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Impacts of river fragmentation on the genetic population structure of the chub *(Squalius cephalus)*

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Zusammenfassung

Weltweit sind unzählige Arten gefährdet durch den anthropogenen Verlust an Lebensraum, dessen Fragmentierung und Qualitätsminderung. Siedlungen, Infrastruktur und Industrie beanspruchen immer grösserer Flächen, die intensive landwirtschaftliche Nutzung formt eine grüne Wüste und aquatische Lebensräume geraten durch die ansteigende Energieproduktion zunehmend unter Druck. Die dramatischsten Veränderungen haben die aquatischen Lebensräume erfahren. Im europäischen Durchschnitt wurden 50% der Feuchtgebiete trockengelegt, wobei die Schweiz bei den Auen mit einem Verlust von 95% einen Spitzenwert erzielt. Dass 72% der Fischarten der Schweiz auf der Roten Liste fungieren und acht ausgestorben sind, erstaunt daher nicht.

Den acht ausgestorbenen Arten wurde zum Verhängnis, dass ihre Migration zwischen Meer und Laichgründen unterbunden ist. Langfristig sind durch die Einschränkung der Fischdurchgängigkeit selbst stationär lebende Arten gefährdet. Ehemals zusammenhängende Populationen werden in kleinere Subpopulationen aufgeteilt. Dies hat zwei nachteilige Auswirkungen auf die Struktur der Population. Erstens sind kleine Populationen durch Katstrophenereignisse jederzeit vom Aussterben bedroht. Zweitens verringert sich in kleinen Populationen die genetische Diversität durch Drift bei gleichzeitig reduziertem Genfluss. Dies kann langfristig die Adaptation an sich ändernde Umweltbedingungen beeinträchtigen oder gar verhindern.

Der Fokus dieser Doktorarbeit lag auf den Auswirkungen der Fragmentierung auf die Konnektivität. Der Alet (*Squallius cephalus*) dient dabei als Modellorganismus. Ausschlaggebend für diese Wahl waren drei Aspekte: Erstens ist der Alet eine häufige weit verbreitete Art des schweizerischen Mittelandes. Das erlaubt grosse Probezahlen

mit vertretbarem Aufwand zu sammeln. Zweitens sind Alet unempfindlich gegenüber Lebensraumveränderungen wie reduzierter Strömung und Sedimentation, die bei der Fragmentierung durch Wasserkraftwerke auftreten. So kann am Alet eher der Effekt der Fragmentierung studiert werden, ohne den Einfluss der Lebensraumveränderung. Drittens wurde der Alt nie besetzt. Demnach sollte seine genetische Struktur mehrheitlich natürlich sein. Dies führte zur Annahme einer panmiktischen Populationsstruktur oder einer mit Isolation über die Distanz.
Ziel aller genetischen Analysen war es Abweichungen von den genannten Populationsstrukturen als Effekt der Fragmentierung zu finden. Die angewendeten Analysen basieren auf Allelfrequenzdaten neutraler nuklearer DNA (Mikrosatelliten).

Im Kapitel 1 wurde der Einfluss der 37 Laufwasserkraftwerke am Hochrhein mit den Zuflüssen Aare, Reuss und Limmat untersucht, die einen bedeutenden Anteil an der schweizerischen Energieproduktion haben. Das Ziel war die Wirkung dieser Hindernisse auf die genetische Populationsstruktur des Alets zu erfassen. Gleichzeitig konnte erstmals die Wirksamkeit der Fischaufstiegshilfen evaluiert werden, mit welcher die meisten, aber nicht alle Laufkraftwerke ausgerüstet sind. Die wichtigste Erkenntnis ist der signifikante negative Effekt der Laufkraftwerke auf die genetische Struktur des Alets, der jedoch bei Kraftwerken mit Fischaufstiegshilfen wesentlich weniger ausgeprägt ist. Die existierenden Fischaufstiegshilfen verhindern teilweise die Unterbrechung der Konnektivität. Solche Anstrengungen zur Wiederherstellung der Konnektivität weisen den richtigen Weg, das Ziel ist jedoch längst noch nicht erreicht.
Im Kapitel 2 wurde die genetische Populationsstruktur in den der drei Flüssen Thur, Galt und Broye verglichen. Die Thur inklusive einem Rheinstück ist nicht

fragmentiert, die Glatt ist stark mit Abstürzen zur Sohlstabilisierung verbaut und die Broye enthält einen Vielzahl an Schwellen die Individuell passierbar sind. In der Thur war auf 76 km Flusslänge keine genetische Differenzierung erkennbar. Hingegen war die Alet-Population über das 50 km lange Glatt stark genetisch differenziert und zusätzlich konnte eine starke Abnahme der genetischen Diversität festgestellt werden. Die ebenfalls nicht vorhanden genetische Differenzierung in der Broye zeigt, dass der Alet problemlos kleinere Hindernisse überwinden kann. Dieses Ergebnis bestätigt die angenommene natürliche panmiktische Populationsstruktur des Alets über grössere Distanzen, wenn keine Hindernisse diese stören.

Im Kapitel 3 wurde der Einfluss der historischen Besiedelung und des aktuelleren anthropogenen Einfluss auf die Populationsstruktur des Alets. Der Alpenraum mit den nahe beieinanderliegenden Quellen von Rhone, Rhein, Donau und Po ist dazu besonders geeignet. Zudem war die Artabgrenzung von Alet (*S. cephalus*) und dem kürzlich als eigene Arte abgespaltenen Cavedano (*S. squalus*) von Interesse. Eine kombinierte Analyse von mitochondrialen Sequenzen, nukleären Mikrostelliten und morphologischen Messungen unterstützt die Auftrennung in zwei Arten. Trotz dieser Trennung wurde eine mitochondriale Introgression von Alet in Cavedano gefunden. Die wahrscheinlichste Erklärung dafür ist eine Hybridisierung von Alet mit Cavedano, der als Köderfisch ins Tessin mitgebracht wurde. Die Alet-Linien waren mehrheitlich konsistent mit der nacheiszeitlichen Kolonisierung der Einzugsgebiete von Rhein, Rhone und Donau. Die interessanten Ausnahmen ist die Kolonisierung des Genfersees und seiner Zuflüsse über den Rhein und starke Donau-Präsenz im Rhein mit einer Einwanderung vermutlich über die Donauversickerung bei Tuttlingen. Der

Schutz dieser evolutionär unterschiedlichen Linien und dem Cavedano als Art, bedingt die zukünftige Prävention vor Verschleppung und Freisetzungen.

Summary

Numerous species are threatened by habitat loss or the fragmentation and alteration of their habitat through human influence. Land is lost to settlements and industry or strongly modified by intensive agriculture, aquatic habitats are increasingly impacted by energy production. Water bodies have indeed changed radically with an average wetland loss of 50% in Europe and even a 95% loss of riverine floodplains in Switzerland. The sad result is that 72% of all fish species are Red-listed in Switzerland and eight species are known to have become extinct. These eight species were all migratory and their disappearance is considered to be a direct consequence of river fragmentation by weirs and hydroelectric power plants. Over the longer term, fragmentation also impacts non-migratory fish species by reducing population connectivity and subdividing previously contiguous populations into smaller subpopulations. These are more susceptible to stochastic extinction and the combination of increased genetic drift and reduced gene flow may lead to the loss of genetic diversity, possibly hampering their capacity to adapt to environmental change.

The impact of habitat fragmentation on population connectivity of riverine fish is the focus of this thesis. I chose the European chub (*Squalius cephalus*) as a model organism to address this issue because it offers three important advantages: First, the chub is so common and widespread in the Swiss midlands that large samples can be obtained with reasonable effort. Second, chub populations are little affected by habitat alterations that are concomitant with river fragmentation, such as reduced flow and increased sedimentation. This allows an assessment of fragmentation that is really due to obstructed dispersal rather than unsuitable habitat. Third, the chub has never been stocked. Its genetic structure should thus be largely unconfounded by human

translocations. I assumed that this species showed a natural population structure that was essentially panmictic or showed isolation-by-distance at a large geographic scale. The goal was to test for deviations from these patterns caused by fragmentation by applying population genetic analyses to allele frequency data from neutral molecular markers (microsatellites).

Chapter 1 is concerned with the fragmentation of the large lowland rivers in the upper Rhine catchment by hydroelectric power stations. The lower parts of the rivers Rhine, Aar, Reuss and Limmat are fragmented by 37 hydroelectric power stations that make an important contribution to the Swiss energy industries' production of electricity. My goal was to assess the effect of these barriers on the population connectivity of the chub and to infer whether the fishpasses installed at many (but not all) of these stations do achieve the desired mitigation of fragmentation. The main result is that man-made barriers had a significant effect on the genetic structure and diversity of chub in the upper Rhine drainage, but that stations with fishpasses had a weaker effect than those without. Thus, existing fishpasses do have the desired effect of at least mitigating fragmentation, but these results also show that on-going efforts to restore connectivity in river systems remain necessary and are heading in the right direction.

In Chapter 2 I compared the genetic structure and diversity of chub along the unfragmented river Thur (including a stretch of the free flowing Rhine) with that of the nearby river Glatt, which is heavily fragmented by impassable barriers, and the river Broye, which is fragmented by a large number of individually passable barriers. Chub along the unfragmented stretch of Thur and Rhine showed no genetic differentiation over 76 km and the highest allelic diversity overall. On the contrary, chub along 50 km of the Glatt river system exhibited clear differentiation associated with barriers and a steep upstream decline of allelic diversity. Finally, chub from the

Broye also showed no genetic substructure, which suggests that chub disperse readily across low-height barriers when waterflow allows. These results also confirm that chub can maintain a panmictic genetic population structure over considerable distances if dispersal is not restricted by migration barriers.

In Chapter 3 I took a broader-scale perspective to examine historical and human-influenced patterns in the chub's genetic structure in central Europe. This was facilitated by the nearby origins of the Rhine, Rhone, Danube and Po rivers in the Swiss Alps. Of particular interest was whether molecular data would support the recent distinction of the Italian chub or cavedano (*S. squalus*) from the European chub (*S. cephalus*). A combined approach using mitochondrial sequences, nuclear microsatellite data and morphometrics clearly supported the separation of European chub and cavedano.However, there was evidence for mitochondrial introgression from European chub to cavedano in southern Switzerland, presumably the result of hybridization following human-mediated translocations, possibly as live bait. The desirable preservation of evolutionarily distinct lineages will thus require the prevention of such translocations in the future. The nuclear genetic structure of the European chub was consistent with postglacial recolonizations from multiple refugia along the major rivers, but interestingly, it was modified by a watershed crossing between Rhine and Rhone near Lake Geneva and possibly by a drainage capture via the Danube sinkhole between Danube and Rhine near Lake Constance.

Introduction

Foreword

The attentive naturalist observes a species' extinction without the obvious indicators in its everyday surroundings. The reason is obscured by our distorted perception caused by the time lag between cause and reaction. This obscure cause is the topic of my thesis.

Background

Population Fragmentation

Many species today face three major threats to their habitat; habitat loss, alteration, and fragmentation (Sala et al. 2000; Foley et al. 2005; Fischer and Lindenmayer 2007). The first two factors reduce the size of their populations, fragmentation further divides them into smaller subpopulations. Our immediate perception and concern is often the reduction in population size because of habitat loss and alteration, but habitat fragmentation strongly influences the fate of the remaining populations as well. Therefore, habitat fragmentation has become a major research area in conservation biology at the ecosystem and species levels (Fischer and Lindenmayer 2007). Species responses to fragmentation are currently well documented in terrestrial ecosystems with a zoological (reviewed by (Cushman 2006; Watling and Donnelly 2006; Banks et al. 2007; Fleishman and Mac Nally 2007; Keyghobadi 2007) and a botanical focus (Aguilar et al. 2006; Aguilar et al. 2008). In the short term, fragmentation does not reduce the overall population size, it just divides population into smaller units. However, a large overall population is key to long-term survival. Small populations face a higher risk of extinction from stochastic events, and local extinctions are irreversible where fragmentation prevents recolonization. Small

populations will also lose genetic diversity by drift. Genetic drift in combination with local extinctions leads to an overall loss of a species' genetic diversity (Keyghobadi 2007). The loss of genetic diversity, on the other hand, may lead to inbreeding depression and a reduced adaptability to environmental changes, hampering population recovery and making populations more susceptible to the next disturbance, which will erode genetic diversity even further. That is, populations can get caught in a so-called extinction vortex (Gilpin and Soulé 1986).

Habitat fragmentation in aquatic ecosystems

Although habitat fragmentation, which is one of the key negative human impacts on animal populations, is particularly severe in streams and rivers (Vörösmarty et al. 2010), its effects on fish and the resulting genetic consequences are poorly studied (Fazey et al. 2005). Most obvious is the impact on migratory species like salmon or eel, whose lifecycles get interrupted by barriers to migration such as hydroelectric power stations or weirs, typically resulting in rapid extinction (Liermann et al. 2012). The impact of fragmentation on non-migratory fish species has received little attention. Many of these species still manage to reproduce in our fragmented rivers and are therefore not of urgent conservation concern, but fragmentation may nevertheless influence their genetic structure and diversity. To counter the negative effects of river fragmentation, technical solutions like fishpasses are the method of choice and such migration aids are nowadays mandatory for damming projects in Switzerland (Hefti 2012). Although monitoring of fish moving through fishpasses is used to evaluate the success of installing fishpasses (Guthruf 2006, 2008), it remains unclear if and to what extent fishpasses really achieve re-establishment of genetic connectivity in fish populations along rivers.

Study region and study organism

Fragmentation of the study region

Rivers in Switzerland are strongly modified and have lost 95% of their flood plains (Tockner and Stanford 2002). The need for hydroelectric power and, to a lesser extent, irrigation water, as well as the necessity to protect settlements and agricultural land from flooding have resulted in the construction of numerous barriers to control flow. A survey by the Swiss Federal Office for the Environment (FOEN) has revealed that Swiss streams and rivers are fragmented by approximately 101,000 barriers over 50 cm in height (Zeh Weissmann et al. 2009). These barriers are not distributed evenly over the country. The majority of the Swiss population lives at low elevation in the Swiss midlands, where river modification and fragmentation is thus most severe, although even at high elevation in the Alps the modification by impounding reservoirs is very conspicuous. I chose to study the effects of river fragmentation of fish genetic population structure and diversity in the Swiss Midlands as an example of a landscape where this fragmentation is particularly severe.

Study organism

To investigate these effects I chose the European chub (*Squalius cephalus*) as a model organism. The chub has the advantage that it is a common species for which large samples can be caught with reasonable effort, and that its genetic population structure is not affected by stocking. Also relevant was the comparatively good swimming capacity of chub, which means that it should be able to benefit from the installation of fishpasses around migration barriers designed to enable passage of migratory salmonids, which are very strong swimmers. Monitoring has indeed shown that chub can pass all fishpasses installed in the large midland rivers Aar and Rhine (Guthruf 2006, 2008). Similar to other cyprinid species, little is known about its broad-scale

genetic structure in Switzerland, which must be influenced by postglacial recolonizations. My research opened the opportunity to also investigate this issue and to clarify with genetic data the status of chub from Ticino in southern Switzerland, which are treated as a separate species; the Italian chub (*S. squalus*).

The European chub's (*S. cephalus*) natural range covers large parts of Europe with an eastern frontier reaching the Caspian Sea and northern frontier reaching the 56th parallel, excluding Scotland, Ireland, Greece, the Iberian Peninsula, the Adriatic region and Italy (Kottelat and Freyhof 2007). In contrast, the range of the Italian chub (*S. squalus*) is limited to Italy, which includes the most southern parts of Switzerland (Kottelat and Freyhof 2007). In Switzerland the European chub's range covers the Swiss plateau where it lives in high numbers in rivers of the Barbel and Grayling Region as well as in lakes (Zaugg et al. 2003). Chub reach an average length of 40 to 50 cm and a weight of 1 kg (Zaugg et al. 2003). They reach maturity at 2-4 years for males and 4-6 years for females and live for up to 15 years (Kottelat and Freyhof 2007). For spawning, which females typically do twice a year, they need gravel with current flow (Fredrich et al. 2003). Their high abundance can be explained by the ability to cope with altered habitats, in particular their ability to thrive in lentic as well as lotic water, by omnivory and their acceptance of altered spawning grounds as long as a stony bottom is available (Arlinghaus and Wolter 2003; Kottelat and Freyhof 2007). The Italian chub, which has been recently segregated from chub and identified as its own species, has a similar life history (Kottelat and Freyhof 2007).

Study aims and basic approach

The aims of my research were to understand the influence of man-made river fragmentation on the genetic structure and diversity of chub populations in

Switzerland, and to describe the large-scale genetic and morphological variation of chub across the main watersheds originating in the Swiss Alps. In chapter 1 I investigated fragmentation of the large Swiss midland rivers Rhine, Aar, Reuss and Limmat by hydropower stations, many of which are equipped with a fishpass. Specifically, I asked whether these fishpasses do indeed mitigate the effects of fragmentation by power stations and improve chub population connectivity along these rivers. This is novel because the success control of fishpasses is normally restricted to counting the passing fish without any genetic evaluation, and published genetic studies of the consequences of river fragmentation typically deal with impassable barriers. In chapter 2 I took advantage of one of the few remaining unfragmented river stretches in the Swiss midlands, the lower parts of the river Thur, and compared the genetic structure and diversity of chub with that in an adjacent river that is heavily fragmented (Glatt), as well as a third river that is fragmented by a large number of low-height barriers that are passable for chub at least under high water levels. It is unclear whether such barriers could exert a cumulative effect on chub population connectivity. Chapter 3 is finally concerned with the genetic and morphological diversity of chub in the main watersheds of the Swiss Alps. The distribution of chub lineages on a Europe-wide scale is is reasonably well documented (Durand et al. 1999; Seifertova et al. 2012), although at a low geographic resolution that excluded Switzerland. This gap was covered by characterizing chub at the top of the major European rivers, namely the Rhone, Rhine, Danube and Po with nuclear and mitochondrial genetic markers as well as traditional and geometric morphometrics. Of particular interest was whether these data supported the presence of Italian chub as a distinct species in Swiss rivers of the Po drainage south of the Alps.

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Chapter I: Fish population genetic structure shaped by hydroelectric power plants in the upper Rhine catchment

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Evolutionary Applications: in press

Abstract

The Rhine catchment in Switzerland has been transformed by a chain of hydroelectric power stations. We addressed the impact of fragmentation on the genetic structure of fish populations by focusing on the European chub (Squalius cephalus). This fish species is not stocked and copes well with altered habitats, enabling an assessment of the effects of fragmentation per se. Using microsatellites, we genotyped 2133 chub from 47 sites within the catchment fragmented by 37 hydroelectric power stations, two weirs and the Rhine Falls. The shallow genetic population structure reflected drainage topology and was affected significantly by barriers to migration. The effect of power stations equipped with fishpasses on genetic differentiation was detectable, albeit weaker than that of man-made barriers without fishpasses. The Rhine Falls as the only long-standing natural obstacle (formed 14'000 to 17'000 years ago) also had a strong effect. Man-made barriers also exacerbated the upstream decrease in allelic diversity in the catchment, particularly when lacking fishpasses. Thus, existing fishpasses do have the desired effect of mitigating fragmentation, but barriers still reduce population connectivity in a fish that traverses fishpasses better than many other species. Less mobile species are likely to be affected more severely.

Introduction

The ongoing landscape modification by humans leads to massive destruction or alterations of pristine ecosystems by a combination of fragmentation, habitat loss and degradation (Sala et al. 2000; Foley et al. 2005; Fischer and Lindenmayer 2007). In riverine ecosystems, fragmentation is considered a key threat for aquatic biodiversity because organisms are restricted to linear dendritic habitats and cannot avoid anthropogenic barriers (Fagan 2002; Vörösmarty et al. 2010). Despite the recognized importance of fragmentation in aquatic conservation, the fragmentation literature is currently biased towards terrestrial ecosystems (Fazey et al. 2005).

River catchments suffered from heavy floodplain losses of up to 90% in the USA and even more than 90% in Europe, as for example in Switzerland where 95% of the floodplains have been lost (Tockner and Stanford 2002). Concurrent with this destruction the same regions are also the most fragmented by dam-building (Tockner and Stanford 2002; Nilsson et al. 2005; Lehner et al. 2011). An inevitable consequence of the many barriers in rivers has been that currently, diadromous fish species are the most threatened at the global (Liermann et al. 2012) and local scale (Kirchhofer et al. 2007). Rieman and Dunham (2000) reviewed the situation for salmonids that are structured in metapopulations and concluded that river fragmentation is frequently the reason for population collapse.

Since the early days of dam construction, fishpasses have been constructed to mitigate negative effects on fish migration (Katopodis and Williams 2012). Noonan et al. (2012) published a meta-analysis of fishpass efficiency assessments from 1960 to 2010. They found 122 articles published over 50 years reporting such assessments - astonishingly few considering the high costs of fishpasses - and most of these assessments focused on salmonids. The conclusions were that the design of fishpasses

affected their efficiency and that they worked better for salmonids than for other fish, but that the overall efficiency was too low to avoid the negative effects of habitat fragmentation on the fish community (Noonan et al. 2012). The focus on salmonids is warranted due to their economic value and their life-histories making them particularly vulnerable to fragmentation (e.g. anadromous salmon, trout and char forms). Nevertheless, even non-migratory species require between 1 to 100 km river length for their entire life history and at this scale habitat changes due to major flooding events occur in the order of every 5 to 50 years (Fausch et al. 2002). It is thus important that fishpasses are designed to also facilitate the migration of fish other than salmonids and that their efficiency in doing so is evaluated.

The long-term persistence of species depends on sufficient genetic diversity to adapt and survive in variable or changing environments (Hughes et al. 2008). If local populations are small, gene flow is the key factor to prevent the stochastic loss of genetic diversity (Palstra and Ruzzante 2008) and to provide the required alleles to subpopulations under selection that lack favourable genotypes (Kinnison and Hairston 2007). While an effective population size of just 50 may be sufficient to avoid the negative effects of inbreeding in the short term, the long term maintenance of adaptive potential requires an effective population size in the range of at least 500 (Franklin and Frankham 1998; Jamieson and Allendorf 2012). Some authors argued that the required number may be even higher (Lynch and Lande 1998). Unfortunately the ratio between the effective and the total population size is difficult to predict and often species-specific in freshwater fish. Published values range from less than 0.01 for introduced bottlenecked pike (*Esox lucius* Linnaeus) (Aguilar et al. 2005) over intermediate values like 0.11 in natural population of brown trout (*Salmo trutta* Linnaeus) (Charlier et al. 2011) to very high ratios approaching 1 in the case of the

endangered copper redhorse (*Moxostoma hubbsi* Legendre) (Lippe et al. 2006). Therefore, fishpass efficiency is not simply a matter of the number of climbing or descending fish. The goal has to be to allow sufficient gene flow for a species to maintain its evolutionary potential in a fragmented habitat.

Previous studies investigated fragmentation caused by barriers that were impassable for fish in the upstream direction, and these studies showed that such barriers had a strong impact on the genetic population structure. Examples include yellow perch (*Perca flavescens* Mitchill) (Leclerc et al. 2008), Macquarie perch (Faulks et al. 2011) brown trout (Horreo et al. 2011; Stelkens et al. 2012), bullhead (*Cottus gobio* Linnaeus) (Hanfling and Weetman 2006; Junker et al. 2012), three-spined stickleback (*Gasterosteus aculeatus* Linnaeus) (Raeymaekers et al. 2008), grayling (*Thymallus thymallus* Linnaeus) (Meldgaard et al. 2003), chub (*Squalius cephalus* Linnaeus) (Dehais et al. 2010) and a four species comparison of chub, dace (*Leuciscus leuciscus*), gudgeon (*Gobio gobio*) and minnow (*Phoxinus phoxinus*) by Blanchet et al. (2010).

Here we present a large-scale study on the effects of dams in a strongly fragmented system where a large proportion (89 %) of dams enable migration through fishpasses. We selected the common chub *(Squalius cephalus* Linnaeus) as our model species, a European cyprinid species that is very common in the Swiss midlands, where it lives in high number in rivers of the Barbel and Grayling Region [categorization according to Huet (1949)] as well as in lakes (Zaugg et al. 2003). Chub reach an average length of 40 to 50 cm in the study region (Zaugg et al. 2003). Due to their ecological generalism, omnivory and behavioural flexibility, chub cope relatively well with the ongoing habitat alteration. For spawning grounds they prefer shallow gravel banks (0.1 - 1.0 m) with some current (0.15 to 0.35 m / s) (Fredrich et al. 2003). They

readily use alternative spawning grounds in altered habitats, as long as some stony bottom is available (Arlinghaus and Wolter 2003). Chub are relatively mobile and show an upstream spawning migration of up to 16 km (river Spree, Fredrich et al. 2003) or even in excess of 25 km (River Meuse, De Leeuw and Winter 2008). Chub are iteroparous, spawn twice a year and may migrate to different spawning grounds between spawnings (Fredrich et al. 2003). The upstream spawning migration may compensate for downstream drift in the larval stage. Chub larvae actively swim into open water to go into drift, which is a common behaviour in many cyprinid species (e.g (Reichard et al. 2002; Reichard and Jurajda 2004; Sonny et al. 2006). Female chub reach maturity in their second to fourth year (Raikova-Petrova et al. 2012) and can reach a maximum age of up to 20 years (Busst and Britton 2014). Because of their low commercial value, chub are not stocked. The genetic structure of chub populations should thus be unconfounded by stocking, making this species especially useful as a sentinel for changes in population connectivity resulting from obstructions to dispersal.

In this study we describe the genetic population structure of the chub in the main Swiss lowland rivers of the Rhine catchment and assess the effects of barriers such as hydroelectric power plants on population connectivity and genetic diversity. In particular, we ask whether fishpasses that are present at most (but not all) barriers do have the desired effect of mitigating fragmentation.

Methods

Study area

Our study area comprises the larger Swiss midland rivers of the upper Rhine catchment (length 376 km, catchment area 35897 km^2, discharge 1037 m^3/s). In addition to the Rhine, the Rhine catchment includes the river Aar (length 288 km, catchment area 17620 km^2, discharge 560 m^3/s) with its two tributaries Reuss (length 158 km, catchment area 3425 km^2, discharge 140 m^3/s) and Limmat (length 140 km, catchment area 2416 km^2, discharge 101 m^3/s) (Verdon et al. 2009) (Fig. 1). The first dam was built in 1830 for textile manufacturing, followed by many hydroelectric power stations from 1861 to 1975. At present, the studied river sections are fragmented by 37 hydroelectric power stations, of which four do not have a fishpass, and two adjacent weirs also lacking a fishpass. The two adjacent weirs are considered as a single barrier in all analyses. When fish sampling started in 2010, the median age oft the artificial obstacles was 82 years and that of the fishpasses 77 years (age known for 23 fishpasses, for all others information was not publicly available) (Supplementary Table S1 and Fig. S1). The Rhine Falls are the only natural barrier in the study area and estimated to be between 17,000 and 14,000 years old (Fig. 1). According to monitoring data from the hydroelectric power stations, chub are able to use all fishpasses in the study area (Guthruf 2006, 2008).

Site selection and fish sampling

Chub from 47 sites were sampled from spring to autumn 2010 and 2011 in the Rhine, Aar, Reuss and Limmat catchments, which include also three lakes (Fig. 1). The goal was to sample each fragment, defined as a river section between two barriers, at least once, and to sample two or more sites from some fragments to break the pairwise

correlation between waterway distance and the number of barriers between sampling sites. The fish were caught by electro fishing (FEG 1700, EFKO comm., Leutkirch, Germany), wading along the river shoreline. The exact locations were chosen in consideration of accessibility and safety. At three sites we could not catch in the main river but managed to obtain samples from the mouths of tributaries (A13, L27, R29). At two sites where electrofishing was impossible, fish were caught with rod and line (sites A19 and L28). Sampling site coordinates are provided in Table 1. The goal was to catch 50 chub per site which was achieved at most sites.

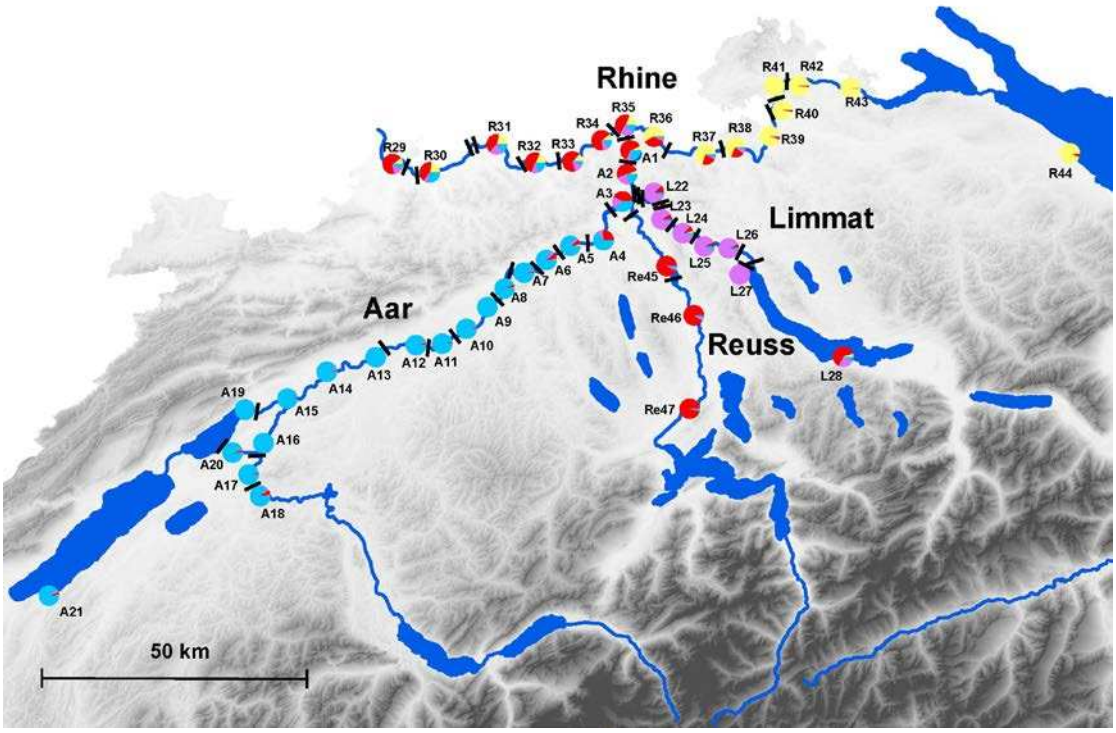

Figure **1** Map of the Swiss midland with the rivers Rhine, Aar, Reuss and Limmat. Pie charts depict mean assignment probabilities of chub genotypes to each of the four inferred genetic clusters, averaged over 28 TESS runs. Black bars represent migration barriers. These are hydroelectric power stations except for the Rhine falls (between site R40 and R41), and two weirs (represented as one bar between L26 and L27**).**

Most individuals caught were young of the year (70%), the remaining fish were juveniles and a few adults. Because chub undergo a larval drift phase (see Introduction), we do not expect the fish from a single sampling site to be related (e.g. siblings). Clove oil was used to lightly anesthetize the fish before total length measurement and tissue sampling from the caudal fin. Fin clips were stored in 99.9% ethanol and all fish were released at the sampling sites after recovering from anesthesia.

Genotyping

DNA was extracted following the salting-out protocol of Sunnucks and Hales (1996) adapted to a 96 deep well format. Fin clips (1 mm^2) were air dried in 8-strip microtubes before adding 300 µl of TNES buffer **(**50 mM Tris, pH 7.5, 400 mM NaCl, 20 nM EDTA, 0.5% SDS) and 5 µl of 10 mg/ml proteinase K (Roche inc., Basel, Switzerland). After incubation for 60 min in an incubation shaker (Thermomixer Comfort, Eppendorf inc, Hamburg, Germany) at 300 rpm, the proteins were precipitated by adding 85 µl of 5M NaCl and shaking the tubes for 10 s. Proteins were pelleted in a centrifuge (Heraeus Megafuge 40R, Thermo Fisher Scientific inc, Waltham, MA, USA) at 4700 rpm for 10 min. The supernatants were carefully transferred into a 96 deep well block. Afterwards the DNA was precipitated by adding 400 µl of ice cold 100 % ethanol and gentle mixing with a pipette. The DNA was pelleted by centrifugation for 10 min at 4700 rpm, washed with 70% ethanol, centrifuged again for 10 min at 4700 rpm and air dried. Finally the DNA was resuspended in 100 µl of TE buffer (100 mM Tris-HCl,10 mM EDTA) and stored at -20 °C.

Table 1 Collection information and measures of genetic diversity for 47 samples of chub (*Squalius cephalus*) from the Swiss Midland rivers Aar (A), Limmat (L), Rhine (R), and Reuss (Re). *N*: sample size, H_e: expected heterozygosity; H_o: observed heterozygosity; *AR:* allelic richness standardized for the smallest sample size (20), F_{IS}: inbreeding coefficient; N_e: effective population size.

Site	Coordinates WGS84	*N*	*He*	*Ho*	F_{IS}	*AR*	*Ne* estimate (95% CI)
A1	N 47° 33' 32.97" E 8° 13' 53.06"	50	0.748	0.753	-0.007	7.126	∞ (301 - ∞)
A2	N 47° 32' 38.45" E 8° 13' 46.80"	50	0.737	0.724	0.017	6.934	439 (118 - ∞)
A3	N 47° 29' 07.19" E 8° 12' 58.84"	48	0.747	0.742	0.007	7.244	∞ (641 - ∞)
A4	N 47° 25' 03.88" E 8° 09' 43.08"	48	0.728	0.709	0.026	7.034	351 (69 - ∞)
A5	N 47° 24' 29.97" E 8° 04' 01.72"	49	0.744	0.755	-0.014	7.632	205 (91 - ∞)
A6	N 47° 23' 01.72" E 8° 00' 50.45"	50	0.746	0.719	0.035	7.155	∞ (349 - ∞)
A7	N 47° 21' 57.55" E 7° 59' 37.84"	49	0.739	0.745	-0.007	7.172	629 (174 - ∞)
A8	N 47° 18' 53.87" E 7° 53' 29.30"	49	0.734	0.750	-0.022	7.115	283 (113 - ∞)
A9	N 47° 18' 37.57" E 7° 52' 09.98"	25	0.756	0.769	-0.017	7.274	∞ (121 - ∞)
A10	N 47° 15' 49.35" E 7° 48' 24.77"	50	0.728	0.747	-0.026	6.637	401 (110 - ∞)
A11	N 47° 14' 16.29" E 7° 44' 42.19"	49	0.734	0.750	-0.021	7.249	3962 (111 - ∞)
A12	N 47° 14' 07.67" E 7° 40' 37.44"	54	0.746	0.761	-0.021	7.216	369 (132 - ∞)
A13	N 47° 12' 51.54" E 7° 34' 24.28"	28	0.753	0.732	0.028	6.869	∞ (602 - ∞)
A14	N 47° 11' 19.92" E 7° 26' 51.32"	48	0.723	0.715	0.012	6.736	551 (113 - ∞)
A15	N 47° 08' 30.69" E 7° 20' 46.29"	36	0.727	0.734	-0.009	6.977	∞ (1893 - ∞)
A16	N 47° 02' 44.27" E 7° 16' 28.32"	50	0.729	0.748	-0.027	6.534	∞ (264 - ∞)
A17	N 47° 00' 24.12" E 7° 14' 49.34"	54	0.727	0.740	-0.018	6.822	202 (93 - ∞)
A18	N 46° 58' 24.58" E 7° 15' 35.76"	36	0.714	0.739	-0.035	6.812	651 (104 - ∞)
A19	N 47° 07' 20.34" E 7° 14' 13.74"	32	0.720	0.677	0.060*	6.669	232 (74 - ∞)
A20	N 47° 02' 47.60" E 7° 12' 21.73"	42	0.725	0.711	0.020	6.715	8270 (96 - ∞)
A21	N 46° 47' 35.08" E 6° 44' 17.48"	49	0.639	0.665	-0.041	5.851	∞ (160 - ∞)
L22	N 47° 29' 03.39" E 8° 17' 25.28"	50	0.713	0.721	-0.011	6.192	59 (40 – 99)
L23	N 47° 27' 22.38" E 8° 18' 45.74"	50	0.724	0.720	0.005	6.176	125 (72 – 345)
L24	N 47° 24' 31.51" E 8° 24' 37.39"	48	0.715	0.666	0.069*	6.107	50 (25 – 164)
L25	N 47° 24' 16.86" E 8° 26' 09.85"	50	0.701	0.698	0.004	5.812	187 (79 - ∞)
L26	N 47° 24' 03.46" E 8° 29' 05.16"	49	0.711	0.676	0.049*	5.851	∞ (101 - ∞)
L27	N 47° 20' 54.09" E 8° 30' 57.75"	48	0.660	0.654	0.010	4.720	69 (34 – 281)
L28	N 47° 12' 26.98" E 8° 46' 35.03"	30	0.695	0.688	0.010	6.504	∞ (127 - ∞)
R29	N 47° 33' 12.70" E 7° 37' 6.017"	48	0.727	0.730	-0.004	6.761	169 (69 - ∞)
R30	N 47° 32' 19.35" E 7° 42' 51.34"	47	0.742	0.747	-0.006	7.787	∞ (280 - ∞)
R31	N 47° 35' 15.08" E 7° 53' 16.80"	50	0.738	0.769	-0.042	7.414	474 (183 - ∞)
R32	N 47° 33' 17.43" E 7° 59' 14.33"	49	0.736	0.701	0.048*	8.178	256 (136 – 1372)
R33	N 47° 33' 24.52" E 8° 05' 01.38"	48	0.730	0.710	0.027	7.358	1694 (114 - ∞)
R34	N 47° 35' 34.95" E 8° 09' 38.66"	50	0.728	0.702	0.036	7.241	165 (79 – 3223)
R35	N 47° 36' 23.24" E 8° 13' 21.66"	56	0.736	0.732	0.006	7.690	1114 (214 - ∞)
R36	N 47° 35' 55.13" E 8° 17' 43.24"	49	0.706	0.692	0.020	7.381	264 (88 - ∞)
R37	N 47° 33' 59.08" E 8° 25' 43.14"	49	0.712	0.712	0.001	7.269	763 (182 - ∞)
R38	N 47° 34' 42.14" E 8° 30' 20.53"	53	0.725	0.715	0.014	7.226	548 (125 - ∞)
R39	N 47° 35' 49.36" E 8° 35' 44.49"	49	0.669	0.655	0.021	7.502	∞ (322 - ∞)
R40	N 47° 39' 09.40" E 8° 37' 46.12"	33	0.658	0.653	0.008	7.027	314 (62 - ∞)
R41	N 47° 41' 05.50" E 8° 37' 35.20"	50	0.651	0.625	0.040	6.763	1274 (128 - ∞)
R42	N 47° 41' 05.17" E 8° 40' 22.69"	48	0.661	0.651	0.016	7.578	250 (90 - ∞)
R43	N 47° 40' 43.48" E 8° 48' 25.57"	26	0.667	0.671	-0.006	7.057	130 (52 - ∞)
R44	N 47° 33' 22.20" E 9° 21' 58.62"	21	0.721	0.734	-0.018	7.908	∞ (2538 - ∞)
Re45	N 47° 22' 16.54" E 8° 19' 29.01"	49	0.735	0.725	0.014	6.998	1216 (161 - ∞)
Re46	N 47° 17' 02.82" E 8° 23' 35.59"	39	0.742	0.751	-0.012	6.651	1694 (177 - ∞)
Re47	N 47° 07' 07.11" E 8° 23' 26.11"	48	0.730	0.725	0.007	6.602	437 (83 - ∞)

* $P < 0.05$

Ten microsatellite loci amplified in two multiplex PCR reactions were used to genotype the individuals: LC128, LC27, LC290, LC32, LC93 (Vyskocilova et al. 2007), LceA149, LceC1, LceCb (Larno et al. 2005), and N7G5, N7K4 (Mesquita et al. 2003). The 10 µl PCR-reactions contained 5 µl Qiagen Multiplex PCR Master Mix (Qiagen inc., Hilden, Germany), 3 µl of ultrapure water, 1 µl of DNA template and 1 µl of primer mix. The forward primers for the two multiplex reactions were labeled with fluorescent dyes (Microsynth inc., Balgach, Switzerland), such that loci with the same dye had non-overlapping allele size ranges. To balance peak heights, labeled forward primers of strongly amplifying loci were partially supplemented with unlabelled primer. Multiplex 1 contained the following primers (only forward primer concentrations given, reverse primer concentrations were equivalent to the sum of labelled and unlabelled forward primers): N7K4 0.4 nM labeled (YYE) and 14.5 nM unlabeled, N7G5 0.9 nM labeled (AT550) and 30.5 nM unlabeled, LC128 4.7 nM (AT550), LC 27 1.8 nM (FAM), LC 290 1.2 nM (YYE). Multiplex 2: LceA149 0.5 nM labeled (FAM) and 10 nM unlabeled, LC32 0.8 nM labeled (AT565) and 13.7 nM unlabeled, LceC1 1.6 nM (AT550), LceCb 6.8 nM (FAM), LC93 1.9 nM (AT565). PCR thermal conditions were as follows: initial *Taq* polymerase activation and denaturation at 95°C for 15 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C (multiplex 1) or 57°C (multiplex 2) for 90 s, extension at 72°C for 90 s, followed by a final extension at 72°C for 10 min (Labcycler Sensoquest, Göttingen, Germany).

PCR products were analyzed on an ABI 3730 capillary sequencer (Applied Biosystems, Foster City, CA, USA). For loading, 1 µl of PCR product was mixed with 8 µl Hi-Di Formamide and 1 µl size standard diluted 1: 7 (GeneScan 500 LIZ, ABI).

The genotyping accuracy was tested by extracting and genotyping a subset of samples twice. Locus LC290 showed a drop-out of large alleles, due to its wide allele size range (allele size 223 to 309 bp). To avoid genotyping errors resulting from this problem, all homozygotes with low peak heights were reamplified with more cycles and scored again. All other loci had scoring errors below 1%. Nevertheless, the scoring was done three times to minimize mistakes. For the final dataset only individuals that were genotyped successfully at nine of the ten loci at least were considered (132 out of 2133 genotyped individuals had one missing locus). That resulted in an average of 45 individuals per site. For the sample sizes per site see Table 1.

Population genetic analyses

Estimators of genetic diversity such as observed heterozygosity (H_O), expected heterozygosity (H_E), mean number of alleles and allelic richness (AR) standardized for smallest N were calculated using FSTAT 2.9.4 (Goudet 2002) (Table 1). We tested for the presence of null alleles with the software Micro-Checker (Van Oosterhout et al. 2004). FSTAT was also used to test for deviations from linkage and Hardy-Weinberg equilibrium (for F_{IS} see Table 1). Pairwise population differentiation (F_{ST}) was calculated according to Weir and Cockerham (1984) as implemented in FSTAT 2.9.4, using 10000 bootstraps for significance tests. The use of F_{ST} to infer population structure has been criticized because of its dependency on within-population diversity, and alternative measures such as F'_{ST} or D have been proposed (Hedrick 2005; Jost 2008). However, we decided to focus on F_{ST} because it is the appropriate measure of deviations from panmixia (Whitlock 2011), which is our main interest in the context of potential barriers to fish migration. The software NEESTIMATOR v. 2.0.1 (Do et

al. 2014), was used to obtain point estimates of the effective population size N_e. We used the method based on linkage disequilibrium restricted to alleles with frequencies > 0.02 as recommended by Do et al. (2014). Confidence intervals were obtained with the jackknife method of Waples and Do (2008). To obtain a general overview of the genetic structuring of chub in the Swiss midland rivers we used the individual-based Bayesian clustering approach implemented in TESS 2.3.1 (Chen et al. 2007). Neighbouring individuals are more likely to belong to the same genetic cluster, and TESS uses the Hidden Markov Random Field approach to take into account the spatial distribution of individuals (Francois et al. 2006). The interaction parameter ψ was set at the default value of ψ = 0.6 for the analyses. ψ can range from zero (no spatial interaction) to one (strongest spatial interaction). The neighbouring system is done by Voroni tessellation (Guillot et al. 2009), which requires individual geographic coordinates for each genotype. To obtain these, the coordinate creator implemented in the software was used. It randomly samples from a normal distribution around the initial coordinate of the population sample. A standard deviation of 25 m was used. As fish can only have up- or downstream neighbours, the automatically generated neighbour system was changed to reflect the dendritic network of our river system. This was done with the neighbourhood modifying option. All overland links were deleted and missing links were added (Neighbourhood TESS 2.3.1 output see Fig S2. Supporting information).

Using the no-admixture model, each number of genetic clusters (K_{max}) was tested with 20 runs, for K_{max} = 2 to 9. The total number of sweeps per run was 300’000 after a burn in of 50’000 sweeps. The best-supported number of genetic clusters was determined by plotting the mean Deviance Information Criterion (*DIC*) of the 20 runs over the respective K_{max} (Spiegelhalter et al. 2002). The best-supported K_{max} is at the

point where the *DIC* curve switches from a sharp decrease to a plateau without further changes (for details see the TESS2.3.1 Manual). Best K_{max} was run an additional 20 times. For averaging the results and taking into account 'label switching' the programme CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) was used. The calculations were done by using the Greedy algorithm in CLUMPP 1.1.2. The cluster visualization was done with the software DiSTRUCT (Rosenberg 2004).

Attempts to estimate contemporary gene flow among sampling sites using the software MIGRATE-N (Beerli and Felsenstein 2001) failed due to non-convergence, presumably because of the very shallow overall population structure of the chub in the study area (see Results), and for the same reason inferring recent migration rates with BAYESASS (Wilson and Rannala 2003) produced nonsensical results. It appears that a global F_{ST} of >0.05 would be required to use this approach profitably (Meirmans 2014). This requirement of at least moderately strong population structure combined with the requirement of individuals with migrant ancestry being frequent enough to be included in realistic sample sizes may well be a general limitation of this approach and could possibly explain the alarming observation by Meirmans (2014) that most of the published estimates of the proportion of nonmigrants produced by BAYESASS cluster at the upper and lower bounds of the prior distribution.

Fragmentation effects

Our main interest was to investigate the impact of barriers on population structure and genetic diversity. First we assessed the general impact of barriers, treating all barriers equally, and second we tested whether different types of barriers had different effects, focusing particularly on the presence or absence of fish ladders.

The overall impact of barriers on genetic differentiation was assessed using an isolation-by-distance (IBD) approach, correlating the matrix of pairwise F_{ST} (n = 1081 pairwise comparisons) with the matrix of pairwise waterway distances extracted with ArcMap10 (Esri inc., Redlands, CA, USA) between sampling sites and the matrix of the pairwise number of barriers (barrier count) between sites. Partial Mantel tests were carried out according to Smouse et al. (1986), using the software Arlequin 3.5 (Excoffier and Lischer 2010) with 10000 permutations. The Partial Mantel test assesses the effect of the number of barriers between sites on pairwise differentiation while controlling for pairwise waterway distance.

Assessing the impact of different barrier types on gene flow is important for assessing the effectiveness of the fish ladders. To evaluate the impact of the different types of barriers we split the 39 barriers in our system into different groups: the Rhine Falls as the only major natural barrier (14000-17000 years old), hydroelectric power stations with fishpass (n = 33), and power stations without fishpass (n = 5). The last group comprised one barrier (the two adjacent weirs) that was not a power station but was included because it could be assumed to be equally obstructive to fish migration. The age of artificial barriers could also influence the differentiation among fish populations they separate. However, we found that the pairwise number of artificial barriers separating two sampling sites and their cumulative age were strongly correlated (r = 0.97). This collinearity precluded an independent assessment of the age of barriers, thus we restricted our analyses on the number of barriers of each type. We constructed six linear models for comparison that had pairwise F_{ST} as the dependent variable and the following predictors:

(i) distance

(ii) distance + barrier count

(iii) distance + count of barriers without fishpass (including Rhine Falls) + count of barriers with fishpass

(iv) distance + Rhine Falls

(v) distance + Rhine Falls + count of all other barriers

(vi) distance + Rhine Falls + count of barriers with fishpass + count of barriers without fishpass

The relative support for the different models was assessed by model selection using the AIC criterion (Burnham and Anderson 2002), and we followed Koizumi et al. (2006) in using the number of populations (47) rather than the number of pairwise comparisons (1082) as *n* in the calculation of AIC to account for non-independence inherent in pairwise data. R 2.15.1 was used to perform the analyses (R Core Team 2012). The significance of individual parameters in the models was evaluated with permutation tests using the R package lmPerm (Wheeler 2010). Because of the unavoidable collinearity between distance and the counts of the different barrier types between sampling sites we applied commonality analysis to the best-supported models (Nimon et al. 2008), using the R package MBESS (Kelley and Lai 2012). This approach was recently advocated for landscape genetics by Prunier et al. (2015) and allows an assessment of the extent to which individual predictors contribute via unique and shared effects to the explained variance in the response variable.
To complement these analyses and to account for the dendritic structure of the river network we also applied the STREAMTREE algorithm by Kalinowski et al. (2008) to map genetic differences among chub subpopulations onto the stream sections

connecting them. This approach uses least-squares estimation to model pairwise genetic distance (here F_{ST} calculated by the STREAMTREE software) as the sum of genetic distances for the stream sections between them. The coefficient of determination R^2 is used to assess the fit of the resulting model to the data. Because a canal between the Aare river and Lake Biel (see site A20 in Fig. 1) creates a loop in the drainage structure that is incompatible with the STREAMTREE model, we had to exclude sites A16-A21 from this analysis.

The genetic diversity expressed as allelic richness (*AR*), was investigated with a similar model selection approach as used for genetic differentiation. Six linear models of *AR* were constructed with the same predictors as above, but here distance was calculated from an arbitrary reference point below the most downstream site, namely where the Rhine crosses the Swiss border, and the numbers of the different types of barriers were counted along the river(s) between this reference point and each site.

Results

Microsatellite variation

The initial analysis with Micro-Checker indicated the presence of null alleles at locus LceCb in 12 sites. We therefore excluded this locus from all further analyses. Without locus LceCb, there was no strong evidence of deviations from HWE within populations. Although 29 out of 423 single-locus tests within populations were significant at $\alpha = 0.05$, this is close to the approx. 21 significant tests expected by chance, and the deviations were spread erratically over loci and populations, including similar numbers of heterozygote excesses and deficits. No deviation was significant after Bonferroni correction. Accordingly, only four out of 47 multilocus estimates of F_{IS} differed significantly from 0 at $\alpha = 0.05$, and none of these deviations was

significant at a Bonferroni-corrected α of 0.001 (0.05/47 sites) (Table 1). There was no evidence of linkage disequilibrium among loci.

Sample sizes and genetic diversity statistics for all collection sites are summarized in Table 1. Expected heterozygosities ranged between 0.639 and 0.756 and the global F_{ST} estimate was 0.028 (0.022 – 0.036 95% C.I.). Table 1 also contains the estimates of N_e obtained with NeEstimator. These are generally very high with most values in the hundreds or thousands. For many samples N_e was estimated as infinite and the upper confidence limit reached infinity in the majority of cases. The lowest estimates tended to come from the river Limmat, which was the only river for which estimates <100 were obtained (sites L22, L24 and L27).

Bayesian clustering

The DIC plot indicated four as the most likely number of genetic clusters present in our chub samples. These clusters corresponded well to the populations in the four rivers we sampled from above their confluence (Fig. 1; for individual cluster membership coefficients see Fig. S3 Supporting information). Samples from the river Rhine downstream of the confluence of the four rivers show admixture of the four clusters up until the most downstream site, whereas mixing upstream of confluences appears very restricted (Fig. 1).

General effects of barriers

The IBD plot shows a monotonic positive relationship between pairwise F_{ST} and distance with increasing scatter (Fig. S4A Supporting information). This corresponds to a case I relationship as defined by Hutchison and Templeton (1999) and is indicative of a regional equilibrium between gene flow and drift. Genetic differentiation increased with increasing waterway distance between sampling sites

(Mantel $R = 0.595$, $P < 0.001$) as well as with increasing numbers of barriers separating the sites (Mantel $R = 0.617$, $P < 0.001$; Fig. S4B Supporting information). Despite our efforts to break the collinearity when choosing sampling sites, waterway distances and barrier counts between sites were positively correlated with $R = 0.827$. However, the partial Mantel tests indicated that the number of barriers between sites is a somewhat better predictor of genetic differentiation than distance *per se* (barrier count corrected for distance: $R = 0.278$, $P = 0.002$; distance corrected for barriers: $R = 0.190$, $P = 0.048$).

Effects of different barrier types

The best-supported model predicting pairwise F_{ST} between samples (model iii, AIC weight = 0.550, Table 2) included barriers in the predictors in addition to distance and distinguished between barriers with and without fishpasses, the latter including the Rhine falls. The second-ranked model (model vi, AIC weight 0.290) additionally distinguished the Rhine falls from all other barriers without fishpass.

Table 2 Results of the model selection procedure based on AIC to assess the relative support of six candidate linear regression models predicting pairwise F_{ST} between chub samples from the Rhine drainage in Switzerland.

Model		Slope	R^2	AIC	Δ AIC	AIC weight
i)	distance	2.731E-4***	0.354	-216.549	10.657	0.003
ii)	distance	1.243E-4***	0.404	-218.349	8.857	0.007
	all barriers	1.974E-3***				
iii)	distance	1.122E-4***	0.527	-227.206	0.000	0.550
	barriers with fish bypass	1.392E-3***				
	barriers without fish bypass (including Rhine falls)	1.149E-2***				
iv)	distance	2.319E-4***	0.423	-219.848	7.358	0.014
	Rhine falls	1.878E-2***				
v)	distance	3.904E-5*	0.498	-224.423	2.783	0.137
	Rhine falls	2.476E-2***				
	all barriers (excl. Rhine falls)	2.458E-3***				
vi)	distance	8.191E-5***	0.534	-225.929	1.277	0.290
	Rhine falls	1.808E-2***				
	barriers with fish bypass	1.720E-3***				
	barriers without fish bypass	9.402E-3***				

∗ indicates $p = 0.051$, ∗∗∗ indicates $p < 0.001$

The other models had a Δ AIC > 2 from the best-supported model. Most notably, the model including distance only (model i, Δ AIC =10.657, AIC weight 0.003) and the model not distinguishing between different types of barriers (model ii, Δ AIC = 8.857, AIC weight 0.007) received virtually no support from the data. These results provide strong evidence that barriers influence the genetic structure of chub in the large Swiss lowland rivers and that fishpasses appear to mitigate the negative effects of barriers on population connectivity. The parameter estimates from the best-supported model iii allow gauging the effects of barriers with and without fishpass relative to free flowing stretches of river. According to this model, the effect of a barrier without fishpass is

equivalent to approx. 102 km of uninterrupted river distance, while barriers with fishpass correspond to approx. 12 km. These values are in a similar range when calculated from the second-ranked model vi (114 km and 20 km, respectively), which assigns an even stronger effect to the only long-standing natural barrier, the Rhine Falls, corresponding to approx. 220 km of uninterrupted river. Unsurprisingly, commonality analysis of the two best-supported models showed substantial contributions of all predictors via common effects, reflecting the multicollinearity of distance and the numbers of different barriers between sites, but it also showed that in both models the barriers without fishpasses had the largest unique effect on genetic differentiation (Table 3a).

To visualize the effects of barriers in more detail we have plotted the genetic differentiation (F_{ST}) from the most downstream site (R29, Fig. 1) along each of the four rivers as a function of distance and added the position and type of barriers to these plots (Fig. 2). Although the effects are not strikingly obvious, more rapid increases of genetic differentiation tend to be associated with a high density of barriers (Fig. 2C) or with barriers that lack a fishpass (Figs. 2A, C & D).

With an R^2 of 0.836 the STREAMTREE model provided a fit to the actual genetic differentiation in the river network that, according to the authors of the algorithm (Kalinowski et al. 2008), is not very good and adverts to caution in interpreting the results. We report the results nevertheless because they provide an alternative visual representation of the resistance to migration in these rivers (Fig. 3) and because it highlights a potential additional factor contributing to genetic differentiation that was not considered in any other analyses. The STREAMTREE analysis predicted a genetic distance of 0 for more than half of all river sections considered, and although the non-zero values tended to be assigned to sections with a high density of barriers or

fishpass-free barriers, the match was not very good (Fig. 3). Especially two of the highest values were assigned to sections without barriers but where major tributaries run into the Rhine river (from site R35 to R36 and R38 to R39, see Figs. 1 & 3). The highest value was assigned to a section comprising a barrier without fishpass (short section below site L27, see Figs. 1 & 3), yet this section is not part of the main course of the river Limmat. It represents the lowest part of an important tributary (river Sihl) from which sample L27 was taken upstream of the weirs separating the Sihl from the Limmat. Although certainly to be taken with caution, these observations suggest that immigration from unsampled tributaries may also contribute to genetic differentiation of chub along the main rivers of the upper Rhine catchment.

Table 3 Commonality Analysis of the best-supported regression models (see Table 2) predicting genetic differentiation expressed as F_{ST} (a) and genetic diversity expressed as *AR* (b). Unique refers to each predictor's unique effect and Common refers to the sum of effects in common with other predictors in the model. Total is the sum of unique and common contributions to the explained variance in the response variable.

Predictor	R^2	*B*	*P*	Unique	Common	Total	% of R^2
(a) Genetic differentiation (F_{ST})							
Model iii (AIC weight = 0.550)	0.527						
Distance		1.122E-4	< 0.001	0.019	0.334	0.354	67.1
Barriers with fish bypass		1.392E-3	< 0.001	0.024	0.268	0.292	55.4
Barriers without bypass (incl. Rhine Falls)		1.149E-2	< 0.001	0.162	0.149	0.311	59.0
Model vi (AIC weight = 0.290)	0.534						
distance		8.191E-5	< 0.001	0.009	0.345	0.354	66.2
Rhine Falls		1.808E-2	< 0.001	0.048	0.146	0.194	36.3
barriers with fish bypass		1.720E-3	< 0.001	0.031	0.261	0.292	54.7
barriers without fish bypass		9.402E-3	< 0.001	0.062	0.176	0.239	44.7
(b) Genetic diversity (*AR*)							
Model vi (AIC weight = 0.853)	0.548						
distance		0.005	0.072	0.037	0.060	0.096	17.6
Rhine Falls		0.349	0.227	0.016	0.020	0.037	6.7
barriers with fish bypass		-0.109	0.001	0.127	0.146	0.273	49.8
barriers without fish bypass		-0.544	< 0.001	0.195	-0.031	0.165	30.0

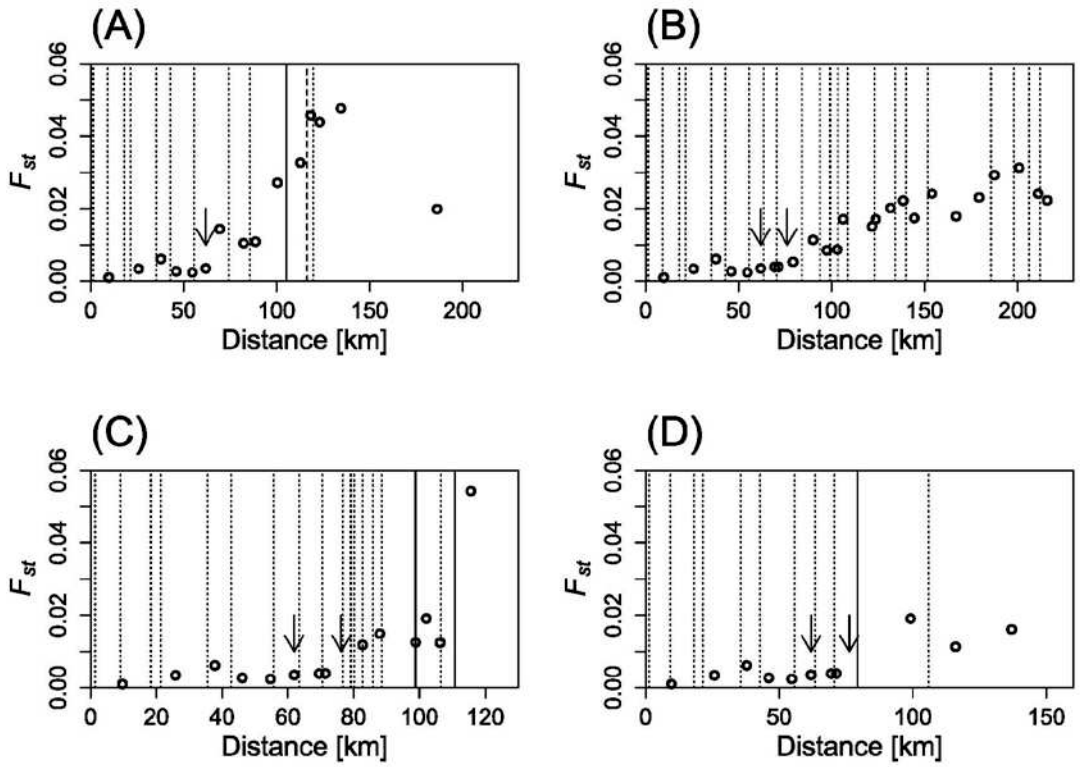

Figure 2 F_{ST} plotted against distance along the rivers Rhine (A), Aar (B), Limmat (C) and Reuss (D) from the most downstream sampling site in the Rhine, R29 (Fig. 1). Dotted lines are barriers with fish bypass, solid lines are barriers without fish bypass and the dashed line represents the Rhine Falls. Confluences are indicated by arrows.

There is also correlative evidence that hydroelectric power plants and weirs affect the upstream decline of *AR* in chub populations. By far best-supported in our set of candidate models (AIC weight = 0.853) was the most complex model vi that fitted separate effects for distance, the Rhine falls, barriers with fishpasses and barriers without fishpasses (Table 4). In this model, the number of both types of man-made barriers between the most downstream point of the Rhine in Switzerland and the sampling sites have significantly negative effects on *AR*, but the effect of barriers without fishpass is about fivefold stronger (Table 4). Again, commonality analysis showed that barriers without fishpass make the largest unique contribution to the

explained variance in *AR*, followed by barriers with fishpass (Table 3b). The estimated effect of the Rhine falls was non-significant but positive in this model, presumably just reflecting that the three samples from the Rhine above the Rhine falls had a higher allelic richness on average than most samples from the other rivers. More interestingly, the effect of distance in this model, which accounts for the effects of barriers, was also positive, although not significantly so.

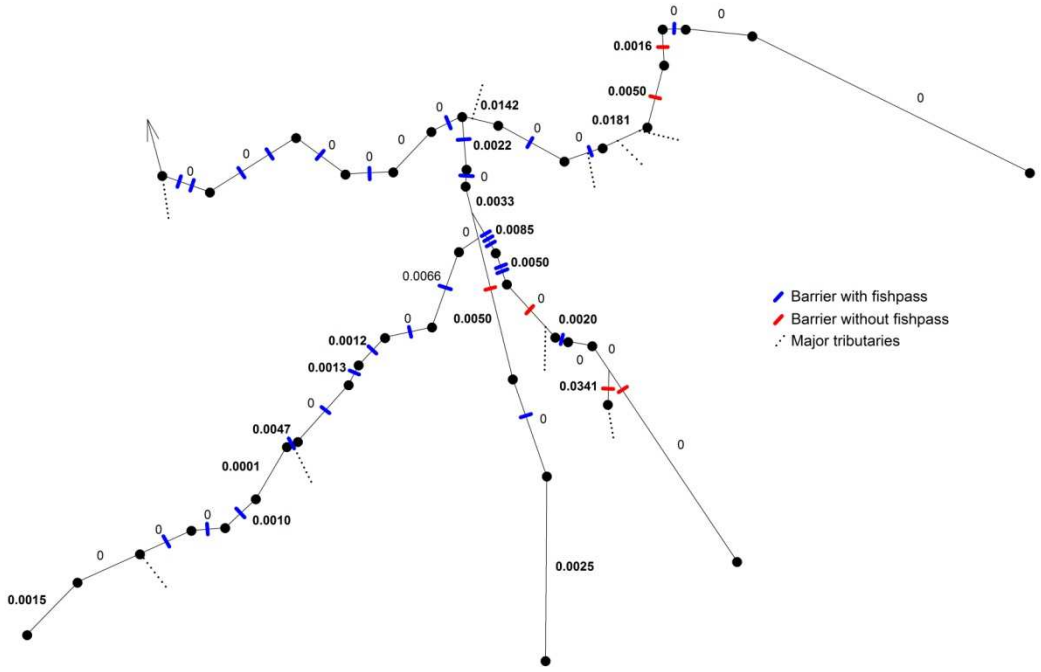

Figure 3 Genetic distance according StreamTree is mapped onto stream sections between the sampling sites. On the river network are sampling sites marked by black dotes, barriers with fishpass as blue bars, without fishpass as red bars, inflow major tributaries are symbolized by dashed lines and the numbers are the genetic distance values.

Figure 4 illustrates the role of barriers. Allelic richness is better predicted by the number of barriers ($\beta = -0.069$, $R^2 = 0.307$, $F_{1,45} = 19.924$, $P < 0.001$) than by distance ($\beta = -0.004$, $R^2 = 0.096$, $F_{1,45} = 4.789$, $P = 0.034$), and this is to a large extent due to the samples from the river Limmat (Fig. 4, purple symbols). These are relatively close to the downstream sites of the Rhine river but separated by many

barriers (Fig 1), including two without fishpasses (see Fig. 2C). Note that the river Limmat also produced the lowest estimates of N_e.

Table 4 Results of the model selection procedure based on AIC to assess the relative support of six candidate linear regression models predicting allelic richness (*AR*) of chub in the Rhine drainage as a function of distance upstream from the most downstream point of the Rhine in Switzerland and the number of differently categorized barriers along this distance.

Model		Slope	R^2	AIC	Δ AIC	AIC weight
i)	distance	-0.004*	0.096	88.758	26.543	0.000
ii)	distance	0.009**	0.440	68.277	6.062	0.041
	all barriers	-0.151***				
iii)	distance	0.009**	0.481	66.698	4.483	0.091
	barriers with fish bypass	-0.152***				
	barriers without fish bypass (including Rhine falls)	-0.322**				
iv)	distance	-0.004*	0.153	87.726	25.511	0.000
	Rhine falls	0.478				
v)	distance	0.009**	0.440	70.273	8.058	0.015
	Rhine falls	-0.134				
	all barriers (excl. Rhine falls)	-0.150***				
vi)	distance	0.005	0.548	62.215	0.000	0.853
	Rhine falls	0.349				
	barriers with fish bypass	-0.109**				
	barriers without fish bypass	-0.544***				

* indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$

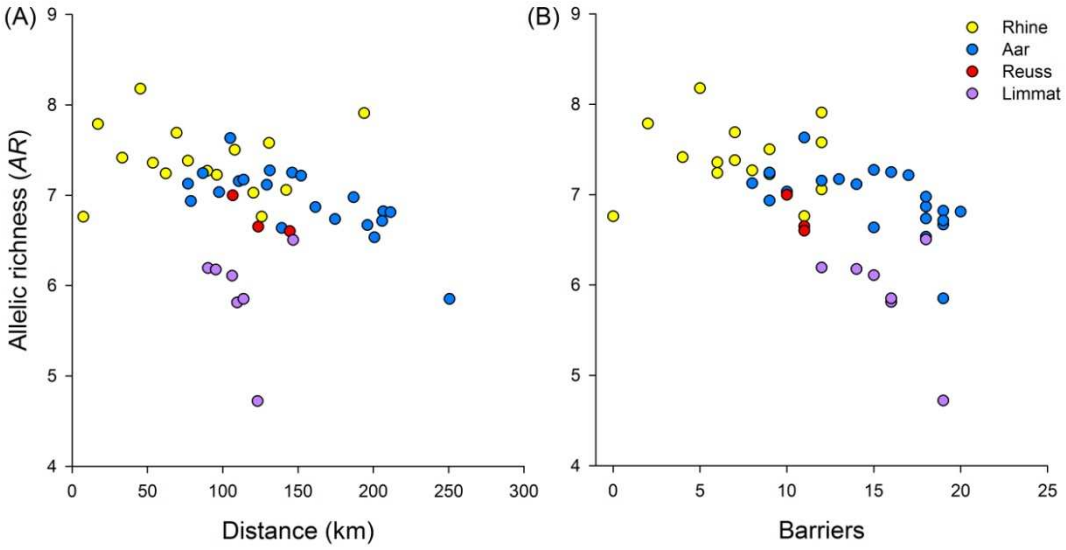

Figure 4 Plots of allelic richness against waterway distance (A) and the number of barriers (B) between the point where the Rhine river leaves Switzerland and the sampling sites. Symbol colours indicate rivers: yellow: Rhine; blue: Aar, red: Reuss, purple: Limmat**.**

Discussion

Genetic differentiation in a fragmented drainage

As expected for a mobile and abundant fish, the overall genetic differentiation of chub populations in the Swiss lowland rivers was low, with a global F_{ST} of only 0.028, and a large-scale genetic structure shaped by drainage topology. The four genetic clusters identified by Bayesian clustering analysis corresponded well to the chub populations in the four rivers contributing to the drainage (Fig. 1). Similar observations were made in other fish species like bullhead (Junker et al. 2012), bluehead sucker (Hopken et al. 2013) or brook trout (Kanno et al. 2011). We further observed significant effects of migration barriers on the chub's genetic structure. The Rhine Falls as the only long-standing obstacle to migration seem to have a marked effect, but hydroelectric power stations and weirs also influence genetic differentiation detectably. Anthropogenic barriers thus contribute to the population genetic structure of the chub as also

documented in other river-dwelling fish species (Meldgaard et al. 2003; Hanfling and Weetman 2006; Leclerc et al. 2008; Raeymaekers et al. 2008; Blanchet et al. 2010; Dehais et al. 2010; Faulks et al. 2011; Horreo et al. 2011; Junker et al. 2012; Stelkens et al. 2012). Importantly, stations equipped with fishpasses impair population connectivity less than those without fishpasses. Depending on the model used (see Table 2), we estimated that isolation by barriers equipped with fishpasses on average corresponds to about 12 km (model iii) or 20 km (model vi) of uninterrupted waterway distance, markedly less than by barriers without fishpasses, estimated as 102 km and 114 km, respectively. For comparison, in a study of chub in the French river Durance, it was estimated that each dam adds a virtual distance of 34 km in terms of genetic differentiation (Dehais et al. 2010). An important additional insight from the present study is that fishpasses do indeed mitigate the negative effects of hydroelectric power stations on population connectivity, but that they do not annihilate this effect. The effect of barriers with fishpasses was significant in both of the best-supported models predicting pairwise F_{ST} (Table 2). Notably, this concerns a fish that due to its size and swimming ability is predisposed to traverse fishpasses readily, and that occurs in huge populations (Zaugg et al. 2003). The chub is indeed the only fish species that has been observed to pass all fishpasses present in our study area (Guthruf 2006, 2008). Thus, the negative effects on population connectivity of dams and hydroelectric power stations – even when equipped with fishpasses – are likely to be more pronounced for many other fish species having lower dispersal abilities. That the permeability of many fishpasses in Switzerland is insufficient was noted by Guthruf (2006), because not all size classes of fish and species were able to pass. A recent observation from the hydroelectric power station in Rheinfelden on the Rhine river (the first barrier upstream of site R30 in Fig. 1) supports the notion that

most fishpasses are not as effective as they could be. In 2010, that is during our sampling campaign, this power station was equipped with a new fishpass of an improved design (a more or less naturally structured fishpass stream with high discharge). Counts revealed an upstream migration of approx. 40'000 fish from 33 species in one season, including species known as poor swimmers such as bullheads (*Cottus gobio*) (Energiedienst/PFA 2013). This is approximately four times more individuals than in the best-frequented of the other fishpasses in our study area (Guthruf 2006, 2008), illustrating that there is indeed much room for improvement of the existing structures aiding fish migration across artificial barriers.

Specific recommendations for particular barriers are more difficult to make because the shallow population structure overall required a global analysis to detect their influence on population structure. Nevertheless, inspection of Figure 2 suggests that some barriers such as the fishpass-free power station at the bottom of the river Reuss (Fig. 2D) or the most upstream weir without fishpass in the river Limmat (Fig. 2C) are particularly influential. Their equipment with a fishpass would thus likely have a substantial positive effect on longitudinal connectivity of fish populations, and possibly also on effective population size, which tended to be lower in the river Limmat compared to other rivers. However, it is important to consider whether such measures would not conflict with other conservation goals. Nowadays we are in the unfortunate situation that man-made barriers can be the only thing protecting upstream reaches of a river from the invasion of non-indigenous species such as the Black Sea gobies or the North American crayfish species currently expanding along the Rhine river (Leuven et al. 2009; Mombaerts et al. 2014), or to shelter autochthonous from stocked fish populations (Fausch et al. 2009). In this context, re-establishing the longitudinal permeability of river networks for aquatic organism to a

state prior to human influence may not be desirable.

The STREAMTREE model suggested that unsampled tributaries may also contribute to the genetic differentiation of chub along the main rivers we sampled. Considering that Bayesian clustering distinguished the populations from the four largest rivers of the drainage rather well (Fig. 1), it is reasonable to assume that populations from smaller rivers of the same drainage would also exhibit some genetic differentiation. Their influence is likely to act in combination with barriers to migration. If fish from such tributaries disperse into the main river but contribute predominantly to populations downstream of the confluence because obstacles prevent upstream movement, it is easy to see how river sections receiving major tributaries affect differentiation along the main stream and are assigned larger genetic distances by the STREAMTREE algorithm.

Genetic diversity

An upstream decline in genetic diversity is generally expected in organisms inhabiting a dendritic river network because of the accumulation of allelic diversity below confluences of tributaries containing genetically differentiated populations (Morrissey and de Kerckhove 2009; Paz-Vinas and Blanchet 2015). That is indeed what we observed when we quantified genetic diversity as allelic richness. However, our analyses indicated that the upstream decline of allelic richness is exacerbated by man-made barriers. This was particularly obvious from the comparatively low allelic diversity of chub from the sampling sites along the river Limmat, which are relatively close to the most downstream sites we considered but separated by many barriers (Fig. 2C). Note that based on the (uncertain) estimates we obtained with NEESTIMATOR, N_e of chub in the Limmat might also be lower than in the other

rivers (Table 1).

In the best-supported model predicting *AR*, which accounted for the negative effect of man-made barriers, the parameter estimate for distance was in fact slightly (and non-significantly) positive (Table 4). A possible explanation for this observation is that the midland rivers we studied all pass through large lakes upstream of our sampling sites. We suspect that the large lake populations of chubs may act as a reservoir of allelic diversity that feeds into the lowland rivers from above and partially compensates for the upstream loss that is otherwise observed along these fragmented rivers. This conjecture is tentatively supported by the two population samples we obtained from Lake Constance (R44 in Fig. 1) and Lake Zurich (L28 in Fig. 1). They are represented in Figure 4A by the most upstream yellow and purple points, respectively, which show a high allelic diversity compared to the general trends along the rivers Rhine and Limmat.

Conclusions

We show that the chub in the Swiss lowland rivers has a shallow population structure that is shaped by drainage topology, but also significantly affected by man-made barriers to migration, most of which are hydroelectric power stations. From a management perspective it is important to note that the fishpasses installed at many of these stations do indeed improve fish population connectivity across the barrier. Nevertheless, even barriers with fishpasses have a detectable effect on genetic differentiation, in a fish that is relatively large and mobile and copes equally well with lotic and lentic environments, i.e. a species that should be among those least susceptible to habitat fragmentation. The negative effects of river fragmentation are likely to be more severe for many other river-dwelling fish species. Fortunately,

improvements in the design of fishpasses show great promise in reducing these effects further, as seen for the Rheinfelden power station mentioned above. It is to be hoped that similar improvements will soon be realized for other barriers as well.

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Data accessibility

Data available at Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.n41nk

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Supplementary Information

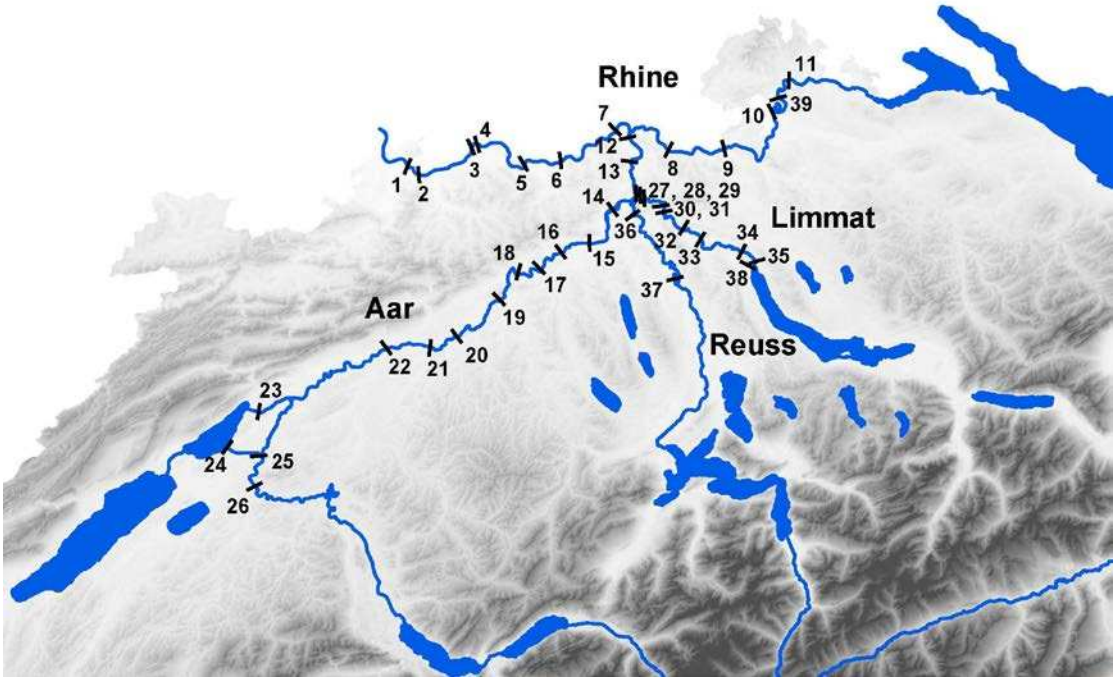

Figure S1 Map showing the location of all barriers included in Supplementary Table S1.

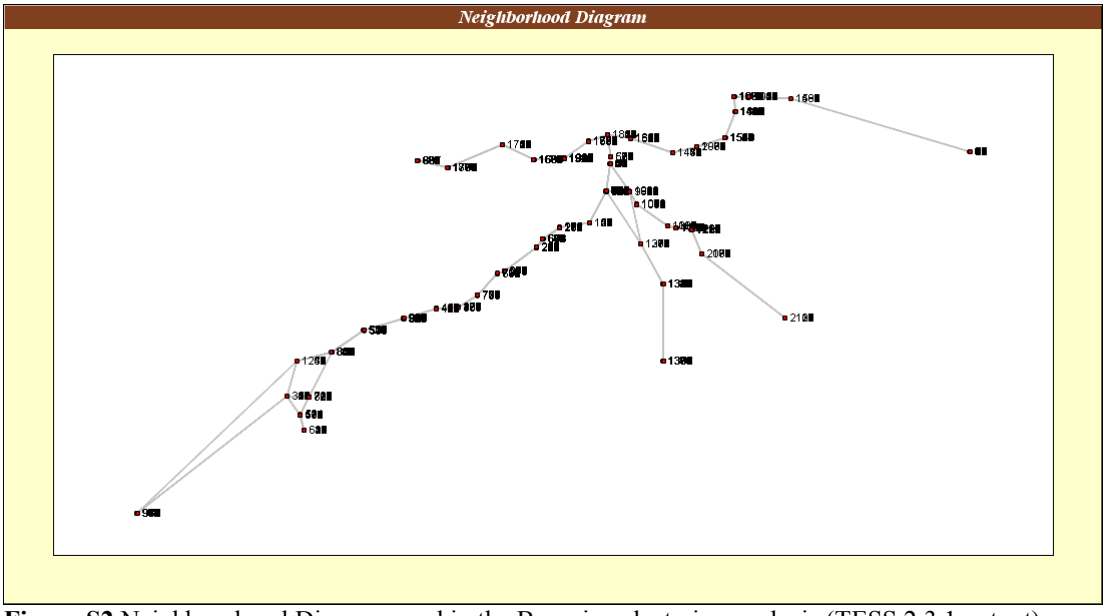

Figure S2 Neighbourhood Diagram used in the Bayesian clustering analysis (TESS 2.3.1 output).

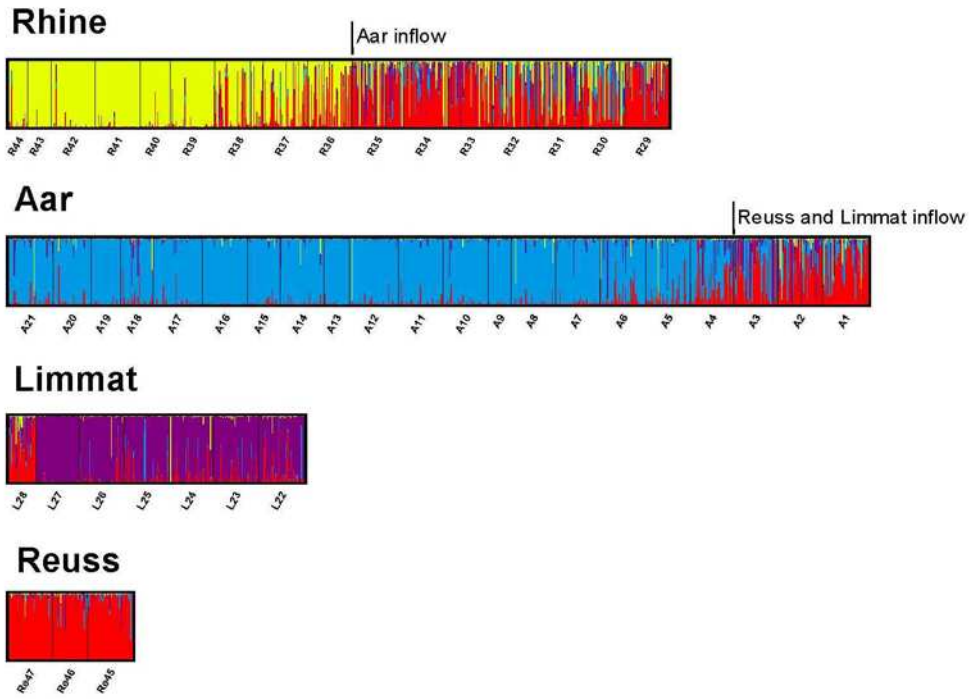

Figure S3 Genetic cluster affiliations of individual fish estimated at K_{max} = 4 in TESS 2.3.1 and visualized with DiSTRUCT.

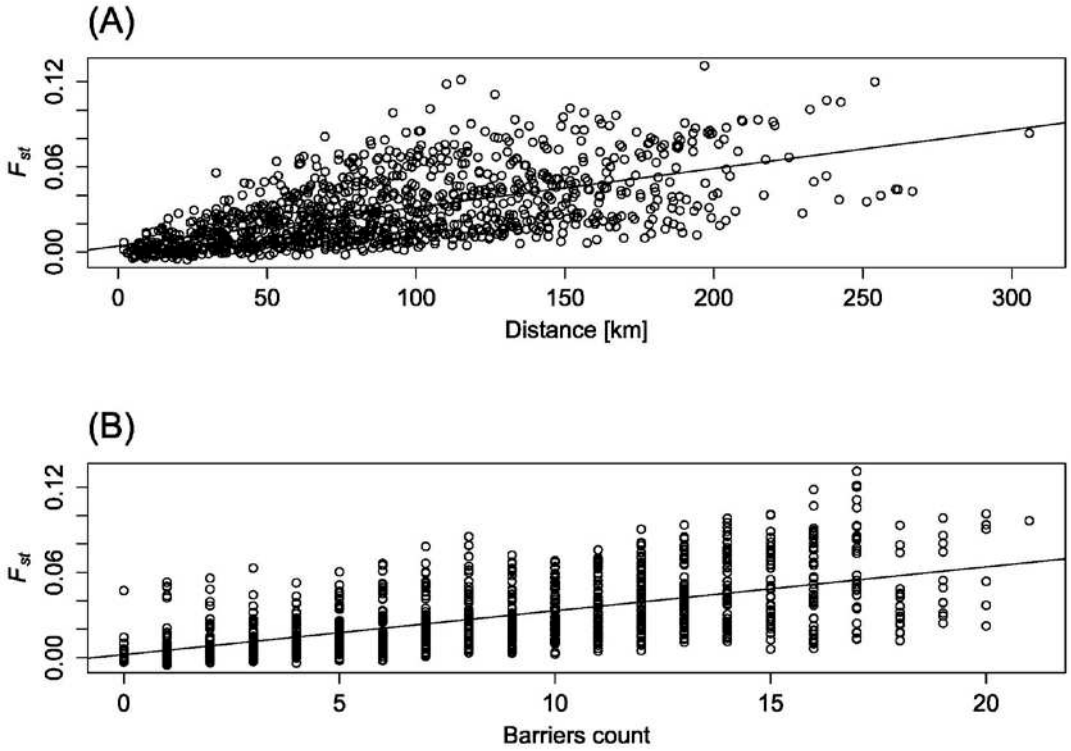

Figure S4 Isolation-by-distance and 'isolation-by-barriers' of chub in the Swiss Lowland rivers depicted as linear regression plots of pairwise F_{ST} against waterway distance (A) and the number of barriers (B) between sampling site

Table S1 Table with age of the hydropower stations, the bypasses, the weir and the Rhine Falls. The numbers correspond to the barrier numbering in supplementary

Hydropower station number	launch of electricity production	bypass since	comments
1	1955		no publicly information about bypass building year available
2	1912	1912	
3	1898	1898	
4	1931	< 1959	
5	1966	1966	
6	1914	1914	
7	1933	1933	
8	1941	1941	
9	1920	1920	
10	1956	no	bypass next 10 years
11	1964	1964	
12	1935	1952	
13	1902	1902	
14	1953	1953	
15	1945	1949	
16	1929	1929	
17	1893	1893	
18	1917	1917	
19	1896	1896	
20	1896	1896	
21	1970		no publicly information about bypass building year available
22	1970	1982	
23	1939		no publicly information about bypass building year available
24	1900		no publicly information about bypass building year available
25	1968	1995	
26	1963		no publicly information about bypass building year available
27	1861		no publicly information about bypass building year available
28	1902		no publicly information about bypass building year available
29	1896		no publicly information about bypass building year available
30	1892	2006	
31	1909	1909	
32	1933	no	2010 bypass after sampling
33	1933	1933	
34	1898		no publicly information about bypass building year available
35	1877	no	2010 bypass after sampling
36	1830	no	bypass next 10 years
37	1975	1975	
Weir			
38	1927	no	block ramp next 10 years
Rhine Falls			
39	formed 14'000 to 17'000 years ago		

Chapter 2: River fragmentation and fish population structure: a comparison of three Swiss midland rivers

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Abstract

Anthropogenic river fragmentation has the potential to alter fish population structure and diversity, but it can be difficult to infer whether observed effects reflect the obstruction of fish movement or population responses to altered flow regimes or habitat structure concomitant with fragmentation. We addressed the influence of habitat fragmentation on the European Chub (*Squalius cephalus*), a large-bodied fish with strong swimming ability and a ubiquitous habitat generalist that occurs at high densities in lentic and lotic habitats. European Chub populations have never been stocked and are little affected by habitat alterations. Thus, they are good sentinels for the effects of fragmentation per se. We used microsatellite genotyping of 1726 fish from 38 sites to compare the genetic structure and diversity of European Chub populations from 3 Swiss midland rivers that are unequally affected by man-made fragmentation. The Thur is unfragmented, the adjacent Glatt is strongly fragmented by tall barriers, and the Broye is fragmented by a large number of low barriers that are individually traversable. European Chub from the Thur possessed the highest allelic diversity overall and showed no significant isolation-by-distance and only a weak nonsignificant upstream decline in genetic diversity. European Chub from the Glatt were less genetically diverse and showed significant isolation-by-distance that was explicable by existing barriers and the presence of a lake in the system and a steep upstream decline of allelic diversity. European Chub from the Broye showed no genetic substructure, suggesting that barriers of low height, even when numerous, had little effect on population connectivity. The detectable effects of anthropogenic fragmentation of rivers on a highly mobile fish like the European Chub suggests that less mobile species, such as habitat specialists or small benthic species, are potentially more vulnerable to isolation.

Introduction

Pressure on river landscapes is enormous in areas with high human population density. Demand for agricultural land or building grounds and their protection from flooding have necessitated river corrections with a concomitant loss of floodplains, and regulated rivers are frequently interrupted by hydroelectric power stations, dams, and weirs. Switzerland is a case in point: 95% of floodplains have been lost (Tockner and Stanford 2002), and Swiss streams and rivers are fragmented by >100,000 artificial barriers (Zeh Weissmann et al. 2009).

Habitat fragmentation is considered a key threat to fish populations worldwide (Vörösmarty et al. 2010). The most obvious reason is that barriers interrupt migratory life cycles, and most globally and locally threatened species have diadromous life histories (Penczak et al. 1998, Kirchhofer et al. 2007, Limburg and Waldman 2009, Liermann et al. 2012). In Switzerland, for example, the Atlantic salmon has been considered extinct since the 1950s because its migration route along the Rhine is obstructed by numerous barriers (Gerster 1991). Barriers also affect the distribution of nonmigratory fish species in rivers (McLaughlin et al. 2006), and they may diminish river restoration potential by hampering recolonization (Stoll et al. 2013). Even without migration to or from the sea, some fish species require up to 100 km of river to complete their entire life history (Fausch et al. 2002).

The genetic effects of habitat fragmentation on riverine fish are another important concern. Reduced gene flow among populations is expected to result in reduced within-population genetic diversity, which may eventually affect the ability of populations to respond to environmental change (Stockwell et al. 2003). Construction of migration barriers tends to be accompanied by changes in flow regime and habitat characteristics. Thus, inferring whether reduced population connectivity and loss of

genetic variation are a consequence of physical obstruction to dispersal or of the creation of inhospitable habitat can be difficult. For example, construction of reservoirs may isolate fish populations among streams that feed into the reservoirs even without physical barriers because the lentic habitat represents a barrier to dispersal by fishes adapted to lotic conditions (Hudman and Gido 2013, Fluker et al. 2014). Impoundments also may degrade or reduce access to specific habitats required by specialists. Man-made structures affect population differentiation and genetic diversity of the endangered Macquarie Perch in southeastern Australia (Faulks et al. 2011), but whether the structures acted by obstructing dispersal or by reducing availability and access to riffle habitat required by this species was not clear. Isolation by habitat alteration is a lesser issue for habitat generalists, like the European Chub (*Squalius cephalus*), which therefore, is a suitable sentinel for the effects of physical obstruction to dispersal per se. The European Chub has coped well with the massive alteration of river habitats taking place in the densely populated areas of Switzerland and is highly abundant in virtually all lotic and lentic waters of the Swiss midland. However, like any other fish, it is likely to be affected by physical barriers to migration. The European Chub is of no commercial interest. Its population and genetic structure are likely to be affected by habitat fragmentation, but any effects are unconfounded by stocking. We used this situation as an opportunity to investigate how man-made obstacles to migration affect the genetic population structure and diversity of fish populations. We sampled European Chub along 3 rivers in the Swiss midlands that differ in their level of anthropogenic fragmentation, and we genotyped them based on microsatellites to compare population connectivity and genetic diversity among the 3 rivers.

METHODS

Study species

The European Chub is distributed across Europe from the Pyrenees to the Urals, excluding the Iberian, Italian, and Greek peninsulas. Its northern distribution extends to ~56°N (Kottelat and Freyhof 2007). It occurs at relatively low altitudes (typically <800 m asl), where it is a habitat generalist that occupies lotic and lentic habitats (Zaugg et al. 2003). European Chub grow to an average maximum length of 50 cm and reach maturity after 2 to 3 y. Spawning takes place in spring on gravel substrate. As fractional spawners, European Chub spawn more than once during a season (Kottelat and Freyhof 2007). In a radio-tagging study at the River Spree in Germany, females spawned twice after spawning migrations of up to 16 km, and some females changed spawning grounds between the 1st and 2nd spawning event (Fredrich et al. 2003). In the French River Meuse, rapid spawning migrations of up to 25 km were observed, suggesting that European Chub are powerful swimmers (De Leeuw and Winter 2008).

Study rivers

We investigated European Chub populations in 3 rivers of the Swiss midlands. The Broye is in the western part of Switzerland, and the Glatt and Thur are in the eastern part (Fig. 1A, B). All 3 rivers are channelized over much of their length, but they differ in the degree of anthropogenic fragmentation. The Glatt and Thur are adjacent tributaries to the Rhine. Their confluences with the Rhine are 14 km apart (riparian distance). Our choice of these 2 rivers enabled us to make the most direct comparison to assess fragmentation effects because the Thur is unfragmented by any migration barriers along the ~80 km we investigated, whereas the Glatt is heavily fragmented by

a total of 35 barriers (Fig. 1C). European Chub cannot transverse most of these barriers in the upstream direction except for twelve 50 to 100-cm barriers that may be traversable when water levels are high. The Broye differs from both the Thur and the Glatt in that it is heavily fragmented by 35 weirs of low height (40–50 cm) and one small natural drop. European Chub can pass each of these barriers, but the barriers may exert a cumulative effect on population connectivity (Fig. 1B). The Thur is the largest of the 3 rivers (average discharge = 47 m^3/s), followed by the Broye (11.50 m^3/s) and the Glatt (8.42 m^3/s). For comparison, the Rhine has an average discharge of 441 m^3/s where it is entered by the Thur and the Glatt.

The upper part of the heavily fragmented Glatt drainage is somewhat complex in structure (Fig. 1C). It includes Lake Greifen (8.45 km^2), which feeds the Glatt and 2 main tributaries named Aabach-U (1.60 m^3/s) and Aabach-M (1.04 m^3/s) (U and M stand for the towns Uster and Mönchaltorf, which the 2 streams traverse). The smaller Lake Pfäffikon (3.3 km^2) east of Lake Greifen drains into Aabach-U and, thus, also is connected to the Glatt. Anthropogenic fragmentation of the Glatt system began in 1822 with the construction of the most downstream barrier (Fig. 1C), and increased gradually with the construction of mills and several flood-protection projects, including numerous weirs, during the 19th and 20th centuries (Vischer 2003). The only natural barrier (age unknown) is the uppermost, just below Lake Pfäffikon (Fig. 1C).

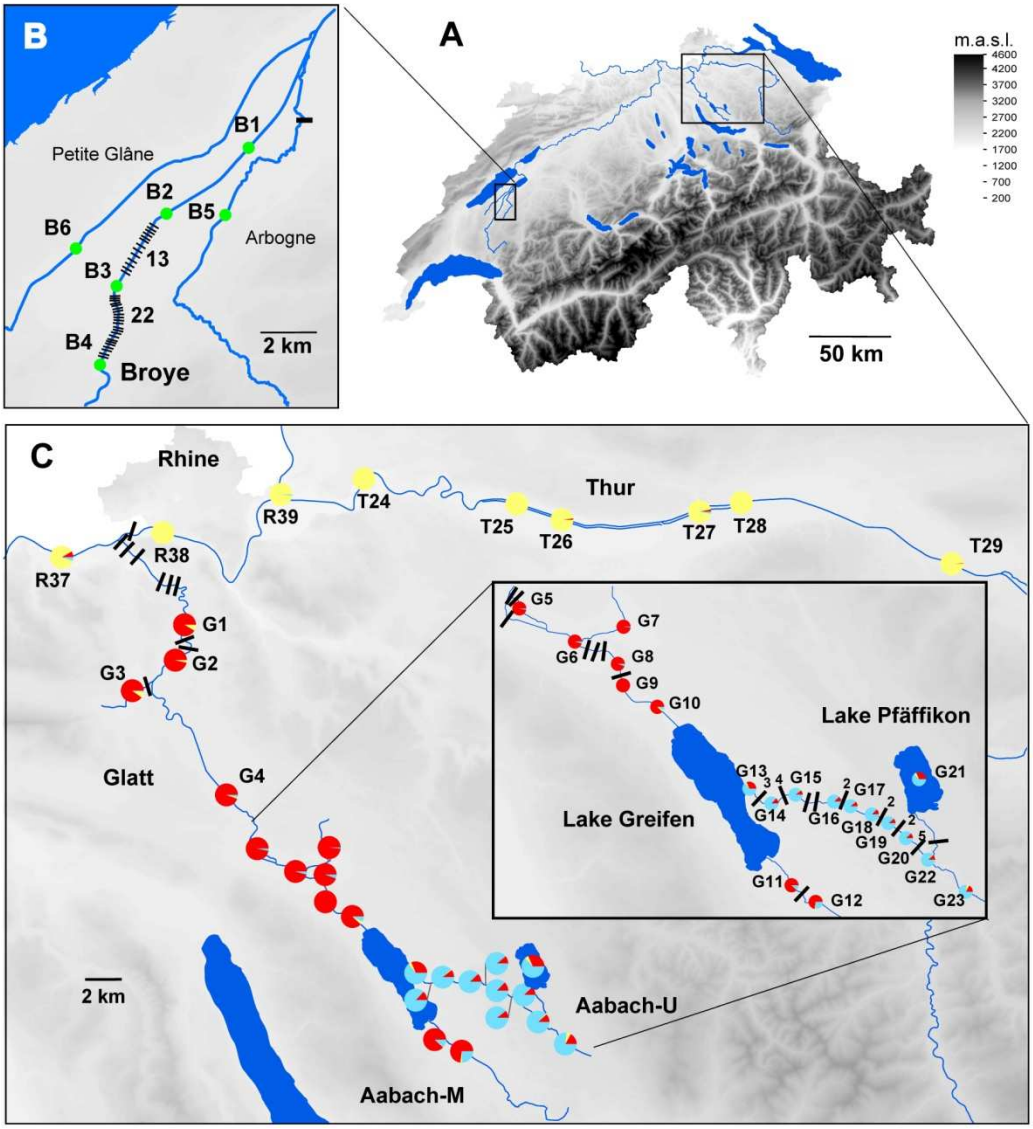

Figure 1 Map of Switzerland indicating the locations of the river systems studied (A) and details of sampling sites in the Broye (B) and the Thur and Glatt systems. In panel A, the thin bars represent fragmentation by a series of barriers of low height (40–50 cm) with the exact number indicated to the right. The only natural barrier is indicated by the wider black bar. In panel C, the thin bars represent single barriers or multiple adjacent barriers when accompanied by a figure indicating their number. The only natural barrier is between sites G21 and G22, a natural drop in terrain that is also obstructed by a hydroelectric power station. Colored sectors in pie charts represent the average assignment probability of European Chub from a given sampling site to each of the 3 genetic clusters inferred with a Bayesian clustering analysis done with the software TESS (see Results). Labelling of sampling locations corresponds to Table 1.

Sampling procedure

We used European Chub from 38 collection sites, including 23 from the Glatt system, 9 from the Thur and Rhine, and 6 from the Broye (Table 1). We caught European Chub from spring to autumn in 2010 and 2011 by electro-fishing (FEG 1700; EFKO, Leutkirch, Germany), except for sites G9 and G21 (Fig. 1C), where rod and line were used. We anaesthetized fish lightly with clove oil before cutting ~1 mm^2 of the caudal fin as a tissue sample. After recovery, we released all fish at the place of catch. We stored fin clips in 99% EtOH until use. Sample sizes ranged from 19 to 53 individuals with an average of 45 fish/site.

Microsatellite genotyping

We followed the salting-out protocol of Sunnucks and Hales (1996) adapted to a 96-deep-well format (Gouskov et al. in press) to extract DNA from fin clips. We used 10 microsatellites amplified in 2 multiplex reactions to genotype individuals: LC128, LC27, LC290, LC32, LC93 (Vyskocilova et al. 2007), LceA149, LceC1, LceCb (Larno et al. 2005), N7G5, and N7K4 (Mesquita et al. 2003). Detailed protocols are available in Gouskov et al. (in press). We visualized the polymerase chain reaction (PCR) products on an ABI 3730 capillary sequencer (Applied Biosystems, Foster City, California), and we used GeneMapper® software (version 4.0; Applied Biosystems) to score them. We used Micro-Checker (Van Oosterhout et al. 2004) to test for the possible occurrence of null alleles. Locus LceCb showed evidence of null alleles at the 4 most downstream sites of Thur and Rhine. Therefore, we excluded this locus from all analyses. For the final data set, we considered only individuals with missing data at ≤1 of the remaining 9 loci (1726 genotypes, 32 with 1 missing locus).

The numbers of individuals fulfilling this criterion at each site are provided in Table 1.

Descriptive population genetic analyses

We used the software FSTAT (version 2.9.3.2; Goudet 2002) to calculate expected (H_E) and observed (H_O) heterozygosities and to test for deviations from linkage and Hardy–Weinberg equilibria. We also used FSTAT to calculate allelic richness (AR) standardized for the smallest sample size (n) and F-statistics (Weir and Cockerham 1984). The size of the rivers and their permeability to fish migration are expected to affect the effective population size N_e. We used the point-estimation method based on linkage disequilibrium (NeEstimator, version 2.0.1; Do et al. 2014) restricted to alleles with frequencies >0.02 as recommended by Do et al. (2014) to compare rough estimates of N_e without having temporal samples. We obtained confidence intervals with the jackknife method of Waples and Do (2008).

We used the Bayesian clustering approach implemented in TESS (version 2.3.1; Chen et al. 2007) to obtain a general overview of the genetic structure of European Chub populations. We used this analysis independently for the samples from the Broye and for all samples from the Glatt and the Thur together because these rivers are connected by the Rhine (3 Rhine samples also were included; Fig. 1C). TESS offers the possibility to include the network position of individuals as a spatial prior on cluster membership on the basis of hidden Markov random fields (HMRF) (Francois et al. 2006). This approach can account for the fact that in a dendritic system, individuals are restricted to move along the waterway to the next population. The spatial relationships among individuals are represented in TESS by a neighborhood system, which requires geographic coordinates for each genotype. We obtained these

by using the coordinate creator implemented in the software and sampled randomly from a normal distribution around the initial coordinate of the population sample with a standard deviation of 15 m. The initial neighborhood system was generated automatically with Voroni tessellation (Guillot et al. 2009) and had to be modified manually with the neighborhood modifying option to reflect the actual river networks. We set the spatial interaction parameter ψ to the default value of $\psi = 0.6$ for all analyses. We ran the no-admixture model 20 times for each potential number of clusters (K_{max}) with K_{max} = 2 to 6 for the Broye and K_{max} = 2 to 9 for the Thur–Glatt system, with 250,000 sweeps after a burn-in of 50,000 sweeps for each run. The best-supported number of clusters was determined by plotting the mean deviance information criterion (DIC) of the 20 runs against K_{max} and choosing the value at which the DIC curve reached a plateau (TESS manual). We processed TESS outputs with CLUMPP (version 1.1.2; Jakobsson and Rosenberg 2007) to account for label switching, and we visualized cluster membership of individuals thereafter with DiSTRUCT (Rosenberg 2004).

Effects of fragmentation on genetic differentiation

Reduced population connectivity caused by fragmentation should result in a steeper increase of genetic differentiation with distance along the river (isolation-by-distance [IBD]). Therefore, for each river, we calculated the matrices of pairwise F_{ST} among sampled subpopulations in FSTAT. As recommended for populations along linear habitats, we regressed $F_{ST}/(1 - F_{ST})$ on untransformed riparian distance (Rousset 1997) and used Mantel tests to assess whether isolation-by-distance (IBD) was significant for each river. For the 2 barrier-interrupted rivers (Broye and Glatt) we also carried out partial Mantel tests to assess whether the number of barriers between sites had a

significant effect on pairwise differentiation after controlling for riparian distance. Last, we used an Akaike Information Criterion for small sample size (AICc)-based model-selection approach (Burnham and Anderson 1998) to identify the most parsimonious models explaining the genetic structure along the 2 fragmented rivers. We constructed linear models containing all additive combinations of the predictors, distance and barriers, on linearized F_{ST} (i.e., $F_{ST}/[1 - F_{ST}]$), including an intercept-only model, and compared their AICc values. We used the number of sampled populations rather than the number of pairwise combinations as N in the calculation of AICc to account for the nonindependence of pairwise data (Koizumi et al. 2006). The Bayesian clustering in TESS suggested a possible effect of Lake Greifen on genetic structure, so we included the presence or absence of the lake between 2 sampling sites as an additional factor in the set of models predicting pairwise differentiation along the Glatt system.

Effects of fragmentation on genetic diversity

Some upstream decline of genetic diversity is a general trend in dendritic landscapes (Morrissey and de Kerckhove 2009), but severe fragmentation is expected to exacerbate this decline by cutting off more-upstream populations from the supply of new alleles by immigration. We quantified genetic diversity as *AR* in FSTAT and analyzed its upstream trend in all 3 river systems. For the Thur/Rhine, this analysis extended from sampling site R38 to T29 ($n = 8$) (Fig. 1C), and for the Glatt it extended from site R37 to G23 ($n = 24$). For the Broye, the analysis included sites G1 to G6, but because they are spread over 3 arms of the river, we arbitrarily selected the lowest confluence as the point from which upstream distances were measured. To compare genetic diversity and its upstream trend among rivers, we ran a linear model

on *AR* that included river as a factor and riparian distance upstream as a covariate, and we used the river-by-distance interaction as a test of whether the upstream trend of *AR* differed among rivers. For the most densely sampled and most strongly fragmented Glatt system, we used AICc-based model selection to identify the most parsimonious model predicting *AR* in a set of candidate models comprising all additive combinations of the predictors upstream distance, barriers, and lake. In this case, the factor, lake, expressed whether or not a sample was from or directly connected to 1 of the 2 lakes in the Glatt system; i.e., distinguishing samples G10, G11, and G13 (Lake Greifen, outflow of Glatt, inflows of Aabach-M and Aabach U), and G21 (Lake Pfäffikon) from all other samples.

RESULTS

Microsatellite variation and effective population size estimates

The 9 microsatellite loci analyzed were highly variable and comprised 148 alleles, ranging from 3 to 40 alleles/locus. We found no strong evidence for linkage among loci. Global linkage disequilibrium (LD) was significant for 2 pairs of loci after Bonferroni correction, but the patterns across populations were very inconsistent, and the vast majority of populations showed no evidence of LD at these locus pairs. Therefore, we considered the loci as unlinked in all analyses. Deviations of observed from expected heterozygosities were small in all populations (F_{IS}; Table 1), such that Hardy–Weinberg conditions were largely met. Only 1 comparison was marginally significant at $p < 0.05$ (heterozygote deficit at site T24), and none were significant after Bonferroni correction (Table 1).

Estimates of N_e were variable but generally high with very wide confidence intervals (Table 1). For 8 population samples, N_e was estimated as infinite and the upper bound

of the 95% confidence interval (CI) reached infinity in most cases. Under these circumstances, the lower bound of the CI may be the most informative parameter estimated, providing plausible limits of N_e (Waples and Do 2010). Excluding the Rhine, where only 3 sites were sampled, these lower bounds differed significantly among rivers (Kruskal–Wallis test, $p = 0.028$), following the order Thur (median lower bound = 252) > Broye (99) > Glatt (58).

Genetic clustering

For European Chub from the Broye, Bayesian clustering analysis using TESS did not provide any evidence for distinct genetic clusters. Mean DIC declined from $K = 2$ toward higher values of K, but at $K = 2$, most individuals could not be assigned to one of the clusters with high confidence. This result indicates a lack of detectable substructure.

In contrast, for European Chub from the Thur and Glatt river system, Bayesian clustering analysis indicated $K = 3$ as the most likely number of genetic clusters, and these clusters had relatively distinct geographic distributions (Fig. 1C). The 1st cluster (yellow) essentially represented the fish from the Thur and Rhine, the 2nd cluster (red) comprised the fish from the Glatt below Lake Greifen and most of the fish from the 2 sampling sites in Aabach-M above the lake, and the 3rd cluster (blue) comprised the fish from Aabach-U above Lake Greifen and the single sample from Lake Pfäffikon, although that sample appeared to show some admixture (Fig. 1C). The distinct genetic clusters present in the Glatt and Aabach-U suggest a role of Lake Greifen in restricting gene flow.

Table 1 Collection information and measures of genetic diversity for 38 samples of European Chub (*Squalius cephalus*) from the Broye (B), Glatt (G), and Thur (T) systems, including 3 sites from the Rhine (R) already reported by Gouskov et al. (in revision). N = sample size, H_e = expected heterozygosity, H_o = observed heterozygosity, AR = allelic richness standardized for the smallest sample size (19), F_{IS} = inbreeding coefficient, N_e = effective population size. The tests for deviations from Hardy-Weinberg equilibrium (HWE) used the randomization approach implemented in FSTAT (version 2.9.4). The p-value reported represents the proportion of randomizations producing a smaller F_{IS} value than observed when $F_{IS} < 0$ (heterozygote excess) and the proportion of randomizations producing a larger F_{IS} value than observed when $F_{IS} > 0$ (heterozygote deficit).

Site	Coordinates WGS84	*N*	*He*	*Ho*	*AR*	F_{IS}	HWE test	N_e estimate
G1	N47°31'51.25" E8°31'15.24"	50	0.683	0.707	6.648	–0.036	0.103	115 (69–275)
G2	N47°30'45.67" E8°30'48.33"	47	0.676	0.682	6.370	–0.009	0.378	(314–)
G3	N47°29'58.65" E8°29'34.61"	30	0.649	0.659	5.829	–0.016	0.370	41.6 (21–171)
G4	N47°26 35.40" E 8°33'03.70"	49	0.636	0.632	5.752	0.007	0.407	147 (58–)
G5	N47°24'55.33" E8°34'25.38"	50	0.624	0.612	5.435	0.019	0.250	(179–)
G6	N47°24'12.00" E8°36'09.54"	50	0.623	0.622	5.523	0.001	0.505	236 (88–)
G7	N47°24'30.04" E8°37'41.37"	45	0.623	0.615	5.357	0.014	0.335	(184–)
G8	N47°23'42.60" E8°37'28.77"	46	0.638	0.652	5.408	–0.023	0.251	338 (83–)
G9	N 47°23'15.32" E 8°37' 9.42"	24	0.637	0.638	5.081	–0.001	0.498	359 (43–)
G10	N 47°22'47.66" E 8°38'42.78"	49	0.652	0.652	6.083	0.000	0.478	148 (65–)
G11	N 47°18'57.06" E 8°42'49.33"	48	0.677	0.670	6.041	0.010	0.380	296 (96–)
G12	N 47°18'35.19" E 8°43'34.09"	50	0.674	0.678	6.111	–0.005	0.466	599 (114–)
G13	N 47°21'00.76" E 8°41'32.62"	48	0.665	0.655	5.766	0.015	0.319	115 (53–2375)
G14	N 47°20'41.81" E 8°42'14.48"	50	0.632	0.650	5.137	–0.030	0.170	87 (36–)
G15	N 47°20'52.43" E 8°42'59.48"	48	0.637	0.619	5.107	0.028	0.181	499 (70–)
G16	N 47°20'42.78" E 8°44'11.29"	31	0.630	0.660	5.223	–0.047	0.119	543 (72–)
G17	N 47°20'36.51" E 8°44'42.48"	48	0.633	0.615	5.108	0.030	0.160	72 (36–327)
G18	N 47°20'25.58" E 8°45'23.14"	47	0.627	0.623	5.011	0.006	0.414	44 (24–116)
G19	N 47°20'14.06" E 8°45'53.44"	46	0.633	0.638	5.069	–0.007	0.442	451 (52–)
G20	N 47°19'54.49" E 8°46'25.35"	49	0.633	0.659	5.293	–0.040	0.094	137 (46–)
G21	N 47°21'08.78" E 8°46'50.51"	19	0.667	0.678	6.000	–0.018	0.391	152 (27–)
G22	N 47°19'26.78" E 8°47'06.52"	45	0.639	0.625	5.208	0.022	0.228	29 (20–44)
G23	N 47°18'45.03" E 8°48'15.89"	50	0.592	0.585	4.536	0.013	0.317	32 (17–81)
T24	N 47°36'15.24" E 8°39'34.38"	44	0.719	0.686	7.559	0.046	0.041	340 (112–)
T25	N 47°35'24.65" E 8°46'29.27"	50	0.750	0.738	7.071	0.016	0.271	(467–)
T26	N 47°34'53.36" E 8°48'31.72"	49	0.730	0.740	7.402	–0.014	0.294	(413–)
T27	N 47°35'04.79" E 8°54'48.78"	49	0.728	0.719	7.062	0.013	0.322	(392–)
T28	N 47°35'19.78" E 8°56'42.84"	45	0.745	0.714	6.869	0.042	0.061	235 (85–)
T29	N 47°33'19.05" E 9°06'15.47"	26	0.732	0.714	7.132	0.025	0.261	94 (35–)
R37	N 47°33'59.08" E 8°25'43.14"	49	0.713	0.712	7.165	0.001	0.505	1106 (198–)
R38	N 47°34'42.14" E 8°30'20.53"	53	0.725	0.714	7.127	0.014	0.277	700 (154–)
R39	N 47°35'49.36" E 8°35'44.49"	49	0.669	0.655	7.390	0.021	0.205	416 (142–)
B1	N 46°52'38.94" E 6°59'40.96"	50	0.685	0.658	6.390	0.040	0.073	77 (52–135)
B2	N 46°51'24.14" E 6°57'26.66"	47	0.661	0.662	5.955	–0.001	0.478	(198–)
B3	N 46°50'02.50" E 6°56'05.61"	50	0.685	0.655	6.390	0.044	0.050	447 (121–)
B4	N 46°48'33.56" E 6°55'39.13"	49	0.673	0.696	5.878	–0.033	0.110	147 (77–)
B5	N 46°51'23.08" E 6°59'03.35"	48	0.680	0.680	6.163	0.000	0.502	203 (66–)
B6	N 46°50'44.43" E 6°54'58.48"	49	0.653	0.628	5.965	0.039	0.098	(351–)

Effects of fragmentation on genetic differentiation

A significant IBD pattern was observed only in the strongly fragmented Glatt system (Mantel $r = 0.460$, 1-tailed $p < 0.001$; Fig. 2). The Glatt system comprised 2 genetic clusters, so we also tested separately for IBD along the Glatt below Lake Greifen and along Aabach-U. Both showed significant IBD (Glatt: $r = 0.441$, $p = 0.006$; Aabach-U: $r = 0.446$, $p = 0.026$). Along the unfragmented Thur–Rhine sites, genetic differentiation was lower overall (Fig. 2) and IBD was nonsignificant ($r = 0.115$, $p = 0.293$). In fish from the spatially less extensive Broye system with low-height barriers, pairwise differentiation was extremely low. A trend toward increased differentiation with distance was not significant ($r = 0.505$, $p = 0.059$).

Further support for an association of genetic differentiation with anthropogenic fragmentation in the Glatt system was provided by the fact that we observed an even stronger correlation of $F_{ST}/(1 - F_{ST})$ with the number of barriers between sites ($r = 0.641$, $p < 0.001$), and the fact that partial Mantel tests revealed a significant effect of barriers after accounting for distance ($r = 0.510$, $p < 0.001$), but not for distance corrected for barriers ($r = 0.101$, $p < 0.116$). In the Broye, no significant correlation between pairwise differentiation and barriers was observed ($r = 0.297$, $p = 0.192$), and this correlation remained nonsignificant after correction for distance ($r = 0.309$, $p = 0.213$).

The lack of genetic structure in European Chub from the Broye was corroborated by AICc-based model selection, in which the null model fitting an intercept only was identified as the most parsimonious (Table 2). For the Glatt system, the model-selection procedure supported the importance of barriers and Lake Greifen in structuring European Chub populations. The most parsimonious model by far (AICc weight = 0.831; Table 2) was the model containing these 2 effects, and the only other

model receiving at least some but considerably less support from the data (ΔAICc = 3.187) included these 2 effects and distance (Table 2).

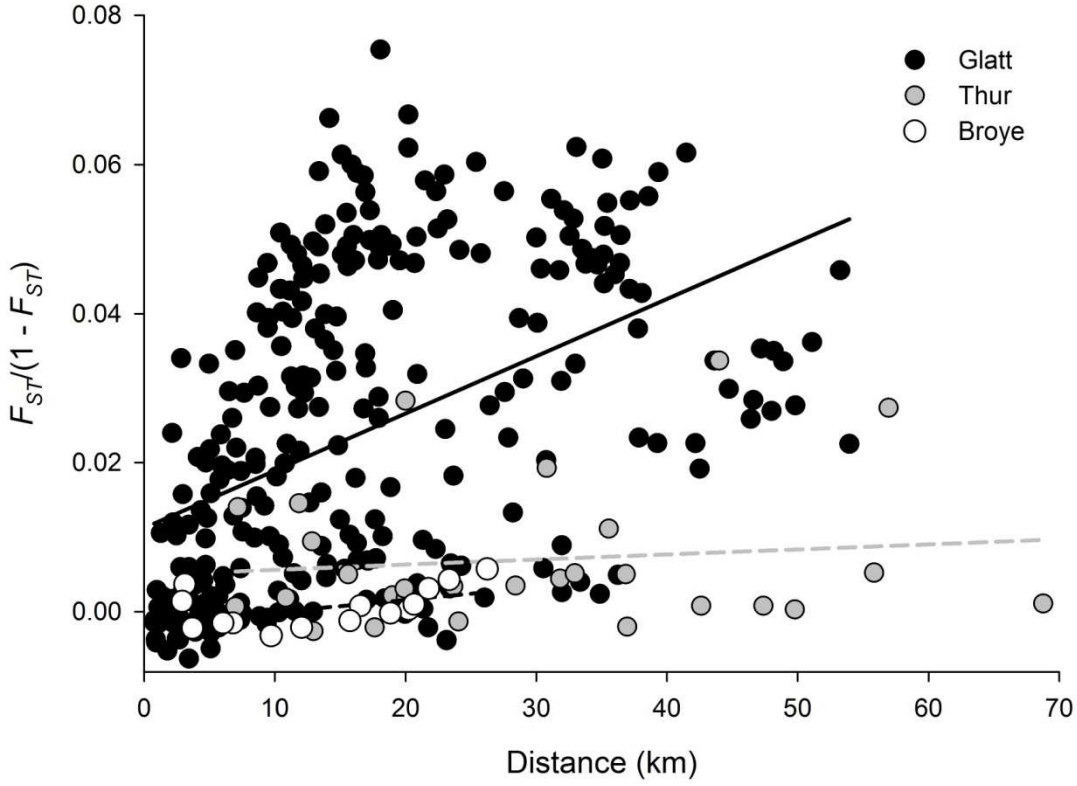

Figure 2 Isolation-by-distance plot depicting the relationship between genetic differentiation of European Chub ($F_{ST}/[1–F_{ST}]$) and distance between pairs of sampling sites for the 3 river systems. Isolation-by-distance was significant only in the Glatt system, as indicated by the continuous line.

Effects of fragmentation on genetic diversity

Genetic diversity expressed as *AR* was highest in European Chub from the large and unfragmented Thur/Rhine system and did not decline significantly upstream (β = −0.003, $R^2 = 0.132$, $F_{1,6} = 0.911$, $p = 0.377$; Fig. 3). In the Broye, the upstream decline of *AR* also was not significant (β = −0.038, $R^2 = 0.316$, $F_{1,4} = 1.848$, $p = 0.246$), but the low number of samples collected over relatively short distances precluded any

Table 2 Results of the model-selection procedure assessing the relative support for candidate models predicting the genetic structure (linearized F_{ST}) in the 2 rivers affected by anthropogenic fragmentation. The best-supported models are in bold typeface. AICc = Akaike's Information Criterion for small sample sizes, Predictors: c = constant, dist = riparian distance between 2 sampling sites, barr = number of barriers between 2 sampling sites, lake = presence or absence of Lake Greifen between 2 sampling sites.

Effects in model	Log likelihood	AICc	Δ AICc	Akaike weight
Broye				
c	**68.164**	**–128.328**	**0.000**	**0.931**
c + dist	70.369	–122.738	5.590	0.057
c + barr	68.854	–119.709	8.620	0.013
c + dist + barr	71.123	–94.246	34.082	0.000
Glatt system				
c	676.478	–1348.384	205.006	0.000
c + dist	709.339	–1411.479	141.911	0.000
c + barr	749.576	–1491.953	61.438	0.000
c + lake	743.187	–1479.174	74.216	0.000
c + dist + barr	750.998	–1491.891	61.499	0.000
c + dist + lake	751.553	–1493.001	60.389	0.000
c + barr + lake	**781.748**	**–1553.390**	**0.000**	**0.831**
c + dist + barr + lake	781.768	–1550.203	3.187	0.169

strong inference (Fig. 3). In the strongly fragmented Glatt system, a significant upstream decline of AR was observed ($\beta = -0.029$, $R^2 = 0.503$, $F_{1,22} = 22.231$, $p < 0.001$). When data from the 3 rivers were analyzed jointly, there was a significant effect of river ($F_{1,32} = 53.754$, $p < 0.001$) as well as distance ($F_{1,32} = 18.830$, $p < 0.001$), and the significant river × distance interaction indicated that the slopes of the upstream decline in *AR* differed among rivers ($F_{2,32} = 5.491$, $p = 0.009$). The 3

samples directly connected to Lake Greifen (outflow of Glatt G10, inflows of Aabach-M G11 and Aabach-U G13) and the only sample from Lake Pfäffikon (G21) in the Glatt system lay substantially above the regression line (Fig. 3), suggestive of an elevated *AR* in lakes.

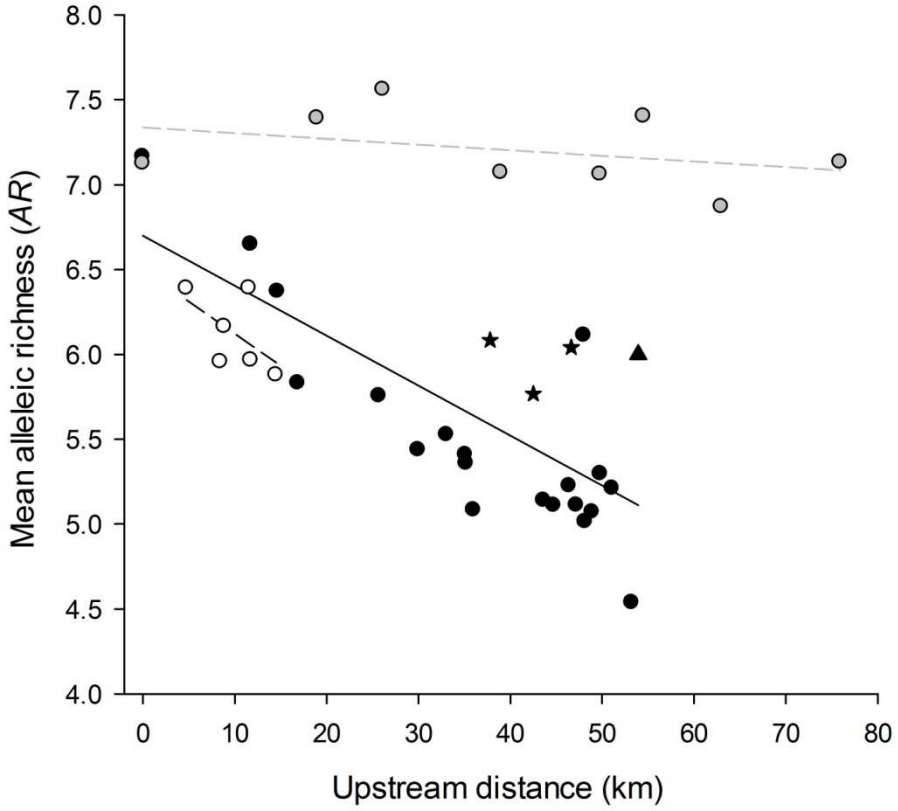

Figure 3 Upstream decline of allelic richness in the 3 river systems. Black symbols represent the Glatt system, grey symbols the Thur, and open symbols the Broye. The dashed regression lines represent nonsignificant relationships. The solid regression line is significant (see text). The black triangle represents the sample from Lake Pfäffikon, and the black stars represent the most upstream sample from the Glatt Rver and the most downstream samples from Aabach-M and Aabach U, which are not separated by any barriers from Lake Greifen.

The model-selection approach used to evaluate the different influences on *AR* in the Glatt system is summarized in more detail in Table 3. The best-supported model contained the effects of distance and lake (Akaike weight = 0.823), and the only other

model receiving any support contained the same effects and barriers. All other models containing the effect of barriers received no support. Overall, these results indicate elevated *AR* in lakes and suggest that for European Chub in the Glatt system, upstream distance from the most downstream site is a better predictor of genetic diversity than the number of barriers along this distance, but these variables are correlated ($r = 0.836$).

Table 3. Results of the model-selection procedure assessing the relative support for candidate models predicting genetic diversity (allelic richness = *AR*) in the Glatt system. The best-supported model is in bold typeface. AICc = Akaike's Information Criterion for small sample sizes; Predictors: c = constant, dist = riparian distance from most downstream site, barr = number of barriers between sampling sites and most downstream site, lake = factor indicating if the sampling site was in a lake or directly connected to a lake (sites G10, G11, G13 and G21) or not (all other sites).

Effects in model	Log likelihood	AICc	Δ AICc	Akaike weight
c	–21.373	47.317	25.576	0.000
c + dist	–12.992	33.184	11.443	0.003
c + barr	–15.233	37.666	15.925	0.000
c + lake	–20.34	47.880	26.139	0.000
c + dist + barr	–12.86	35.825	14.084	0.001
c + dist + lake	**–5.818**	**21.741**	0.000	**0.823**
c + barr + lake	–12.65	35.405	13.664	0.001
c + dist + barr + lake	–5.769	24.871	3.130	0.172

DISCUSSION

Our analysis of European Chub populations in 3 Swiss midland rivers with variable levels of anthropogenic fragmentation revealed marked differences in genetic structure and diversity. Most instructive was the comparison between the adjacent Thur and Glatt, which could be investigated over a similar length and drain into the same river (Rhine) in close proximity. European Chub from the unfragmented Thur possessed higher allelic diversity overall, showed no significant IBD and only a weak and nonsignificant upstream decline in *AR*. Fish from the strongly fragmented Glatt were less genetically diverse overall, exhibited significant IBD and a steep upstream decline of *AR.* Similar patterns were observed in European Chub from the fragmented Durance River in France (Dehais et al. 2010). However, the comparison between the Thur and Glatt was not entirely fair because the unfragmented Thur is considerably larger than the Glatt (~5× discharge; see Methods). Assuming similar habitat suitability for the European Chub, lower population sizes and, thus, a more important influence of genetic drift in the Glatt also would be expected in the absence of any differences in fragmentation. Two lines of evidence indicate that the large numbers of man-made barriers in the Glatt do indeed contribute to the observed differences. First, the partial Mantel tests and the most parsimonious predictive model (Table 2) indicate that the IBD pattern in the Glatt system largely reflects ‘isolation-by-barriers’. Second, the Broye is very similar in size to the Glatt but fragmented only by low-height barriers that are traversable by European Chub. There, the fish show much lower genetic differentiation over similar distances than in the Glatt (Fig. 2), where barriers are mostly impermeable to European Chub migration. We are convinced that the fragmentation-associated genetic structure in the Glatt system comes about by physical obstruction to dispersal rather than any unsuitable habitat created by

impoundments, because the European Chub is a habitat generalist that thrives in lentic and lotic conditions. Accordingly, it is highly abundant along the entire Glatt system. A recent study by Roberts et al. (2013) on the endangered Roanoke Logperch (*Percina rex*) in the eastern USA also found virtually free gene flow along unimpounded river distances comparable to those along the Thur and Glatt in the present study, but strong differentiation across barriers, which were hydroelectric dams unequipped with fish passes.

N_e could not be estimated with high precision, but at least the lower bounds seem to be higher in the Broye than in the similar-sized Glatt, consistent with a positive effect of connectivity on N_e (Palstra and Ruzzante 2008). Thus, low-height drops <~0.5 m, even when very numerous such as those found in the Broye, do not appear to present important barriers to gene flow for the European Chub. We acknowledge that the spatial extent of the Broye system is smaller than that of the Glatt system and this difference may have affected the opportunity to detect genetic structure. On the other hand, *AR* in the Broye is comparable to that in the similar-sized Glatt below Lake Greifen (Fig. 3), but the average level of genetic differentiation over the 0 to 25 km range is lower than in the Glatt and more similar to that in the large and unfragmented Thur (Fig. 2). This result suggests that population connectivity is little affected by the low-head barriers in the Broye. However, the same types of barriers may have a much stronger effect on other species, e.g., fishes with small body size like sticklebacks (Raeymaekers et al. 2009) or ground-dwelling species with poor swimming ability like Bullhead (*Cottus gobio*) (Junker et al. 2012).

The Bayesian clustering analyses revealed an interesting role of the 2 lakes for the genetic structure and diversity of European Chub in the highly fragmented Glatt system. That European Chub from the Glatt and from Aabach-U are mostly assigned

to 2 distinct genetic clusters (Fig. 1C) is suggestive of Lake Greifen acting as an obstacle to dispersal, even though European Chub clearly thrive in the lake and in the river habitat. On the other hand, the inflow of Aabach-M is even further away from the outflow of the Glatt, and European Chub from Aabach-M are genetically very similar to those from the Glatt. This result does not support reduced gene flow across the lake. An alternative explanation would be that lakes serve as important source populations that supply large numbers of recruits, which—at least in a highly fragmented system like the Glatt drainage—predominantly pass obstacles in the direction of flow and, thus, influence the genetic composition at downstream locations. This explanation is consistent with the observation that the distinct genetic composition of European Chub from Aabach-U is very similar to that of Lake Pfäffikon, which drains into Aabach-U (Fig. 1C). This lake is well separated from the rest of the Glatt system by a barrier that was originally natural and now is further obstructed by a hydroelecric power station exploiting the natural drop in terrain, but movement downstream from the lake by drifting European Chub larvae is still possible. Lake Pfäffikon seems to show some admixture from the genetic cluster associated with the Glatt further downstream (Fig. 1C). A feasible reason could be the occasional movement from Lake Greifen of small European Chub used as live bait by recreational fisherman. Both lakes can be fished with the same license.

Both lakes appear to act as reservoirs of genetic diversity because the sample from Lake Pfäffikon and the samples from sites directly connected to Lake Greifen showed elevated *AR* compared to river sites. This finding probably reflects that because of their size, the lakes can support very large populations of European Chub, altough the N_e estimtes obtained do not substantiate this claim (Table 1). However, these estimates come with such wide CIs that they cannot be taken as evidence against

higher N_e in lakes.

In contrast to genetic differentiation, genetic diversity (expressed as *AR*) was not clearly associated with fragmentation by barriers in the Glatt system. That distance upstream was a better predictor of *AR* than the number of barriers along this distance was largely a result of the situation in Aabach-U below Lake Pfäffikon. There, many samples collected over a short distance had very similar *AR* (sites G14–G22, the dense cloud of black circles in Fig. 3) even though they are separated by a large number of barriers along this short distance (Fig. 1C). The comparisons with the unfragmented Thur, in which the upstream decline in *AR* was very shallow and nonsignificant, suggest that fragmentation may at least contribute to the significant upstream decline in the Glatt.

Implications for river management

Effects of anthropogenic fragmentation on fish population connectivity have been reported in a number of cases (e.g. Bessert and Orti 2008, Faulks et al. 2011, Junge et al. 2014). That anthropogenic fragmentation has detectable effects on the genetic structure and diversity of European Chub populations is significant because in many respects, the European Chub represents the best case scenario of a fish that should be resilient to fragmentation effects. It occurs at high population densities, it is a strong swimmer able to migrate long distances and to traverse at least low barriers (Broye), and it is very tolerant of habitat alterations that typically accompany river fragmentation (e.g., altered flow regime and substrate). Accordingly, the overall population structure is very shallow in this fish. The global F_{ST} value over all sampling sites across Thur, Rhine, and the Glatt system was only 0.036 ± 0.003 (SE). Thus, improving population connectivity across man-made obstacles in the European

Chub would appear to be relatively straightfoward, e.g., by installment of fish bypasses, which European Chub accept readily (Guthruf 2008). However, the effects of river fragmentation are species-specific (Blanchet et al. 2010), and small ground-dwelling fish like bullheads, loaches, or gudgeons could be more strongly affected by the same level of fragmentation. Thus, the goal of re-establishing free fish migration in the course of river restorations, explicitly requested in the Swiss legislation by the revised Water Protection Act (Könitzer et al. 2012) and in the European Water Framework Directive (European Parliament and Council 2000), must be pursued. However, such restorations also may ease access to previously uninvaded rivers by invasive fish or aquatic invertebrates (Rahel 2013), which are a growing problem worldwide (Strayer 2010). This possibility will require some difficult choices by river managers and calls for informed pragmatism in the face of conflicting conservation goals in river restoration.

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Chapter 3: Postglacial recolonizations, watershed crossings and human translocations shape the distribution of chub lineages around the Swiss Alps

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Abstract

Background: Distributions of European fish species were shaped by glaciations and the geological history of river networks until human activities partially abrogated the restrictions of biogeographical regions. The nearby origins of the Rhine, Rhone, Danube and Po rivers in the Swiss Alps allow the examination of historical and human-influenced patterns in fish genetic structure over a small geographic scale. We investigated these patterns in the widespread European chub from the Rhone, Rhine and Danube catchments (*Squalius cephalus*) and its proposed southern sister species Italian chub (*Squalius squalus*) from the Po catchment.

Results: A phylogenetic tree constructed from mitochondrial Cytochrome b and CO1 sequences showed a clear separation of European chub and the Italian chub. The separation into two distinct species was also supported by microsatellite allele frequencies, morphological traits and shape differences quantified by geometric morphometrics. However, we also observed introgression of the predominant mitochondrial haplotype from the Rhine and Rhone catchments into Swiss populations of the Italian chub, presumably as a result of human translocation. Consistent with postglacial recolonizations from multiple refugia along the major rivers, the nuclear genetic structure of the European chub reflected drainage structure, but it was modified by a watershed crossing between Rhine and Rhone near Lake Geneva and possibly by a drainage capture between Danube and Rhine near Lake Constance.

Conclusion: Our study adds new insights into the cyprinid colonization history of central Europe by showing that multiple processes shaped the distribution of different chub lineages around the Swiss Alps. Interestingly, we find evidence that holocene cross-catchment migration has been mediated by unusual geological events, as well as

evidence that human transport has interfered with the historical distribution of these fish (European chub haplotypes present in the Italian chub). The desirable preservation of evolutionarily distinct lineages will thus require the prevention of further translocations.

Background

Strong climatic fluctuations during the Pleistocene induced extensive population contractions and expansions in the European flora and fauna (Hofreiter and Stewart 2009). The geographic situation of Europe, surrounded by sea and divided by east-west mountain barriers, repeatedly trapped species in glacial refuges until recolonization during the warm interglacial periods. The repeated separation into glacial refuges led to genetic subdivision in many species, with hybrid zones forming where they came into contact again following recolonization (Hewitt 1999). In freshwater fishes, populations are additionally constrained by the geological history of a region. They remain restricted to their hydrographic basins unless new interconnections or chance dispersal over land allow further expansion. Accordingly, the highest species diversity is found in the historically ice-free but isolated river catchments of Peri-Mediterranean and Ponto-Caspian Europe, and the lowest diversity in northern and central Europe ((Reyjol et al. 2007) and references therein).
Human activities have partially abrogated the borders between biogeographical regions. Today, shipping waterways such as the Rhine-Main-Danube canal or the Rhone-Rhine canal connect most important watersheds throughout Europe, and commercial and recreational fisheries spread species of interest either actively by stocking or accidentally by live bait release, increasing the potential for hybridization between related but geographically separated taxa. Indeed, non-native fish have

become one of the major threats for native species of the Mediterranean region by enabling hybridization and introgression, but also by the transmission of novel parasites and diseases or competition and predation (Ribeiro and Leunda 2012). Here we investigate the potentially human-influenced current biogeography of European chub from the *Squalius cephalus* complex in Central Europe, with a particular focus on Switzerland, where four major river systems originate in close proximity (Rhine, Rhone, Danube and Po). The European chub originated during the Pliocene (3-2.5 Myr ago) in the Tigris-Euphrates basin, where secondary contacts and hybridization with other *Squalius* species of previous waves of colonization played an important role in the evolutionary history of the group (Durand et al. 2000). The invasion was at the end of an invasion period of several fish genera with Asian origin, which lasted from the Oligocene to the Pliocene (44 - 2.5 Myr ago), composing the large majority of today's European fish species (Banarescu 1992). A rapid radiation of the chub from its Mesopotamian origin led to four contemporary phylogroups that are represented by four major clades in a mitochondrial cytochrome b (Cyt b) phylogeny, namely a Western, Adriatic, Aegean and Eastern lineage (Durand et al. 1999; Seifertova et al. 2012). Collectively they cover almost the entire European continent (Durand et al. 2000). Italian and Greek haplotypes found by Durand et al. (Durand et al. 1999) are frequently considered a separate species, the Italian chub *S. squalus* (Kottelat and Freyhof 2007; Perea et al. 2010), although this is not universally adopted (Seifertova et al. 2012). Western Europe appears to have been colonized out of the Danubian basin before the last Würm glaciation, probably during the Riss - Würm interglacial period (about 100,000 years ago), and holocene recolonization of the Western lineage presumably started from multiple Würm refuges (Rhone, Rhine, Danube, and tributaries of the Black Sea) (Durand et al. 1999; Seifertova et al. 2012).

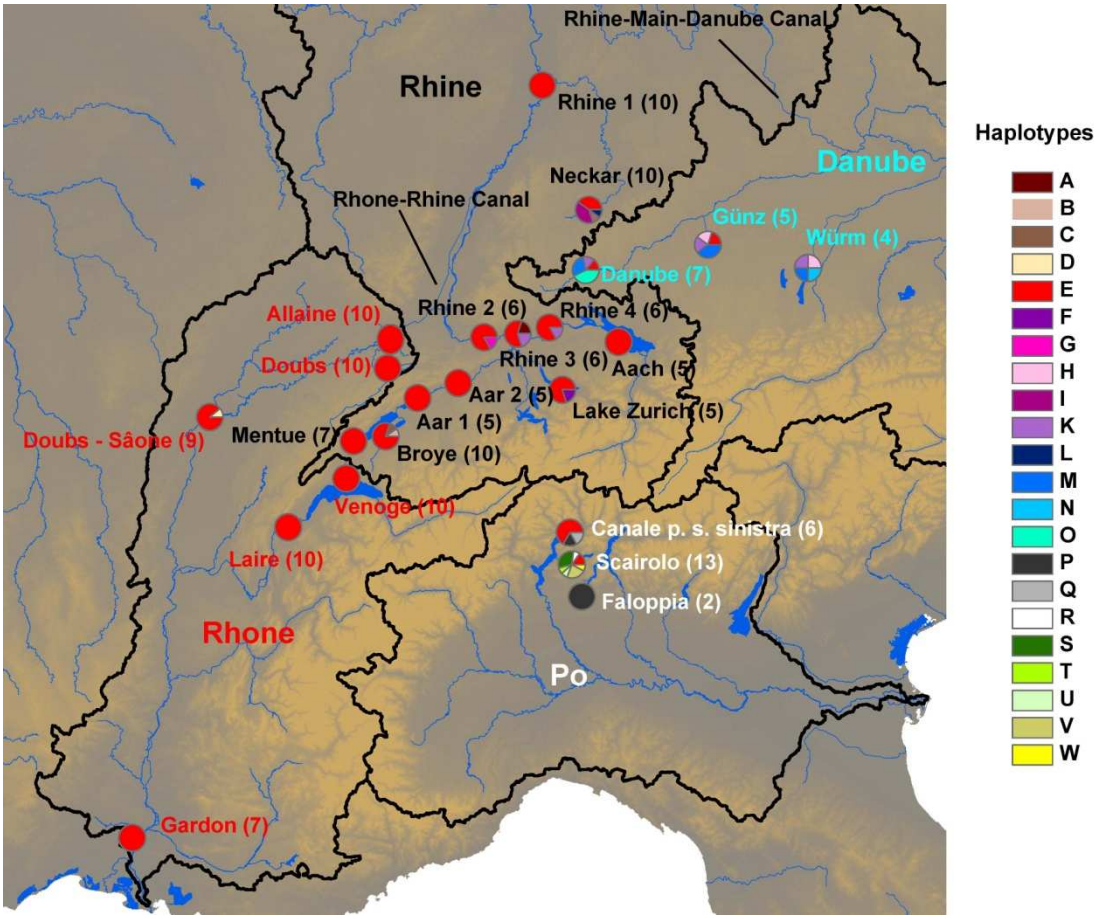

Figure 1 Overview of the study region. Sampling sites are represented by pie charts indicating the relative frequencies of mitochondrial haplotypes found at these sites.Sample sizes are in brackets. Catchments are outlined by black borders and colored annotations indicate to which catchments each sample belongs: Rhine = black, Danube = light blue, Rhone = red and Po = white Shipping channels connecting catchments are annoted with their names in black.

Of particular relevance for the present study is the capture of the upper catchment of the Danube by the Rhine, such that water from the Danube deviates through the karst underground towards the Rhine catchment in the so-called Danube Sinkhole (Rutte 1987). The Rhone catchment below Lake Geneva was disconnected from the remaining catchments by a 60 m deep underground pass of the Rhone called “Pertes du Rhône”, but this natural spectacle was flooded in 1948 after construction of a dam for hydroelectric power production (Lake Genissiat). Furthermore, the different

catchments were connected for shipping by the Rhone-Rhine Canal in 1833 and the Rhine-Main-Danube Canal in 1981 (Fig. 1). This situation provides an opportunity to study how natural and anthropogenic processes shaped the current distribution of chub lineages around the European Alps. We do this by applying genetic analyses of mitochondrial gene sequences and nuclear microsatellites as well as morphological analyses to chub from the four major river catchments originating in the Swiss Alps.

Results and Discussion

Clear mitochondrial separation of European and Italian chub

Based on the concatenated COI and Cyt b sequences, 22 different haplotypes could be distinguished among the 167 chub analyzed. These haplotypes fell into two distinct monophyletic groups (Fig. 2). Haplotypes P-W all came from fish captured in the Swiss canton of Ticino, that is in fish from the Po drainage south of the Alps (Fig. 1), assigned to the Adriatic lineage (Italian chub) by Durand et al. (1999). The other clade of haplotypes A-O, with one exception (see below), represented fish from the Rhine, Rhone and Danube drainages, which are part of the Western lineage according to Durand et al. (1999). A minor split within this clade separates haplotypes A-K from the four haplotypes L-O, of which three (L, N and O) are restricted to the Danube drainage (Figs. 1, 2).

The deep split we observed between the two major mitochondrial clades in our samples support the proposed distinction of the European chub, *S. cephalus*, and the Italian chub, *S. squalus* (Kottelat and Freyhof 2007; Perea et al. 2010).

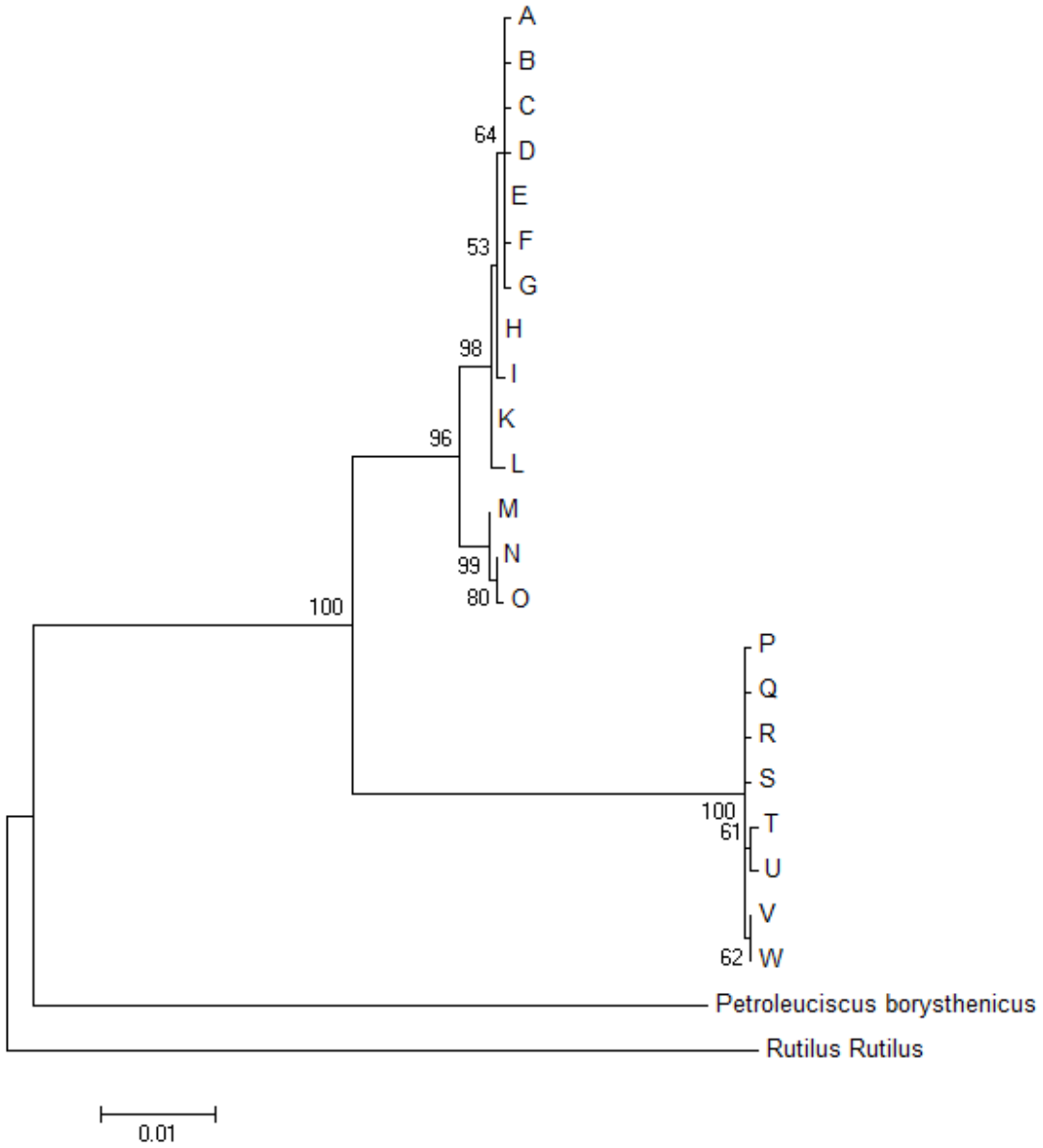

Figure 2 Maximum likelihood phylogenetic tree of chub haplotypes obtained from concatenated mitochondrial *CO1* and *Cyt b* sequences, based on the Tamura-Nei model (Tamura and Nei 1993).*Petroleuciscus borysthenicus* and *Rutilus rutilus* are used as out-groups. Letters A to O are European chub (*Squalius cephalus*) haplotypes and P to W are from Italian chub (*S. squalus*). The tree is scaled with branch lengths indicating amino acid substitutions per site (see scale bar). Numbers at nodes represent bootstrap support from 1000 replications. Lower than 50 percent support values are not shown.

This is further supported by quantifying the sequence divergence at the barcoding gene COI only. In total we could distinguish 8 COI haplotypes that fell into the same two clades with a sequence divergence of 3.8% between clades (Kimura's two-parameter model (Kimura 1980)) and a mean sequence divergence of only 0.5% within European chub sequences and 0.2% within Italian chub sequences. This is strong evidence for a species distinction by commonly applied standards (Hebert et al. 2003).

Of the European chub haplotypes (A-K), haplotype E was by far the most common. It was the predominant haplotype in the Rhine drainage, virtually the only haplotype present in the Rhone drainage, where only one European chub out of 56 had a different haplotype (D), and it was also found in two chub from the Danube drainage (Fig. 1). Most surprisingly, haplotype E was also discovered in Italian chub from two sites south of the Alps in the Po drainage (Fig. 1). Because these chub showed nuclear genotypes as well as morphological traits consistent with the Italian chub *S. squalus*, our interpretation of this finding is a mitochondrial introgression after human-mediated transport across the Alps. Although the European chub is not comprised in the list of 38 introduced fish species in Italy (Gherardi et al. 2008), unrecorded releases cannot be excluded, and because our study region in the Swiss canton of Ticino is well-frequented by tourists, translocation as live fishing bait is a likely route of introduction. Introgression from the European chub has also been reported in the Catalan chub (*S. laietanus*) in southern France (Denys et al. 2013). Depending on how frequently it occurs, such introgression may be of concern for the lineage integrity of the Italian chub in Switzerland, where it has recently been taken up it in the fisheries legislation as a distinct species, in accordance with the modification of the IUCN species list (Freyhof 2013).

Nuclear genetic structure at microsatellite loci

The distinct status of Italian chub from south of the Alps was also supported by the microsatellite data. A first indication was provided by the fact that two microsatellite loci, LC 128 and LceCb, could not be amplified in 14 out of the 20 individuals we genotyped from the Po drainage, suggesting high-frequency null alleles (i.e. mutations in the priming sites) at these loci in the Italian chub. Thus, all following analyses were restricted to eight loci that could be amplified in all 168 individuals and did not show any evidence of null alleles (see Table S1 for sample sizes per site). These loci had an average of 15.6 alleles, ranging from 2 – 42 alleles per locus. Mean expected and observed heterozygosities across loci as well as allelic richness (*AR*) for the four drainages are listed in Table 1, but note that these estimates lump several distinct sites with small sample size for each drainage and should therefore be interpreted cautiously.

Table 1 Genetic diversity measures for European chub (*Squalius cephalus*) from the Danube, Rhine and Rhone catchments, and for the Italian chub (*S. squalus*) from the Po catchment. Ho: observed heterozygosity, He: expected heterozygosity; AR: allelic richness standardized for the smallest sample size (16)

Catchment	H_O	H_E	*AR*
Danube (n = 16)	0.77	0.76	7.43
Rhine (n = 64)	0.71	0.74	5.23
Rhone (n = 47)	0.62	0.68	7.34
Po (n = 18)	0.64	0.68	7.83

Most notable is the comparatively low *AR* observed in the Rhine drainage. Because

AR is more sensitive than heterozygosity to founder effects (Allendorf 1986), this is suggestive of a recent population expansion in this drainage. Pairwise genetic differentiation expressed as F_{ST} was highly significant between all drainages and ranged from 0.05 (Danube-Rhine) to 0.19 (Rhone-Po) (Table 2). These seemingly low values are a consequence of the dependency of F_{ST} on within-population diversity, which biases differentiation towards zero in highly polymorphic markers such as microsatellites (Hedrick 1999). Using Jost's estimator of differentiation *D* (Jost 2008), which does not suffer from this problem, genetic differentiation is strong among all drainages, particularly for the Italian chub from the Po drainage, differentiated by D = 0.44 – 0.52 from chub in the Rhine, Rhone and Danube drainages (Table 2).

Table 2 Genetic differentiation estimated as F_{st} (above diagonal) and D_{est} (below diagonal) among chub from the Rhine, Danube and Rhone catchments (*Squalius cephalu*s) and the Po catchment (*S. squalus)*. All F_{st} and D_{est} values are statistically significant (all $p < 0.001$).

	Danube	**Rhine**	**Rhone**	**Po**
Danube		0.05	0.14	0.09
Rhine	0.22		0.07	0.11
Rhone	0.38	0.18		0.19
Po	0.44	0.44	0.52	

A more detailed picture of the genetic structure was provided by the Bayesian clustering analysis (Fig. 3). The best-supported number of genetic clusters as indicated by the method of Evanno (Evanno et al. 2005) was K = 5. Consistent with an earlier study by Seifertova et al. (2012), chub from the Po, Rhone and Danube drainages are relatively clearly separated. Chub from the Rhine drainage are mostly

assigned to an additional cluster, albeit many with low probability. Several chub from the Rhine are even assigned to the Danube cluster with high probability, indicating extensive introgression between these two drainages. This introgression may be facilitated by the Rhine-Main-Danube canal or the drainage capture in the Danube sinkhole. Considering that introgression is most evident in the sites Rhine 2-4 and Aach (Fig. 2), all located in the vicinity of Lake Constance (Fig. 1), it is likely that the drainage capture played a more important role, because water from the Danube enters the Rhine drainage at the western end of Lake Constance via the Radolfzeller Aa. Of course, an additional role of human-mediated transport, e.g. via the release of bait fish, cannot be excluded. Another interesting observation is that under $K = 5$, chub from the Rhone drainage are split into two clusters, one representing the rivers Venoge and Laire, tributaries of Lake Geneva and the Rhone just below Lake Geneva, and one representing all other sites within the Rhone drainage, from the upper reaches of Doubs and Allaine down to the Gardon near the Mediterranea sea (Figs. 1,3).

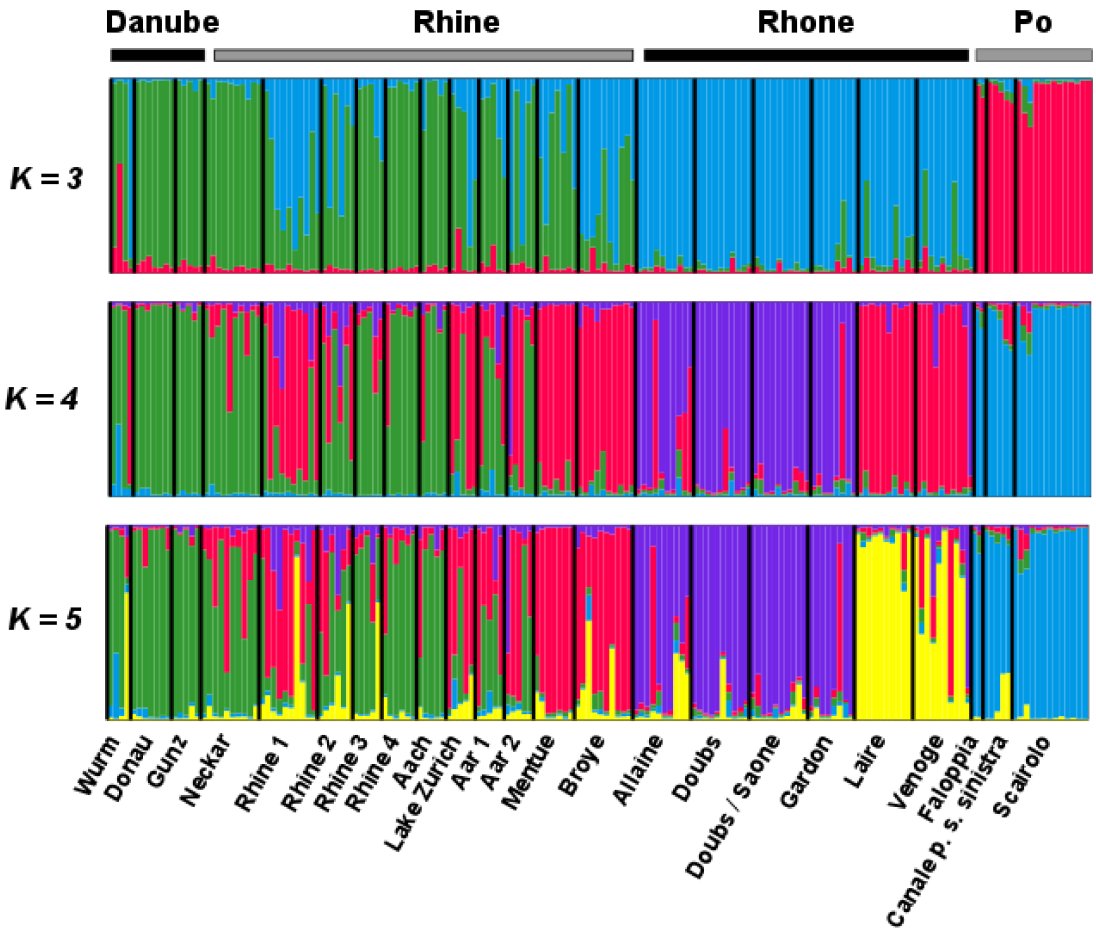

Figure 3 Results from the Bayesian structuring analysis in STRUCTURE using $K = 3$ to $K = 5$ clusters. Individuals aresorted by sampling site and the lengths of colored bars represent individual assignment probabilities to each of the inferred genetic clusters. Individuals from the Po drainage are Italian chub (*Squalius squalus*), all other individuals are European chub (*S. cephalus*) The most likely number of clusterinferred by the method of Evanno REF was $K = 5$.

To understand the situation in the Rhone drainage it is instructive to also inspect individual assignment probabilities for values of K lower than the best-supported $K = 5$. Under $K = 4$, chub from the Rhone drainage remain assigned to two different clusters, but most fish from the Venoge and Laire near Lake Geneva are now assigned to the same cluster as most fish from the rivers Montue and Broye, the two sites in the Rhine drainage that are geographically closest to Lake Geneva (Figs. 1, 3). Thus, chub from the Lake Geneva area seem more closely related to chub from the nearby parts

of the upper Rhine drainage than to other chub from the Rhone drainage. This pattern is remarkable because it is also observed in the brown trout, *Salmo trutta* (Largiadèr and Hefti 2002) in the European grayling, *Thymallus thymallus* (Largiadèr and Hefti 2002) and in the bullhead, *Cottus gobio* (Largiadèr and Hefti 2002; Vonlanthen et al. 2007). It has been explained by a postglacial watershed crossing, facilitated by the retreating Rhone glacier after the Würm glaciation (Vonlanthen et al. 2007). It appears that the same process has also led to the colonization of Lake Geneva by chub from the Rhine drainage, which is the first example of this for a cyprinid fish for this region. These chub remained to some extent distinct because upstream colonization from the lower Rhone was impeded by the "Pertes du Rhône" that was only submerged since the construction of Lake Genissiat in 1948 (see Background). Because the presence of the E haplotype in Italian chub from canton Ticino indicated the possible introduction of European chub in the Po drainage (Fig. 1), we subjected all genotypes to an analysis with the software NewHybrids 1.1 Beta3 (Anderson and Thompson 2002) to test whether there was any evidence for hybridization from nuclear microsatellite genotypes. However, none of the individuals were identified with any confidence as either F1or F2 hybrids or backcrosses. This does not exclude the possibility of nuclear introgression, but there is no evidence for the presence of recent hybrids among the Italian chub we sampled.

Morphological variation

A canonical variate analysis on size-corrected Procrustes coordinates revealed minor and non-significant differences in shape among European chub from the Rhine, Rhone and Danube catchments, but clear and significant differences between Italian chub from the Po catchment and European chub from all other catchments (Table 3).

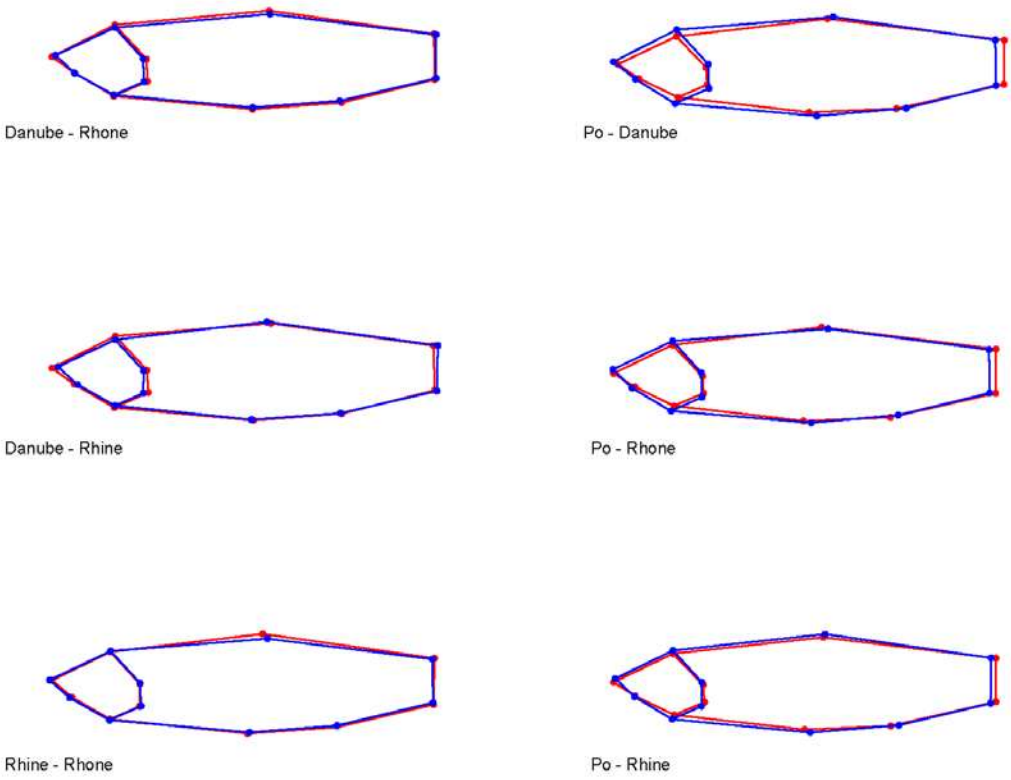

Figure 4 Pairwise shape differences between chub from different catchments, visualized by two-fold enhanced wireframe plots. Dots indicate landmark positions. For all comparisons, the mean shape of fish from the first catchment is colored in red and the mean shape of fish from the second catchment in blue.

The pairwise shape differences between chub from the different catchments are illustrated in Figure 4. Italian chub from the Po catchment have a more shallow and tapered head, more anterior insertions of the dorsal, pelvic and particularly the anal fins, and an elongated caudal peduncule compared to European chub (Fig.4).

Table 3 Differences in body shape expressed as Procrustes distances (above diagonal) and associated *p*-values (below diagonal) among chub from the Danube, Rhine, Rhone (*Squalius cephalus*) and Po catchments (*S. squalus)*.

	Danube	**Rhine**	**Rhone**	**Po**
Danube		0.0136	0.0098	0.0250
Rhine	0.1168		0.0099	0.0181
Rhone	0.3531	0.0832		0.0214
Po	0.0031	0.0209	0.0020	

Traditional morphometrics using size-standardized measurements of body parts, fin ray and scale counts as well as fin color produced a similar pattern in that European chub from the Rhine, Rhone and Danube catchments were very similar but distinguishable from the Po drainage's Italian chub. The trait values for each catchment are summarized in Table S2. Figure 5 illustrates the separation of Italian chub individuals from European chub in a plane described by the first two principal components of a PCA including all traits. The five traits contributing most strongly to the separation were the color of the fins (black in Italian chub, mostly red in European chub), the head length (shorter in Italian chub), the number of anal fin rays (more in Italian chub), the length of the anal fin (longer in Italian chub) and the snout length (shorter in Italian chub). There was no indication that individuals from the Po drainage possessing the European chub mitochondrial haplotype E were morphologically more similar to the European chub (Fig. 5). Because the first two PCs explained only 28 % of the total variance, we also compared chub morphology among catchments with MANOVAs on the PC scores from the first 14 PCs that cumulatively explain 80 % of the variance. These analyses confirmed that the largest morphological differences occur between European chub and Italian chub, but they

also detected significant differences among European chub from different drainages (Table 4).

Table 4 Pairwise tests for differences in morphology between chub from the four drainages, based on MANOVAs on scores from the first 14 PCs (explaining 80% of the variance) from a PCA on all traditional mophological traits (see Table S2). Pillai's Trace and p-values are reported.

	Rhine	**Rhone**	**Po**
Danube	Pillai's Trace = 0.33, p = 0.012	Pillai's Trace = 0.46 p = 0.003	Pillai's Trace = 0.91 p < 0.001
Rhine		Pillai's Trace = 0.39, p = 0.003	Pillai's Trace = 0.87 p < 0.001
Rhone			Pillai's Trace = 0.90 p < 0.001

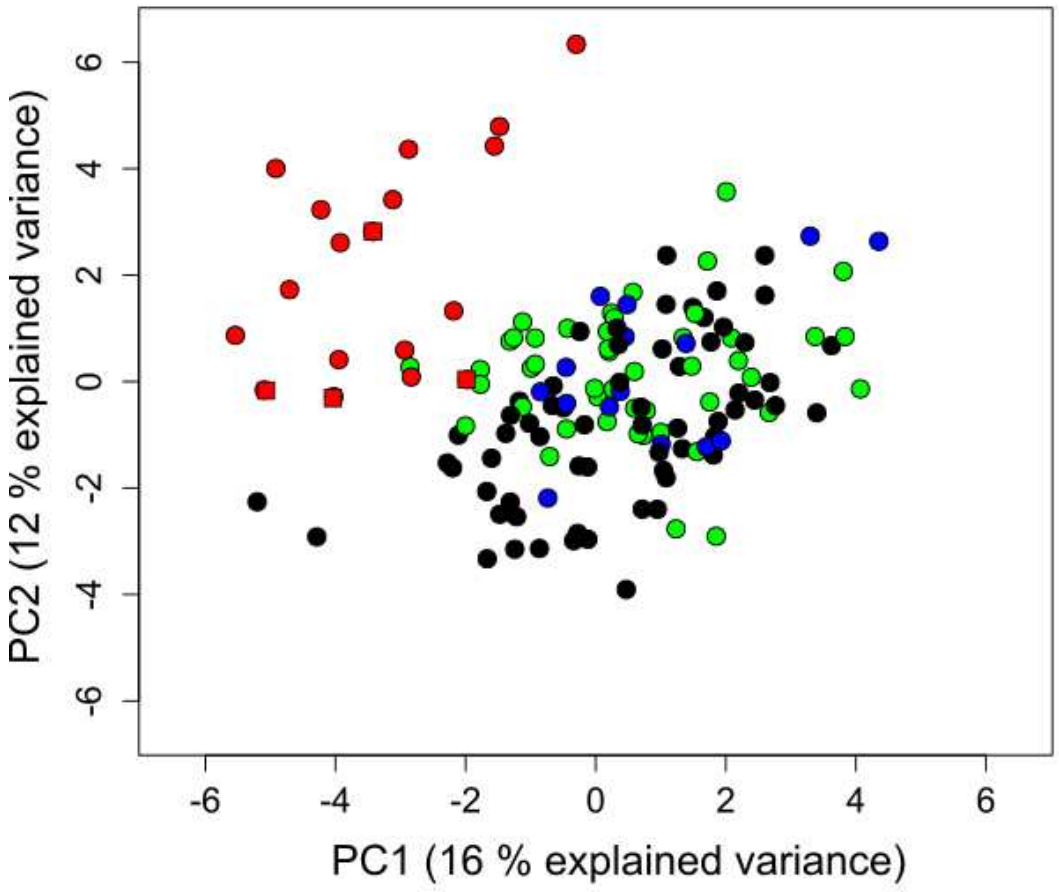

Figure 5 Positions of individual fish relative to the first and second principal component axes derived from the correlation matrix of traditional morphometric traits. Individuals are color-coded by drainage: green = Rhone, black = Rhine, blue = Danube (European chub, *Squalius cephalus*) and red = Po (Italian chub, *S. squalus*). Italian chub possessing the mitochondrial haplotype E are indicated by red squares.

Conclusions

Four major European river catchments originate in close proximity around the Swiss Alps: The Rhone, the Rhine, the Danube and the Po catchment. A combined genetic and morphological analysis of chub from these catchments identifies the chub from the Po drainage, which are part of the Adriatic lineage *sensu* Durand et al. (1999), as clearly distinct. Our analyses thus support its treatment as a distinct species, the Italian chub *S. squalus*, which is commonly but not universally adopted (Perea et al. 2010; Seifertova et al. 2012). The other drainages contain European chub, *S. cephalus*, belonging to the Western lineage as defined by Durand et al. (1999). The higher mitochondrial diversity in the Danube catchment and a nuclear genetic structure coarsely reflecting catchment structure is generally consistent with the proposed scenario of a Pleistocene colonization of central Europe from east to west via the Danube (Durand et al. 1999; Seifertova et al. 2012), followed by post-glacial recolonizations from multiple refugia such as the lower Danube, the lower Rhone and possibly also the Rhine. However, we found evidence that interesting additional processes have shaped the phylogeography of the European chub around the Swiss Alps. These include a watershed crossing between the upper parts of the Rhine catchment and the Lake Geneva area of the Rhone catchment, which has also been inferred from phylogeographic patterns observed in other fish species (Largiadèr and Hefti 2002; Vonlanthen et al. 2007), and possibly a drainage capture through the Danube Sinkhole just north of the Rhine near Lake Constance. We also found introgression of the predominant European chub haplotype from the Rhone and Rhine drainages into the Italian chub in southern Switzerland. This is difficult to explain by any process other than human-mediated transport. Although we do not currently have any evidence for that in the Italian chub, there are deterrent examples of endemic fish

species losing their 'identity' by hybridization, e.g. the Italian barbel *Barbus plebejus* suffering from introgressive hybridization with introduced *B. barbus* (Meraner, Venturi et al. 2013), or Adriatic trout getting dissolved in large populations of stocked Atlantic trout (Meraner, Gratton et al. 2013) The desirable preservation of distinct evolutionary lineages will thus require the prevention of translocations.

Methods

Fish samples

Chub were collected from a total of 23 sites, three from the Danube (sites nr. 1-3), 11 from the Rhine (sites nr. 4-14), six from the Rhone (sites rr. 15-20) and three from the Po catchment (sites nr. 21-23) (Fig. 1, Table 1). Fish were caught by electro fishing with a backpack generator (FEG 1700, EFKO comm., Leutkirch, Germany) or with rod and line. The adult fish designated for morphometric analysis were anesthetized with clove oil and killed according to the animal protection laws by gill cut before photographing and taking fin clips as tissue samples. Juvenile fish were fin clipped (approx. 1 mm^2) for genotyping and released thereafter. Fin clips were stored in 99 % ethanol until DNA extraction.

Genotyping

DNA extraction

We used the salting-out DNA extraction protocol developed by Sunnucks and Hales (1996), adapted to a 96 deep well plate format. Fin clips were first air dried in 8-strip microtubes. Thereafter, 300 µl of TNES buffer **(**50 mM Tris, pH 7.5, 400 mM NaCl, 20 nM EDTA, 0.5% SDS) and 5 µl of 10 mg/ml proteinase K (Roche Inc., Basel, Switzerland) was added, followed by incubation on a shaker (Thermomixer Comfort,

Eppendorf Inc., Hamburg, Germany) for 60 min at 300 rpm. Protein precipitation was performed by adding 85 µl of 5M NaCl and shaking for 10 s. After the proteins were pelleted in a centrifuge at 4700 rpm for 10 min (Heraeus Megafuge 40R, Thermo Fisher Scientific inc, Waltham, MA, USA), the clear supernatants were transferred into a 96 deep well block. The DNA was precipitated by adding 400 µl of ice cold 100 % ethanol and pelleted by centrifugation for 10 min at 4700 rpm. The DNA pellet was washed with 700 ml of 70% ethanol and air dried. For storage at -20°C the DNA was resuspended in 100 µl of 1× TE buffer (100 mM Tris-HCl, 10 mM EDTA).

Microsatellites

Two multiplex PCR reactions were used to genotype the individuals at ten microsatellite loci: LceA149, LceC1, LceCb (Larno et al. 2005), N7G5, N7K4 (Mesquita et al. 2003) and LC128, LC27, LC290, LC32, LC93 (Vyskocilova et al. 2007). Amplifications were performed in a total reaction volume of 10 µl, containing 5 µl Qiagen Multiplex PCR Master Mix (Qiagen inc., Hilden, Germany), 1 µl of primer mix, 3 µl of ultrapure water, and 1 µl of DNA template. Forward primers were labelled with fluorescent dyes (Microsynth Inc., Balgach, Switzerland) as described below. To balance peak heights, the labelled primer was partially replaced with unlabelled primer for loci with strong amplification. Forward primer concentrations in the two multiplex reactions were as follows (reverse primer concentration was equivalent to the sum of labelled and unlabelled forward primer concentrations): Multiplex 1: LceA149 0.5 nM labeled (FAM) and 10 nM unlabeled, LC32 0.8 nM labeled (AT565) and 13.7 nM unlabeled, LceC1 1.6 nM (AT550), LceCb 6.8 nM (FAM), LC93 1.9 nM (AT565).

Multiplex 2: N7K4 0.4 nM labeled (YYE) and 14.5 nM unlabeled, N7G5 0.9 nM labeled (AT550) and 30.5 nM unlabeled, LC128 4.7 nM (AT550), LC 27 1.8 nM (FAM), LC 290 1.2 nM (YYE).

PCRs were carried out in a Labcycler machine (Sensoquest, Göttingen, Germany) with the following cycling conditions: initial Taq polymerase activation and denaturation at 95°C for 15 min, follwed by 30 cycles of denaturation at 94°C for 30 s, annealing at 57°C (multiplex 1) or 58°C (multiplex 2) for 90 s, extension at 72°C for 90 s and final extension at 72°C for 10 min.

Mitochondrial DNA

The phylogenetic relationships among different chub populations were investigated by sequencing parts of two mitochondrial genes, the cytochrome oxidase subunit I gene (COI) and cytochrome b (Cyt b). To amplify CO1 we used primer pair FishF1 and FishR1 (Ward et al. 2005) and for Cyt b we used primer pair Glu and Thr (Machordom and Doadrio 2001). PCR conditions were as previously described (Perea et al. 2010) and for PCR cleanup, the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel, Düringen, Germany) was used according to the manufacturer's instructions. PCR products were sequenced in both direction using the BigDye Terminator V3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and an ABI 3130 capillary sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were edited with Geneious 6.0 (Biomatters, Auckland, New Zealand).

Genetic analysis

Genetic population indices

Observed heterozygosity (H_O), expected heterozygosity (H_E) and standardized allelic richness (AR) at microsatellite loci as well as pairwise F_{ST} among catchments was calculated with FSTAT 2.9.4 (Goudet 2002). Differentiation among catchments was also estimated with Jost's (2008) differentiation estimator (D_{est}), using the R package DEMEtics (Gerlach et al. 2010). The software MicroChecker (Van Oosterhout et al. 2004) was used to test for the presence of null alleles at the microsatellite loci.

Bayesian clustering

Microsatellite genotypes were subjected to a Bayesian Clustering analysis using the Markov chain Monte Carlo (MCMC) approach developed by Pritchard et al. (Pritchard et al. 2000) and implemented in Structure 2.3.4 (Pritchard et al. 2000). We used the admixture model with uninformative priors. Forty simulations for each number of genetic clusters (K) from K =1 to K=10 were run with a burn-in of 50'000 iterations followed by 300'000 iterations. The most likely number of genetic clusters was inferred according to the method of Evanno et al. (Evanno et al. 2005). The 40 runs for the best-supported values of K were averaged using CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) for visualization with the software DiSTRUCT (Rosenberg 2004).

Hybrid detection

Microsatellite genotypes were also used for detection of potential hybrids among chub from the Po catchment. The algorithm for this MCMC approach was developed by Anderson and Thompson (Anderson and Thompson 2002) and implemented in the

program NewHybrids 1.1 Beta3 (available from http://ib.berkeley.edu/labs/slatkin/eriq/software/software.htm). The analysis were run without prior information and with. Because we had no evidence for introgression of Italian chub mitochondrial haplotypes in the Rhine, Rhone and Danube drainages. The simulation was run with a burn in of 50'000 and followed by 300'000 iterations.

Phylogenetic analysis

Sequences of CO1 (618 bp) and Cyt b (1108 bp) were aligned using Clustal W in MEGA 6 (Klingenberg 2011). All sequences were indel-free and have been deposited in GenBank (accession nrs KU302616 - KU302623, KU302625 - KU302642). Because preliminary tree reconstruction from COI and Cyt b produced very similar results, the sequences of both genes were concatenated. Their evolutionary relationships were inferred by constructing a Maximum Likelihood tree based on the Tamura-Nei model (Tamura and Nei 1993) with 1000 bootstraps in MEGA 6 (Klingenberg 2011). The tree was rooted by including sequences from *Petroleuciscus borysthenicus* downloaded from Genbank (CO1 HM560281.1 and Cyt b HM560111.1 from the same voucher specimen and from *Rutilus rutilus* (accession nr KU302624, KU302643).

For comparability with earlier work inferring species status of fishes based on the DNA barcoding gene COI (Ward et al. 2005; Ward et al. 2009), we also calculated pairwise haplotype differences just for COI using the Kimura 2-parameter model (Kimura 1980) which is the best model for sequences containing transitional an transversional substitutions.

Morphometrics

Procrustes-based geometric morphometrics

Photographs of fish used in morphometric analyses were taken at a fixed distance and identical settings with a Nikon D5000 camera mounted on a tripod. Fish were placed on a white background with a fixed scale on the left side. Each fish was photographed twice but in the end one photograph was chosen at random because all were of sufficient quality. On these images we digitized 11 landmarks: tip of snout, posterior end of maxillary, posterior end of gills, posterior end of head, anterior insertion of fins (pectoral, pelvic, anal and dorsal) and superior and inferior insertion of caudal fin. Photographs were processed at a random order using TPSutil (Rohlf 2008) for creating the input file to the landmark editor program TPS2 (Rohlf 2005). Shape variation was analyzed in the software MorphoJ 2.03b (Klingenberg 2011) using a full Procrustes fit to remove variation in scale, position and orientation. To correct for allometric differences, size correction was done by using the residuals of a regression of the procrustes coordinates on log-transformed centroid size.

Traditional morphometrics

The measurements taken from each fish were a selection of morphological features previously presented (Kottelat and Freyhof 2007). From the left side, the following measurements were taken: standard length, total length, predorsal length, postdorsal length, head length, dorsal head length, prepelvic length, pre-anal length, length of the fins (dorsal, pectoral, pelvic, anal), length of base of anal and dorsal fin, length of caudal peduncle, depth of caudal peduncle, snout length, eye diameter, postorbital length and interorbital width. All measurements were standardized as the percentage of the standard length. Allometric changes were again corrected for obtaining the

residuals from a regression of the standardized measurements on size following the recommendations of Reist (Reist 1985). We also counted the total number of rays of all fins, not discriminating between soft rays, branched rays and spiny rays. The last double ray of the dorsal and anal fin was counted as 1.5. The scales along the lateral line were also counted. Fin color was noted as red, black or mixed (coded as red = 0, black = 1 and mixed = 0.5). A Principal Components Analysis (PCA) executed in R (R Core Team 2012) was used to reduce the dimensionality of the morphometric variables before comparisons. The PCs cumulatively explaining 80 % of the variance were retained for between-catchment comparisons of fish by MANOVAs on the scores of these PCs, carried out with the statistical software R (R Core Team 2012).

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Supplementary Information

Supplementary Table S1

Site overview; Site coordinates and sample size used for nuclear, mitochondrial and morphological analysis

Nr.	Site	Coordinates WGS84	Samples	micro-satellites	*CO1 & Cyt b*	morpho-metrics
1	Würm	N 48° 04' 45.98" E 11° 23' 46.14"	4	4	4/4	4
2	Danube	N 48° 04' 10.35" E 09° 00' 27.61	7	7	7/7	7
3	Günz	N 48° 15' 24.40" E 10° 19' 17.13	5	5	5/5	5
4	Neckar	N 48° 30' 19.16" E 9° 01' 51.57	10	10	10/10	10
5	Rhine 1	N 49° 23' 47.32" E 8° 29' 58.94	10	10	10/10	0
6	Rhine 2	N 47° 36' 23.24" E 8° 13' 21.66"	6	6	6/6	6
7	Rhine 3	N 47° 35' 49.36" E 8° 35' 44.49"	6	5	6/6	5
8	Rhine 4	N 47° 39' 09.40" E 8° 37' 46.12"	6	6	6/6	6
9	Aach	N 47° 33' 22.20" E 9° 21' 58.62"	5	5	5/5	5
10	Lake Zurich	N 47° 12' 26.98" E 8° 46' 35.03"	5	5	5/5	5
11	Aar 1	N 47° 07' 20.34" E 7° 14' 13.74"	5	5	5/5	5
12	Aar 2	N 47° 14' 07.67" E 7° 40' 37.44"	5	5	5/5	5

13	Mentue	N 46° 47' 35.08" E 6° 44' 17.48"	7	7	7/7	7
14	Broye	N 46°50' 02.49" E 6° 56' 05.63	10	10	10/10	10
15	Allaine	N 47°27' 43.15" E 7° 02' 53.35	10	10	10/10	10
16	Doubs	N 47°21' 36.09" E 7° 07' 22.02	10	10	10/10	9
17	Doubs / Sâone	N 46°54' 05.55" E 5° 04' 57.27	10	10	10/9	0
18	Gardon	N 43°51' 10.39" E 4° 36' 09.35	8	7	7/7	8
19	Laire	N 46°08' 48.44" E 5° 58' 02.95	10	10	10/10	10
20	Venoge	N 46°31' 04.47" E 6° 32' 39.08	10	10	10/10	10
21	Faloppia	N 45°49' 53.84" E 9° 00' 42.76	2	2	2/2	2
22	Canale p.s. sinistra	N 46°09' 08.92" E 8° 52' 47.76	6	5	6/6	6
23	Scairolo	N 45°56' 59.70" E 8° 54' 20.75	13	13	13/13	10
	Total		170	167	169/168	145

Supplementary Table S2

Catchment means of metric, meristic and standardized qualitative morphological traits used for traditional morphometric analysis. Metric and meristic traits are presented with their standard errors (SE). Fin color was noted as red, black or mixed (coded as red = 0, black = 1 and mixed = 0.5)

Trait	Rhine	Rhone	Danube	Po
predorsal length	0.53 ± 0.002	0.54 ± 0.002	0.54 ± 0.003	0.53 ± 0.005
postdorsal length	0.37 ± 0.002	0.37 ± 0.002	0.36 ± 0.003	0.38 ± 0.002
head length	0.25 ± 0.001	0.25 ± 0.001	0.26 ± 0.002	0.26 ± 0.003
dorsal head length	0.17 ± 0.001	0.17 ± 0.001	0.16 ± 0.001	0.16 ± 0.002
prepelvic length	0.48 ± 0.002	0.49 ± 0.002	0.49 ± 0.004	0.48 ± 0.004
preanal length	0.70 ± 0.002	0.70 ± 0.003	0.71 ± 0.003	0.69 ± 0.004
lenght of dorsal fin	0.17 ± 0.001	0.17 ± 0.002	0.18 ± 0.002	0.17 ± 0.002
length of pectoral fin	0.17 ± 0.001	0.17 ± 0.002	0.17 ± 0.003	0.17 ± 0.002
length of pelvic fin	0.15 ± 0.001	0.14 ± 0.001	0.15 ± 0.002	0.14 ± 0.002
length of anal fin	0.14 ± 0.001	0.14 ± 0.002	0.14 ± 0.003	0.13 ± 0.003
length of base of anal fin	0.10 ± 0.001	0.09 ± 0.001	0.10 ± 0.001	0.11 ± 0.002
length of base of dorsal fin	0.10 ± 0.001	0.10 ± 0.001	0.10 ± 0.002	0.10 ± 0.002
lenght of caudal peduncle	0.22 ± 0.001	0.22 ± 0.002	0.21 ± 0.003	0.22 ± 0.002
depth of caudal peduncle	0.10 ± 0.001	0.10 ± 0.001	0.10 ± 0.002	0.09 ± 0.002
snout length	0.06 ± 0.001	0.06 ± 0.001	0.06 ± 0.002	0.07 ± 0.001
eye diameter	0.05 ± 0.001	0.05 ± 0.001	0.04 ± 0.001	0.04 ± 0.001
postorbital length	0.15 ± 0.001	0.15 ± 0.002	0.15 ± 0.002	0.15 ± 0.002
interorbital width	0.10 ± 0.001	0.10 ± 0.001	0.10 ± 0.001	0.09 ± 0.002
dorsal rays	9.53 ± 0.031	9.50 ± 0.043	9.50 ± 0.000	9.50 ± 0.000
anal rays	9.48 ± 0.027	9.48 ± 0.021	9.50 ± 0.000	10.44 ± 0.098
pectoral rays	17.49 ± 0.082	17.27 ± 0.085	17.19 ± 0.209	17.06 ± 0.104
pelvic rays	9.06 ± 0.038	9.02 ± 0.038	9.00 ± 0.000	9.06 ± 0.056
caudal rays	18.98 ± 0.027	18.98 ± 0.022	19.00 ± 0.000	19.11 ± 0.076
lateral line scales	45.54 ± 0.114	45.55 ± 0.145	45.13 ± 0.328	44.94 ± 0.243
color	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.86 ± 0.068

Concluding remarks

The work presented in this thesis collectively indicates that anthropogenic fragmentation of rivers has a detectable but moderate effect on the genetic composition of chub populations. It increases genetic differentiation as well as the upstream decline of genetic diversity. Soon after the construction of the first hydroelectric power plants in the 19th century, the ensuing decline of migratory fish species has led to the construction of fishpasses. Fishpasses allow migratory fish to complete their lifecycle, which is a fundamental goal in fish conservation. A second goal of fishpasses is to allow enough gene flow for a natural genetic population structure of all riverine fish species. Conceptually, these are different goals. While migratory species may need near-complete success in passing barriers to maintain their stock size, maintaining a natural genetic population structure is possible with a lower success rate (Lowe and Allendorf 2010). However, the earliest fishpasses were a failure in that not all species and sizes of fish were able to pass them (Guthruf 2006). My data suggest that in the case of the chub, currently installed fishpasses in the large Swiss midland rivers fulfill the goal of maintaining the natural population connectivity only partially. Although they appear to improve population connectivity substantially, barriers with fishpasses still had a statistically significant effect on genetic differentiation in my comparisons. Notably, this is in a fish species that can climb fishpasses relatively easily. The negative effects of river fragmentation are likely to be stronger for many other species such as small benthic fish, even in the presence of fishpasses, and also still not all hydroelectric power stations are equipped with fishpasses. There is clearly still room for improvement and further improvement is indeed possible (see below). Unfortunately the study has an important limitation: In

a heavily impacted system like the Swiss lowland rivers it is impossible to know the natural, pre-fragmentation population structure. An unfragmented network does no longer exist to make a comparison. The best I could do was to investigate the genetic structure along an unfragmented stretch of 76 km length in the Thur and Rhine rivers. I observed neither a significant isolation-by-distance (IBD) pattern nor a significant upstream decline of allelic diversity along this stretch, indicating that in the absence of barriers to dispersal, chub can indeed maintain a panmictic population structure over rather large distances. This was in stark contrast to the heavily fragmented Glatt system, where IBD and a strong upstream decline of allelic diversity occurred over a much shorter distance, providing a strong argument for the restoration of river connectivity. The presence of lakes in some of the river systems I sampled provided an additional interesting insight. The data from chapters 1 and 2 suggest that lakes – presumably because of the large numbers of chub they may contain overall - can act as reservoirs of genetic diversity. This represents an additional aspect to consider for the study of fish genetic population composition in dendritic systems, where the species' life history and habitat geometry are otherwise the main determinants of the genetic structure (Paz-Vinas and Blanchet 2015). One would assume that species that can exploit lotic and lentic habitats should be able to maintain a higher genetic diversity in the Swiss midlands than obligate lotic species, which may even become additionally fragmented by interjacent lakes or reservoirs (Franssen 2012; Hudman and Gido 2013). These assumptions could be evaluated by similar studies on lotic species such as barbel (*Barbus barbu*s) and spirlin (*Alburnoides bipunctatus*).

Implications for management and conservation

This work documented the influence of river fragmentation on chub population connectivity within watersheds (chapters 1 + 2) as well as the broad-scale differences among chub populations from different watersheds, which were shaped by postglacial recolonizations (chapter 3). The latter can be viewed as evolutionary significant units (Crandall et al. 2000; Moritz 2002), and the chub from the Po drainage even represents a distinct species. From a management and conservation perspective, the disruption of population connectivity along rivers is equally undesirable as 'artificial connectivity' resulting from human translocations across watersheds. The discovery of the predominent mitochondrial haplotype of the European chub in some Italian chub from canton Ticino suggests that human-mediated dispersal across the Alps may indeed have occurred, possibly as live bait. I cannot currently judge to what extent this might threaten the genetic integrity of the Italian chub in southern Switzerland, but the preservation of distinct evolutionary lineages is certainly desirable and will thus require the prevention of such translocations. The risk might have diminished since fishing with live bait is principally forbidden for animal welfare reasons, but some exceptions allowing this fishing practice still remain. These exceptions should also be abandoned for conservation reasons.

The method of choice to mitigate the undesirable effects of hydroelectric power stations on fish migration and population connectivity within watersheds has long been the installation of fishpasses. My analyses have shown that the existing fishpasses at hydroelectric power stations in the upper Rhine drainage fulfill this purpose at least partially. However, smaller barriers installed in the course of river corrections for flood protection are often equally impassable for fish and tend not to be be equipped with bypasses. The Glatt river is a case in point, but also the large

lowland rivers still contain several barriers that completely prevent upstream passage by fish. Their equipment with fishpasses would result in a significant improvement of overall population connectivity not just in the chub, but presumably in other species as well. Similarly influential could be an upgrade of existing fishpasses following the model of the one at the Rheinfelden power station on the Rhine installed in 2010 (see chapter 1). There the near-natural river circumventing the power station is passed by many more fish than other fishpasses under monitoring, especially by species known to have a poor ability to climb conventional fishpasses (Energiedienst/PFA 2013). Although a genetic confirmation is lacking so shortly after its opening, it is seems safe to assume that this fishpass will increase population connectivity of many fish species. Unfortunately, such improvements may have to be traded off occasionally against other conservation goals. Especially the Rhine is an invasion highway for invasive aquatic species like black sea gobies or non-native crayfish species. Unwanted invaders are likely to also benefit from an improved permeability of man-made barriers. This is an uncomforable dilemma, likely without a general solution. Management decisions may well require careful case-by-case evaluations of whether obstructed dispersal for native species or invasion corridors for invasives represent the lesser evil.

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