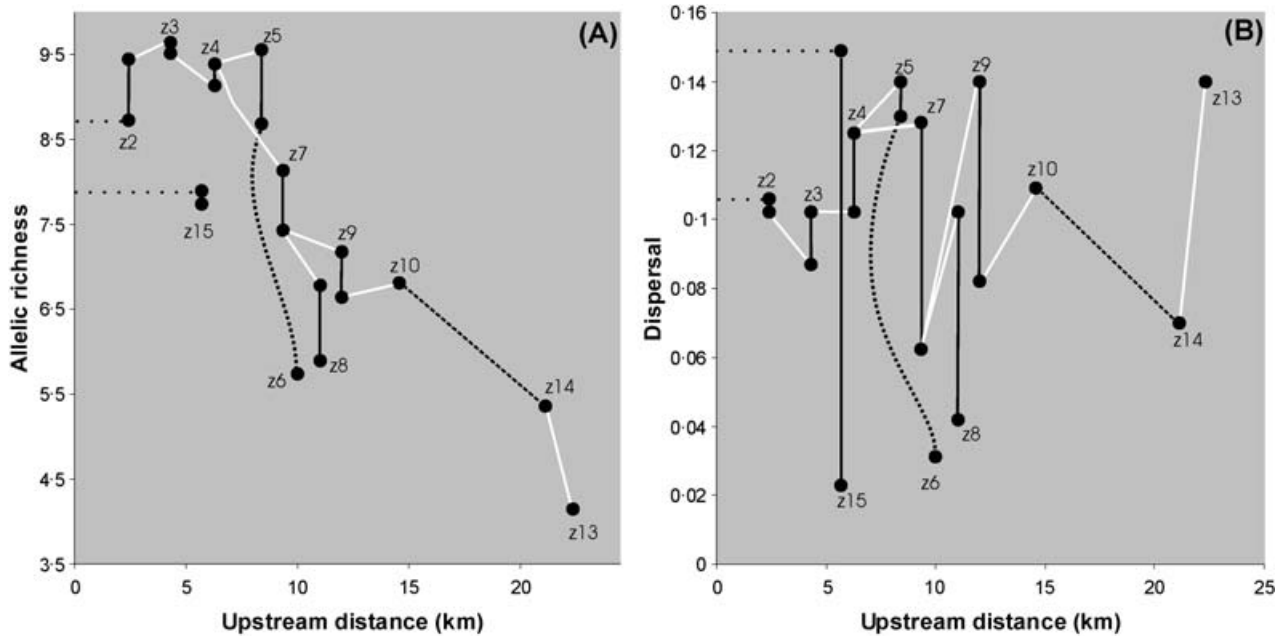


Fig. 2. (A) Allelic richness standardized for 30 diploid individuals and (B) dispersal in populations of three-spined stickleback, plotted against upstream distance. Lines connect neighbouring populations separated by a single water mill (black lines), multiple small barriers (dashed lines), or 1200–2900 m of barrier-free river (white lines). The most downstream river stretches (dotted lines) were not investigated. Population codes are as in Table 1.

of a fish passage would become detectable at the genetic level. Simulations were parameterized with values matching the genetic characteristics of the sticklebacks at the Boembekemolen (Z9). Limited gene flow among two populations separated by a water mill was simulated with 10 replicates using $N_e = 1000$ or 2000 , $\mu = 0.0005$, $m = 0.001$ – 0.003 , random mating, and minimum variation at six loci as the starting point. Populations were allowed to reach equilibrium ($F_{ST} \approx 0.02$) under the K-alleles model or the stepwise mutation model, after which the construction of a fish passage was simulated by switching m to values between 0.01 and 0.2. We then calculated the average number of generations required to reach values of $F_{ST} < 0.005$.

Results

GLOBAL GENETIC STRUCTURE

Mean allelic richness was highest (9.65) at Z3b, one of the most downstream populations, and lowest (4.15) at Z13, the most upstream population (Table 1). Allelic richness strongly decreased with upstream distance ($R = -0.87$, $P < 0.0001$; Fig. 2A). Observed and expected heterozygosity ranged between 0.50 and 0.82. From a total of 300 tests (15 locus pairs in 20 populations), there was no evidence for linkage disequilibrium after Bonferroni correction. Systematic deviations from HWE at each locus were observed in population Z7b due to a heterozygote deficit ($F_{IS} = 0.08242$; $P = 0.0023$). There was no evidence for systematic scoring errors according to Micro-Checker. The biological reason for this deficit remains unclear.

The assignment test for quantifying dispersal identified 93 immigrant genotypes (about 10% of all fish). Dispersal

strongly fluctuated along the river (2.3–14.9%; Table 1; Fig. 2B). Overall genetic differentiation in the Zwalm system was moderate ($F_{ST} = 0.069$; $P < 0.0001$). An NMDS plot of pairwise F_{ST} values (see Supporting Information, Fig. S2) showed that most downstream populations (Z2, Z3, Z4) were genetically similar, whereas the upstream populations (Z6, Z8, Z13, Z14, Z15) were highly differentiated. Neighbouring populations were genetically more similar than non-neighbouring populations.

WATER MILL IMPACT

Allelic richness – AR was lower in 16 upstream populations than in their immediate downstream neighbours (repeated measures ANOVA; *within* effect: $F_{1,14} = 5.64$; $P = 0.0324$). The effect was similar for two kinds of geographical separation (i.e. water mill vs. uninterrupted river). When separated by uninterrupted river, the upstream population lost on average 6% of the allelic richness of the downstream population (Fig. 3A). We therefore estimate the loss of genetic variation in the absence of barriers to be between 2% and 5% per kilometre river stretch. When separated by a water mill (and a negligible distance), the average depletion in allelic richness reached almost 4% (Fig. 3B). Within-pair comparisons revealed that the loss of alleles at water mill Z5 was significant (Wilcoxon matched pairs test between Z5a and Z5b; $Z = 1.99$; $P = 0.0464$). The ratio of above-mill vs. below-mill AR strongly decreased with upstream distance, and, surprisingly, decreased with the year of the water mill's first historical record (Fig. 4).

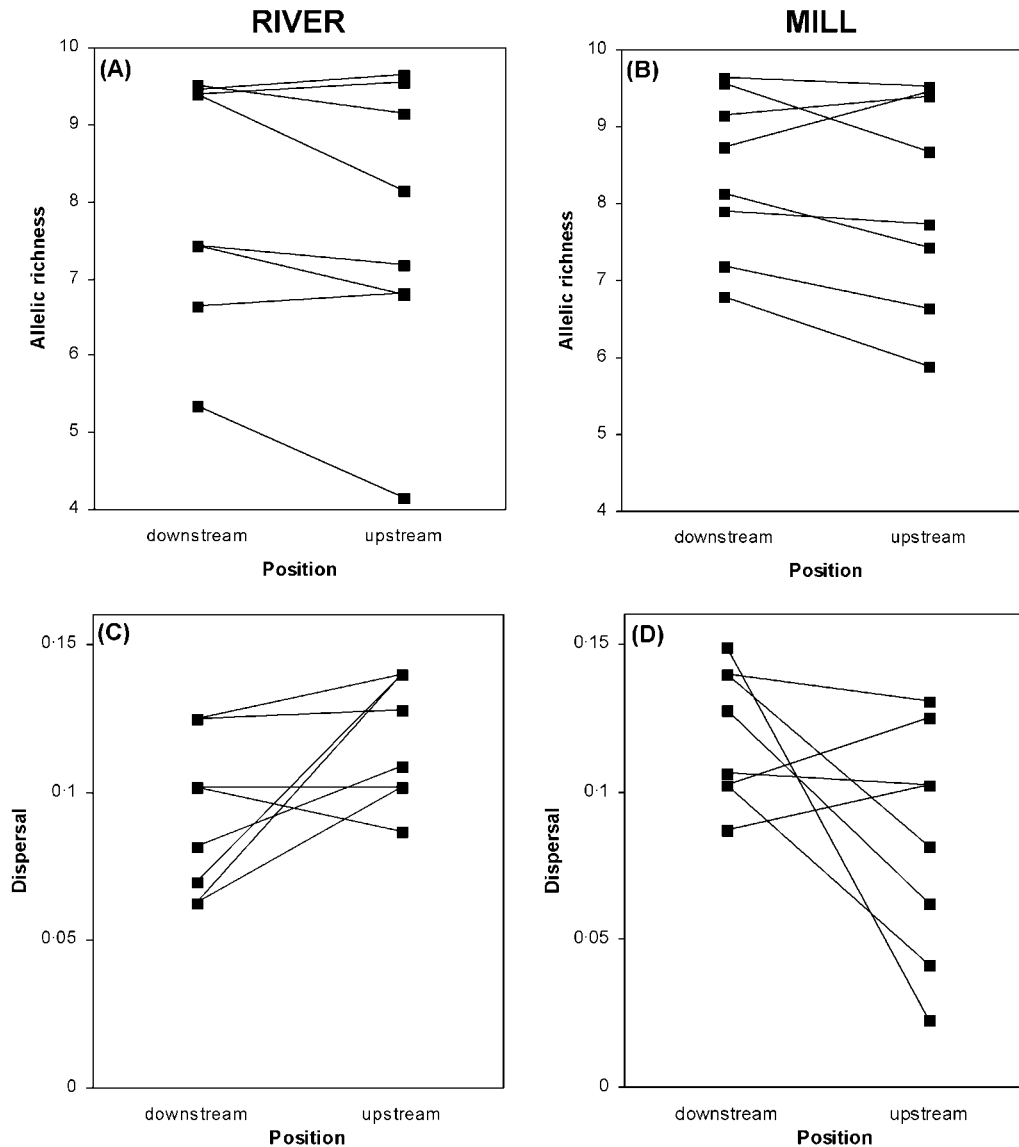


Fig. 3. Allelic richness (A, B) standardized for 30 diploid individuals and dispersal (C, D) in neighbouring population pairs of three-spined stickleback, genotyped at six microsatellite loci. Neighbouring populations spanned 1200–2900 m of barrier-free river (A and C), or a single water mill (B and D).

Dispersal – Uninterrupted river and water mills had, on average, opposite effects on dispersal in neighbouring populations (repeated measures ANOVA; interaction effect: $F_{1,14} = 8.62$; $P = 0.0109$). Along stretches of uninterrupted river, upstream populations showed higher dispersal than downstream populations (Fig. 3C). In contrast, across water mills, upstream populations showed lower dispersal than downstream populations (Fig. 3D). The strongest effects were observed at water mills Z7, Z8, Z9 and Z15 (Table S1). The ratio of above-mill vs. below-mill dispersal significantly decreased with water mill height (Fig. 4).

Genetic differentiation – F_{ST} values among above-mill and adjacent below-mill populations ranged between 0 and 0.020 (Table 2). Differentiation was significant at two mills (Z7:

$F_{ST} = 0.013$; $P = 0.0090$; Z9: $F_{ST} = 0.020$; $P < 0.0001$). Remarkably, Z9 was the only mill to be bypassed by a fish passage at the time of sampling. Values of $F_{ST} = 0$ were observed at four mills. F_{ST} did not correlate significantly with any of the potential determinants of water mill impact (Fig. 4).

ECOLOGICALLY EFFECTIVE RIVER RESTORATION

Starting from an equilibrium $F_{ST} \approx 0.020$ as observed at the Boembekemolen (Z9), simulations revealed a fast decay of genetic differentiation after the construction of a fish passage for values of $m > 0.05$. Values of $F_{ST} < 0.005$ were reached within two ($m = 0.2$) to seven ($m = 0.05$) generations. Assuming a generation time of 1–2 years, the functioning of the fish passage may be detectable after 2 to 14 years.

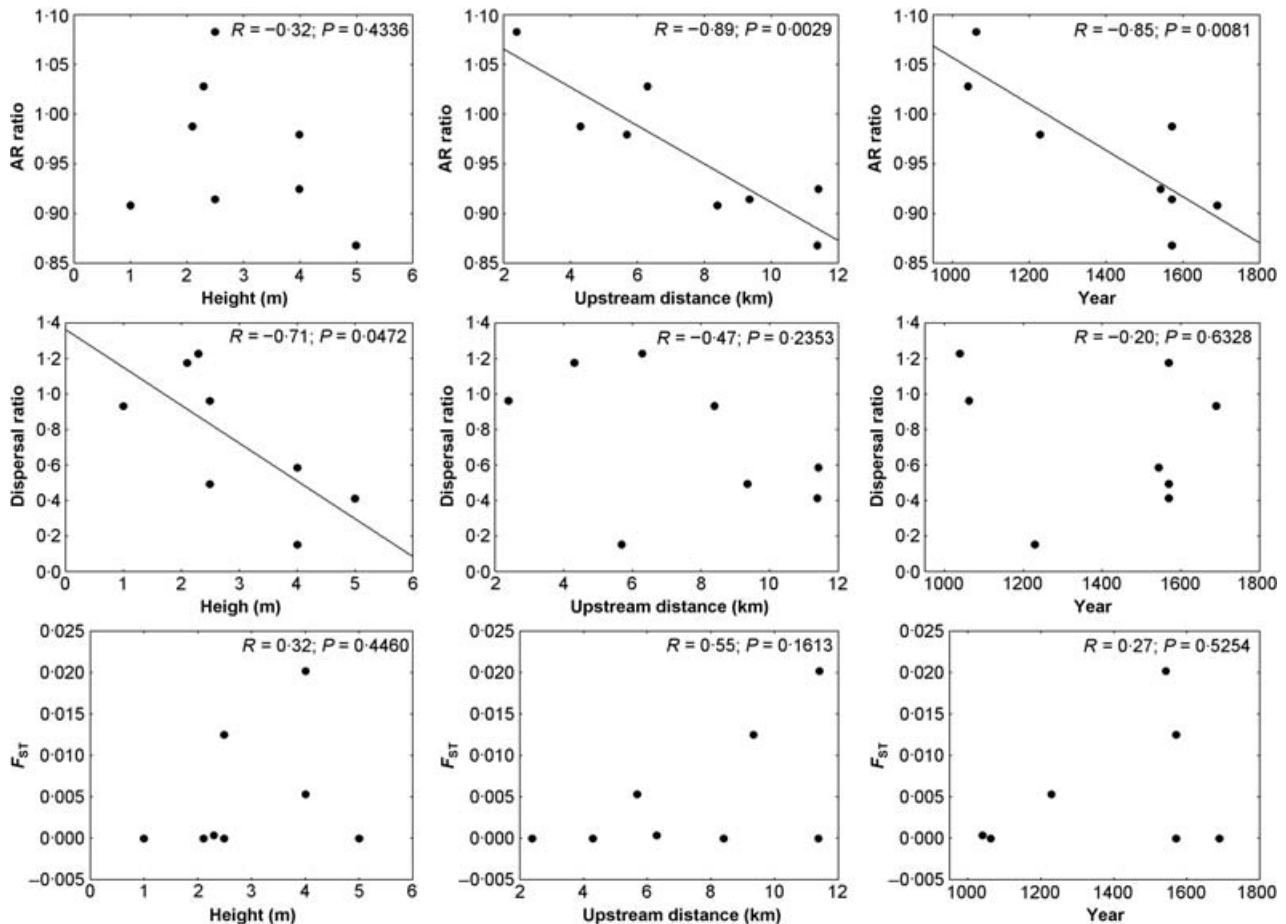


Fig. 4. Relationship between three water mill characteristics (height, upstream distance and year of first historical record) and three genetic measures of water mill impact (AR ratio, dispersal ratio and F_{ST}) for eight mills in the Zwalm basin. AR ratio and dispersal ratio are calculated by dividing above-mill by below-mill values of allelic richness (AR) and dispersal (high values suggest low impact). F_{ST} quantifies pairwise genetic differentiation between above-mill and below-mill populations (high values suggest high impact).

Discussion

This study documents relative connectivity and potential human impact in a river system with high priority for habitat restoration and a significant cultural heritage. We investigated the effect of eight historical water mills on the genetic connectivity of 20 stickleback populations. Providing insight in the causes and consequences of habitat fragmentation and modification, the results are relevant for the basin's depauperate riverine fauna, allowing specific recommendations for river restoration.

WATER MILL IMPACT

Our findings complement many studies documenting various negative impacts of artificial barriers on the riverine environment. Examples include species extinction and decline (Luttrell *et al.* 1999; Endou *et al.* 2006), changes in growth and fitness (Morita *et al.* 2009), decreasing species richness (Reyes-Gavilán *et al.* 1996), genetic differentiation and loss of genetic variation (Yamamoto *et al.* 2004; Wofford *et al.* 2005) and altered parasite communities (Loot *et al.* 2007). The

water mills in this study provoked an average loss of almost 4% of genetic variation. This result is unambiguous, because populations were sampled immediately above and below the same mill. Therefore, every genetic difference spanning a mill can be attributed to its construction and the number of generations over which it has been limiting migration.

Populations of riverine fish often show a gradual loss of genetic variation with upstream distance (Hernandez-Martich & Smith 1997; Castric *et al.* 2001; Crispo *et al.* 2006). Such a strong and predictable pattern could be generated either through historical processes (e.g. past upstream colonization) or by ongoing ecological processes (e.g. downstream-biased gene flow) (Hänfling & Weetman 2006). Based on neighbouring populations spanning a stretch of barrier-free river, we estimated the loss of genetic variation between 2% and 5% per km along the river. The average water mill therefore represents the equivalent of 800 to 2000 m of barrier-free river. The loss in genetic variation along the main channel (Z2 to Z13) due to migration barriers ($N = 13$) alone accumulated to 40%. In a basin with a maximal river length of 23 km, fish populations probably face much lower levels of genetic variation than expected under pristine conditions.

The comparison of contemporary dispersal rates among neighbouring upstream and downstream stickleback populations highlighted another effect of the mills. Barriers and steep stream gradients obstruct upstream dispersal. It is reasonable to assume that any movement would be biased in the downstream direction, regardless of the fish species (Hänfling & Weetman 2006; Caldera & Bolnick 2008). In this study, upstream populations showed lower dispersal than downstream populations in comparisons spanning a water mill, but higher dispersal than downstream populations in comparisons spanning a stretch of uninterrupted river. The first observation suggests that the opportunities to cross the water mills upstream are limited. The second observation may be a side-effect of the first observation, but may also indicate that migration between two water mills is upstream-biased. Mills drastically alter stream conditions, leading to a simplified and slower flow pattern that may facilitate local upstream migration. One may notice that barriers not only alter the population genetic dynamics of riverine fish by fragmenting the habitat, but also by modifying it.

FACTORS EXPLAINING WATER MILL IMPACT

The impact of the water mills on allelic richness increased with upstream distance. This suggests that the mills are particularly influential when populations are already small. Indeed, upstream populations showed reduced genetic variation compared to downstream populations, which is indicative of smaller effective population size (N_e) and increased genetic drift (Frankham 1996). The river section between water mill Z4, Z5 and Z7 represents a breakpoint with respect to these effects (Fig. 2). Below this breakpoint, N_e is probably large, resulting in negligible drift in above- and below-mill populations. Upstream from this breakpoint, genetic drift probably becomes substantial. Discrepancies in above- vs. below-mill allelic richness probably arise from differences in compensatory gene flow, which is smaller in above-mill than in below-mill populations.

The correlation between water mill age and the impact of the mills on allelic richness was negative. This is unexpected, because evolutionary change is expected to be larger over a larger number of years of impact (Yamamoto *et al.* 2004). Coincidentally, however, the first mills were mostly constructed downstream (correlation between water mill age and upstream distance: $R = -0.63$; $P = 0.095$). Upstream position, with effects attributable to differences in N_e and genetic drift, probably largely overrules age as a determinant of water mill impact. Raeymaekers *et al.* (2008) also found that water mills had no stronger impact on the genetic connectivity than more recent constructions (< 100 years), and that barrier impact is mostly location-dependent.

The impact of the mills on dispersal increased with water mill height, ranging between 1 and 5 m (Table 1). The highest mills have a lower probability of inundation, which may leave fewer opportunities to cross the mills upstream. Correlations between F_{ST} and any of the predictors of water mill impact were weak, which was expected. On the one hand, it takes

time for the effects of barriers to appear in F_{ST} values (Yamamoto *et al.* 2004). On the other hand, genetic drift makes F_{ST} values unpredictable (Raeymaekers *et al.* 2008).

APPLICABILITY

The impact of migration barriers on the population genetic dynamics of a species depends on its lifestyle, generation time, and physical (e.g. size and dispersal capacity) and genetic (e.g. N_e) properties (Meldgaard *et al.* 2003; Yamamoto *et al.* 2004; Hänfling & Weetman 2006). The stickleback's high sensitivity to barriers enabled us to generate a detailed picture of the connectivity in the system. If this picture is indicative for the majority of local species, it is relevant for river restoration. This is a reasonable assumption because, in contrast to terrestrial organisms, migratory pathways in rivers are comparable among many riverine organisms, and demographic processes are confined by the same river channel and barriers. In addition, many riverine fishes have comparable population structures, characterized by high N_e in mainstem populations, low N_e in upstream tributaries, and asymmetric gene flow (Wofford *et al.* 2005; Crispo *et al.* 2006; Caldera & Bolnick 2008). Species may differ significantly in effective population size, and we have seen that such differences strongly determine barrier impact. However, what can be generalized across species with a similar genetic structure is the *relative* impact of the barriers: barriers of stronger impact for the stickleback will be more significant barriers in general, and the physical properties of the water mills (height and upstream distance) are likely to affect other species similarly.

We note two limitations to our approach. First, highly migratory species do not directly benefit from an assessment of relative barrier impact, because their survival mostly depends on basin accessibility as determined by the most downstream barriers (e.g. Yamamoto *et al.* 2004; So *et al.* 2006). Limited accessibility contributes to the extinction of such species, as occurred in the Zwalm basin. Secondly, the conclusion that a barrier has no detectable genetic impact on the investigated species does not mean that there is no impact on other species. Populations may not have reached equilibrium since they were separated by the barriers, and thus tests for differentiation and loss of diversity may have been unable to reveal that these processes are indeed occurring. In this case, the relative impact of such barriers remains unknown.

RESTORATION STATUS AND RECOMMENDATIONS

The Zwalm's improving water quality and intrinsic natural values stimulated the construction of four fish passages on the main channel (Z2, Z3, Z4, and Z7) in 2005. Based on the significant genetic differentiation observed in 2004, a fish passage at the Z7 site was highly recommended. In contrast, our tests did not reveal a strong impact on any of the genetic properties of the populations at the three most downstream mills (Z2, Z3 and Z4; Table S1). This implies that we cannot judge the relative impact of these barriers on species with a lower N_e than the stickleback. An evaluation of the Z2

passage in 2007 revealed that it is used by most of the local fish (22 species including stickleback; Buysse *et al.* 2007). The observation of river lamprey, chub and dace underlines the importance of the downstream passages.

The upstream part of the basin remains obstructed by water mills Z5, Z8, Z9 and Z15, limiting access to 12.85 km, 9.95 km, 54.48 km, and 19.36 km of upstream riverine habitat, respectively (see Supporting Information, Table S1). Considerable loss of genetic variation and declining immigration across these barriers underline the necessity for further restoration efforts. Practical management solutions include the construction of effective fish passages. Translocation of individuals from below-mill to above-mill sites represents an alternative solution for restoring genetic variation in above-mill populations (Yamamoto *et al.* 2006). We recommend actions in the context not only of the genetic impact, but also the ecological benefit in terms of accessible riverine habitat, the cost of restoration, and the cultural-economical value of the water mills (Supporting Information, Table S1).

A large ecological benefit and significant genetic differentiation between its above- and below-mill stickleback populations suggest that restoration at water mill Z9 should receive high priority. Although Z9 has been bypassed by a fish passage since 1995, the upper stairs obstruct fish migration (Buysse *et al.* 2007). This can be solved at relatively little cost. The next restoration target should be water mill Z5. While this mill is provoking a significant loss of genetic variation, its preservation is of very little concern as there are no above-ground remains. A fish passage could be constructed at the left bank without the risk of damaging the authenticity of the site (D. Malfroid, personal communication). Water mill Z15 strongly affected dispersal, but did not provoke a significant loss of genetic variation. The reduction in dispersal may represent an important early warning and restoration should be a priority. As a fish passage may compromise the functioning of the mill, its design should be discussed with the stakeholder. Water mill Z8 affected both allelic richness and dispersal, but we could not demonstrate a significant impact. As the size of the upstream habitat is limited and the mill is still operational, we suggest the lowest priority for a fish passage. Translocation of individuals from species of conservation concern could be considered as a temporary solution for sites Z15 and Z8. Finally, several modern constructions including small dams, sluices and culverts, are limiting access to the far upstream sites (Z6, Z13, Z14). Further study should determine how to minimize the impact of these barriers.

ECOLOGICALLY EFFECTIVE RIVER RESTORATION

An important criterion for ecologically effective river restoration is that ecological improvement should be measurable (Palmer *et al.* 2005). Population genetic approaches may be useful here for re-assessing population connectivity. While the success of translocations can be evaluated genetically after one generation (Yamamoto *et al.* 2006), the effect of fish passages will only become detectable when allele frequencies homogenize across barriers, or when differences in genetic

variation fade with time. The speed of these changes depends on N_e and the migration rate. Simulations of population genetic dynamics allowed us to predict the long-term influence of restoration actions. Here we based such simulations on the water mill provoking the strongest genetic differentiation (Z9). We predicted the decay of genetic differentiation within 2 to 14 years. Reassessment of stickleback genetic structure after a decade could therefore reveal whether habitat connectivity has been restored.

CONCLUSION

We have demonstrated the utility of population genetic approaches to infer and complement guidelines for river restoration. Population genetic approaches will not replace ecological studies for documenting crucial ecological processes such as migration or survival, but can provide estimates of the relative connectivity in a river system at any geographical scale. Genetic parameters like allelic richness and dispersal come with a spatial resolution. Moreover, these parameters reveal current effects (e.g. dispersal) and provide information integrated over time (e.g. allelic richness), whereas ecological studies are often snapshots in time. Population genetic studies therefore have a unique explorative value, and may be particularly useful prior to the planning of restoration initiatives and associated ecological studies.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. River restoration status and recommendations at eight water mills

Fig. S1. The Temsche molen in 1935.

Fig. S2. NMDS plot of pairwise F_{ST} values among 20 stickleback populations.

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