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Research Article

Round goby population differentiation across river barriers in Central Europe

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Abstract

River barriers such as hydropower dams and weirs can negatively affect river ecosystems by disrupting connectivity and reducing biodiversity. However, such barriers could also limit the spread of invasive species. Here, we used a spatial population genetics approach to test whether river barriers act as a hindrance to gene flow in the invasive round goby (*Neogobius melanostomus* Pallas, 1814). We sampled gobies from four different rivers across their invasive range in Central Europe (the Danube, Dyje, Morava, and Rhine rivers), with locations on either side of eight major river barriers. Using microsatellite genotyping, we found that round goby populations were differentiated with increasing number of river barriers and with increasing distance between sampling sites, depending on the river system in focus. We found significant population differentiation across three individual barriers, but no clear indication that this was related to barrier type as barriers were highly diverse. We also found reduced genetic diversity in populations that were more recently established. Our findings suggest that successive river barriers can sometimes slow the spread of round goby. Further research on the features of barriers that hinder round goby movement will help to design barrier passage solutions that will both limit spread of this invasive species and maintain connectivity for the native fauna.

Key words: Aquatic habitat invasion, dispersal, genetic differentiation, hydropower dam, invasive species, *Neogobius melanostomus*, river connectivity

Introduction

Aquatic invasive species are a significant driver of global biodiversity loss, especially in freshwaters (Reid et al. 2018; Pyšek et al. 2020; WWF 2020). Invasive species can negatively impact native species via increased predation pressure, heightened resource competition, habitat destruction, and the introduction of diseases and/or parasites that were not previously present in the environment (Pyšek et al. 2020). Invasive species can also have a significant economic cost in invaded regions, especially if they impact the productivity of native ecosystems (e.g., fisheries Marbuah et al. 2014) or damage infrastructure (Booy et al. 2017). It is difficult to eradicate invasive species after their introduction and establishment, and therefore management strategies often involve actions that try to limit the spread of invasive species into new areas. One means to achieve this is through the use of barriers (Krieg and Zenker 2020). Barriers can be particularly effective in riverine systems where an entire invasion corridor can be blocked to limit further spread. While some barriers can be explicitly designed to prevent the expansion of an invasive species (e.g., electrical grids or chemical barriers), others such as hydropower dams and weirs can secondarily act as a means to slow the spread of invasive species – or be equipped with additional barriers to impede the spread of invasive species (Wiegleb et al. 2022). Despite this, river barriers themselves are another contributor towards biodiversity loss in freshwater river systems because they alter environmental flows, fragment previously connected habitats, and disrupt migratory movements for native biota (Mueller et al. 2011; Thieme et al. 2023). Consequently, measures to improve connectivity, such as adding passage solutions or even complete barrier removal, are being implemented, but thereby may also inadvertently facilitate the dispersal of aquatic invasive species. It is therefore important to understand how existing river barriers affect species movements to better inform new developments. Here, we studied the extent to which various riverine barriers restricted the dispersal of a widespread invasive species, the round goby (*Neogobius melanostomus* Pallas, 1814).

The round goby is considered a prime model of a highly invasive species (Cerwenka et al. 2023) with ranges that continue to expand in both Europe (e.g., the Baltic Sea, the Danube, Rhine, and Elbe rivers and surrounding tributaries) and North America (e.g., the St. Lawrence River, all five Laurentian Great Lakes, and surrounding tributaries). The round goby is native to the Ponto-Caspian region of Europe and was first documented in its introduced ranges in Europe and North America in the late 1980's and early 1990's (Kornis et al. 2012; Ojaveer et al. 2015). Its long-distance dispersal to new locations has been mediated by human translocation via ship ballast water discharges (Corkum et al. 2004). Then, over more local spatial scales, range expansions continue primarily via short-distance movement dispersal (Šlapanský et al. 2020) and stochastic transport events, presumably facilitated by human activities (e.g., boating, bait fish) (Bronnenhuber et al. 2011). The round goby was originally thought to have a limited capacity for natural, non-human assisted dispersal due to its small body size, lack of swim bladder, and small home range. However, more recent research indicates that some individuals can disperse longer distances, ranging from 10 to 27 river-km/year (Brownscombe and Fox 2012; Brandner et al. 2013; Cerwenka et al. 2018; Christoffersen et al. 2019; Andres et al. 2020). Moreover, recent work has also demonstrated their ability to climb vertical surfaces and withstand fast flowing waters (Pennuto and Rupprecht 2016; Bussmann and Burkhardt-Holm 2020). It is currently unknown to what extent round goby have the capacity to bypass river barriers, and to what degree river barriers slow their expansion. Some initial work found that upstream range expansion was faster in a river without weirs compared to a river with weirs (Šlapanský et al. 2017). This knowledge is especially relevant given the current global recognition and push for increased river connectivity. Indeed, the European Union's (EU) Water Framework Directive requires that rivers harnessed for hydropower production need to mitigate the damages that barriers cause to their ecosystems, through for example, implementing passage solutions like fishways (Geist 2021).

To assess the extent to which river barriers restrict round goby dispersal, we have used a spatial population genetics approach and estimated pair-wise population differentiation as a proxy of gene flow. This approach captures the effects of migration (or lack thereof) over long time periods, given that cross-barrier movements are probably too infrequent to be tracked in real-time for individual fish. We sampled round goby populations at sites throughout their invasive range in Central Europe where round goby already had established populations on either side of river barriers. We then related genetic population differentiation to geographic distance and the number of river barriers (e.g., weirs, hydropower dams). We predicted that population structure would follow a pattern of isolation by resistance, which would be evidenced by higher levels of population differentiation across multiple river barriers relative to barrier-less river stretches. This would indicate either a limited capacity for the fish to bypass barriers of their own accord and/or limited human-facilitated transfer (e.g., bait fish dumping, boat traffic). The barriers studied here from river systems in the wild are diverse, and they vary in type and potential impact on fish dispersal. We can therefore only preliminarily explore whether certain river barrier types appear to be more impactful. Finally, this data allowed us to investigate if more upstream populations showed reduced genetic variation in relation to downstream populations, as expected when gene flow towards the invasion front is restricted.

Methods

Field sampling

We sampled round goby from four European rivers (Rhine, Danube, Dyje and Morava rivers) in three countries, and all field collections were carried out under the appropriate licenses issued within each country (see section "Ethics and permits" at the end of the publication for details). Sampling sites in the Danube, Dyje, and Morava rivers are thought to have a single invasion front for round goby that is progressing upstream, to the best of our knowledge. In the Rhine river, sampling sites were located between two commercial harbours that receive ships from different shipping corridors, thereby increasing the chances of two potential invasion fronts (implications of which are detailed in the discussion). Sampling sites were located upstream and downstream of pre-existing and diverse river barriers types such as weirs and hydropower dams, some of which were outfitted with fish passages (Table 1, sample sizes are reported in Table 2).

In May 2022, we sampled round goby from sites along the Dyje and Morava rivers in the Czech Republic via electrofishing (Fig. 1). In June 2022, we sampled round goby from sites along the Rhine river in Switzerland via minnow trapping (Fig. 1). In April and July 2022, we sampled round goby from sites along the Danube in Germany via electrofishing based on previous knowledge of the distribution of the species in the upper Danube (Pander and Geist 2010; Cerwenka et al. 2018). In total, 689 adult fish were collected from 16 sampling sites across the four rivers, spanning eight barriers (four hydropower dams with fish passages, one hydropower dam without a fish passage, two weirs with fish passages, and one weir

Table 1. Description of river barriers and the sampling environments, including the estimated year of the arrival of round goby at that site.

River, country	Barrier site	Barrier	Fish pass description	Lock system	River width	Cargo boats	Sampling year, Estimated year of goby arrival
Danube, DE	Poikam	Dam	No fish pass	Yes	120–180 m	Yes	2022, 2009
Danube, DE	Bergheim	Dam	Nature-like fish pass	No	75–240 m	No	2022, 2020
Dyje, CZ	Bulhary	Weir	Boulder ramp	No	50–60 m	No	2022, 2012
Dyje, CZ	Lednice	Weir	Boulder ramp	No	50–60 m	No	2022, 2009
Dyje, CZ	Břeclav	Weir	Boulder ramp	No	50–60 m	No	2022, 2008
Morava, CZ	Morava	Weir	No fish pass	No	30–35 m	No	2022, 2008
Rhine, CH	Birsfelden	Dam	Vertical slot	Yes	200–250 m	Yes	2022, 2013
Rhine, CH	Augst-Wyhlen	Dam	Vertical slot & fish lift[a]	Yes	200–250 m	Yes	2022, 2015

[a] A boulder ramp fish pass is also present on the opposite bank of the Rhine river at this site, which is located in Germany, and was not sampled in this project.

without a fish passage). When possible, we sampled the fish in close proximity to the river barriers, on either side of them, to minimize isolation-by-distance effects (though in some cases, this was not possible; see Fig. 1). Upon capture, round goby were euthanized via an overdose of MS-222 (Danube, Dyje and Morava rivers) or clove oil (Rhine river). Fin clips were taken from the caudal fins of each adult and stored in 99% ethanol for later genotyping. Routine monitoring of fish communities at these sites first recorded the arrival of round gobies (Table 1). Thus, the current study focuses on genetic differentiation among round goby populations that have been very recently founded. We took GPS coordinates of each sampling site location, and measured geographic distances between sites (within each river), by tracing along each river bank in Google Maps (to the nearest 10 m).

Considering previous findings on limited movement of gobies across the banks of larger rivers (Brandner et al. 2015; Šlapanský et al. 2020) we consistently sampled round goby along one bank of each river (both upstream and downstream from various barriers). The implicit assumption is that the opposite side of the river would show a similar pattern of isolation by resistance and/or distance. An exception to this was at the Lednice weir in the Dyje river, Czech Republic, where we captured fish on both banks in order to reach a sufficient sample size (9 fish were caught on one bank, 32 fish were caught on the other bank). Detectable genetic differentiation across the river would be unexpected, given the low level of gene flow necessary to prevent it. Indeed, this expectation was met as population F_{ST} estimates calculated in the software Arlequin (v3.5.1.2, Excoffier et al. 2005, see details below) showed that the fish caught on the two river banks at this site were not differentiated from one another ($F_{ST} = -0.00435$, $P = 0.59$), and we therefore pooled the fish together for this sampling site of the Dyje river.

Microsatellite genotyping and population genetic analyses

DNA was extracted from fin clips using Qiagen DNeasy blood and tissue kits and diluted 1:30 with 10 mM Tris-HCl pH 8, following manufacturer instructions. Each fish was then genotyped at ten microsatellite loci using two multiplex reactions. PCR volumes consisted of 5 µl of Qiagen HotStarTaq Master Mix, 2 µl of template DNA, 1.5 µl of primer mix (0.3 µM concentration per forward and reverse primer), and 1.5 µl of ddH_2O. Forward primers were labelled with one of the following fluorescent dyes: FAM, ATTO550, ATTO565, ATTO532. We used the following PCR programme settings: denaturation at 95°C for 15 min, followed by

35 cycles at 94°C for 30 s, annealing at 56°C (for both multiplexes) for 90 s, extension at 72°C for 60 s and a final extension at 72°C for 30 min. We scored allele sizes against an internal standard (GeneScan 500 LIZ; Applied Biosystems) in an automatic sequencer (ABI3730 Genetic Analyzer; Applied Biosystems).

Table 2. Pairwise population differentiation within each river system. Each river barrier has both an upstream (US) and downstream (DS) site and a sample size of fish collected from each site. F_{ST} values are reported along with corresponding P-values (applying a Benjamini-Hochberg correction for multiple comparisons within each river), and cells are bolded when they indicate significant differentiation between populations on either side of a river barrier.

Danube river						
	Bergheim US	Bergheim DS	Poikam US	Poikam DS		
	$N = 50$	$N = 50$	$N = 50$	$N = 50$		
Bergheim US	NA					
Bergheim DS	-0.0043	NA				
	$P = 0.91$					
Poikam US	0.069	0.058	NA			
	$P < 0.0001$	$P < 0.0001$				
Poikam DS	0.11	0.093	**0.013**	NA		
	$P < 0.0001$	$P < 0.0001$	**$P = 0.0094$**			
Dyje river						
	Bulhary US	Bulhary DS	Lednice US	Lednice DS	Břeclav US	Břeclav DS
	$N = 45$	$N = 32$	$N = 41$	$N = 50$	$N = 48$	$N = 50$
Bulhary US	NA					
Bulhary DS	0.0010	NA				
	$P = 0.41$					
Lednice US	0.025	0.0078	NA			
	$P < 0.0001$	$P = 0.11$				
Lednice DS	0.041	0.020	0.0014	NA		
	$P < 0.0001$	$P = 0.0092$	$P = 0.40$			
Břeclav US	0.034	0.022	0.0012	-0.0026	NA	
	$P < 0.0001$	$P < 0.0001$	$P = 0.40$	$P = 0.78$		
Břeclav DS	0.049	0.021	0.0063	0.0084	**0.012**	NA
	$P < 0.0001$	$P = 0.0021$	$P = 0.11$	$P = 0.039$	**$P = 0.0021$**	
Morava river						
	Morava US	Morava DS				
	$N = 44$	$N = 50$				
Morava US	NA					
Morava DS	0.00009	NA				
	$P = 0.45$					
Rhine river						
	Augst US	Augst DS	Birsfelden US	Birsfelden DS		
	$N = 18$	$N = 28$	$N = 49$	$N = 33$		
Augst US	NA					
Augst DS	0.0054	NA				
	$P = 0.35$					
Birsfeld US	0.0030	0.0092	NA			
	$P = 0.40$	$P = 0.090$				
Birsfeld DS	0.0090	0.013	**0.0092**	NA		
	$P = 0.16$	$P = 0.047$	**$P = 0.047$**			

Genotyping revealed several round goby individuals that had three allele peaks at certain loci. This occurred in seven fish sampled by the Poikham hydropower dam in the Danube river, Germany (two fish had three alleles at locus Nme3, two fish had three alleles at locus Ame17, and three fish had three alleles at locus Ame133). We omitted the allelic information at these loci for these fish.

However, our genotyping also revealed three alleles at locus Ame133 for 17 fish sampled by the Augst and Birsfelden hydropower dams in the Rhine river in Switzerland. In these cases, the third allele was always associated with the genotype 195/219/X (fragment sizes in base pairs [bp]), with X being the third allele. Furthermore, the allele size 219 bp always co-occurred with allele size 195 bp, but was rare amongst the individuals that did not possess a third allele (found in four such individuals). This pattern suggests that a chromosomal region bearing locus Ame133 may have been duplicated in the Swiss population, giving rise to a haplotype carrying alleles 195 bp and 219 bp. We represented this haplotype by the 195 bp allele; that is, we omitted the 219 bp allele from the dataset, which resulted in genotypes 195/219/X being transformed into 195/X, and 195/219 being transformed into 195/195 for locus Ame133 (Note that analyses that exclude this locus entirely from the Rhine sites produce qualitatively similar results).

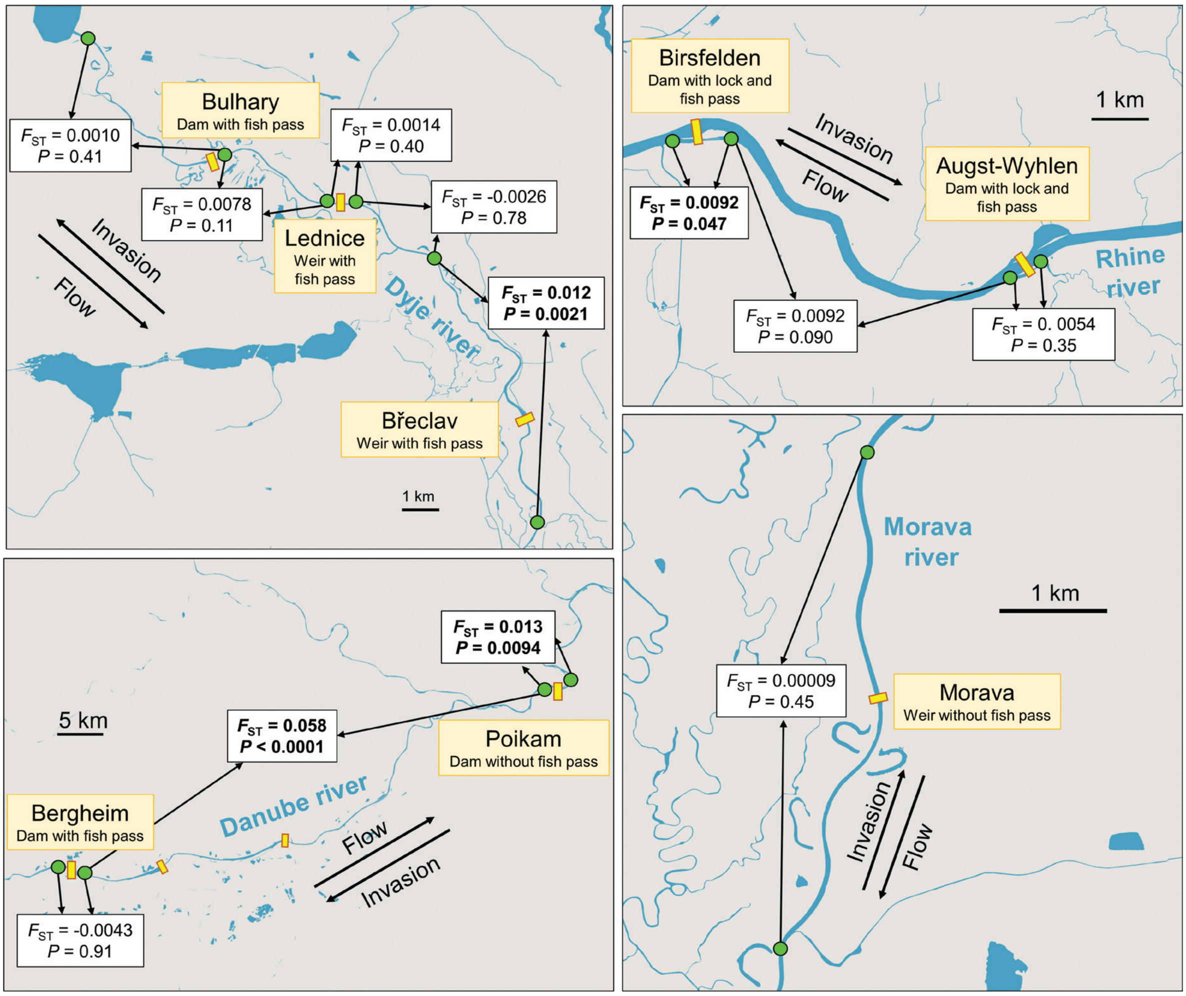

Figure 1. Maps of sections of the Dyje, Rhine, Danube, and Morava rivers where we sampled round goby populations (green circles) on either side of river barriers (yellow rectangles). Estimates of population differentiation, F_{ST}, and the respective *P*-values (after applying a Benjamini-Hochberg correction for multiple comparisons within each river) are given for consecutive sampling sites along each river (white boxes), some of which are separated by a barrier (described in beige boxes with site name). Note that in the Danube, between Bergheim and Poikam, there are two other hydropower dams, each at Vohburg and Ingolstadt (with boat locks).

We used Arlequin to test for Hardy-Weinberg equilibrium (HWE) for each microsatellite marker in each population sample (ten markers each tested in 16 population samples = 160 HWE tests). Several deviations were detected after Benjamini-Hochberg corrections, however, given the recent colonization of these populations, some deviations from HWE are to be expected due to the relatively stronger effects of genetic drift at small (presumed) population sizes (Suppl. material 2: table S1).

Statistical analyses

We first used Arlequin to calculate pairwise differentiation, F_{ST}, between populations within each river. *P*-values were calculated based on 1,023 permutations of the data, and adjusted for multiple comparisons within each river using the Benjamini-Hochberg method. We also used Arlequin to calculate expected heterozygosity (H_e) as a measure of genetic diversity within populations at each of our sampling sites, as well as to calculate the modified Garza-Williamson index (M) as a measure of recent population bottlenecks (Garza and Williamson 2001). M is calculated as the mean ratio of the number of alleles to the range in allele size across multiple microsatellite loci and is expected to be smaller in bottlenecked than in equilibrium populations, with values below 0.68 indicating recent and severe population size reductions (Garza and Williamson 2001).

Within each river system, some pairs of sampling sites straddled river barriers (in relatively close proximity to the barriers), while others were separated by longer stretches of river that may have been free of barriers or may have had several barriers (Fig. 1). When analyzing these data, we tested whether pair-wise F_{ST} within river systems was significantly associated with geographic distance and/or the number of barriers separating sampling sites by fitting a multiple regression model on distance matrices (MRM, using the 'ecodist' R package, v 2.1.3, Goslee and Urban 2007). MRM involves the regression of a response matrix on any number of predictor matrices, where each matrix contains a set of pairwise distances (or similarities, dissimilarities, etc., Lichstein 2007). We fit one model for each river in which we sampled more than two sites and across at least one barrier, i.e., the Rhine, Danube, and Dyje rivers. The Morava river was not included in this analysis as we only sampled at two sites (spanning one barrier) in this river. *P*-values were calculated based on 10,000 permutations of the data.

We next tested whether H_e declined as we sampled further upstream from our most downstream sites, and as we sampled across consecutive river barriers. Expected heterozygosity is predicted to decline further upstream as sampling sites approach the invasion front where the round goby population is more recently founded. Expected heterozygosity is also predicted to decline as additional river barriers are surpassed by small subsets of the downstream populations. We therefore fit a linear mixed effects model (LMM) to the H_e data (means across loci for each population) using the 'glmmTMB' R package, v 1.1.8 (Brooks et al. 2017). As predictor variables, we included 'distance from the most downstream site' (in 0.01 km; the most downstream site in each river was considered the 0 km starting point) and 'number of barriers in between the sampling site and the most downstream site' (this ranged up to 4 barriers). We included 'river' as a random intercept to account for non-independence arising from sampling along stretches of the same rivers.

Results

Our MRM models indicated that population pair-wise F_{ST} in the Danube was marginally associated with geographic distance between sampling sites ($P = 0.05$; Fig. 1A), and significantly associated with the number of river barriers separating sites ($P = 0.001$, Fig. 1B). In the Dyje River, F_{ST} was significantly associated with barrier number ($P = 0.04$, Fig. 1B), but not geographic separation ($P = 0.16$, Fig. 1A). In the Rhine, F_{ST} was not clearly associated with either geographic separation ($P = 0.10$, Fig. 1A) or the number of river barriers ($P = 0.09$, Fig. 2B).

Significant population differentiation was detected across three out of the eight barriers: Poikam hydropower dam in the Danube, Birsfelden hydropower dam in the Rhine river, and Břeclav weir in the Dyje river (Fig. 2; Table 2).

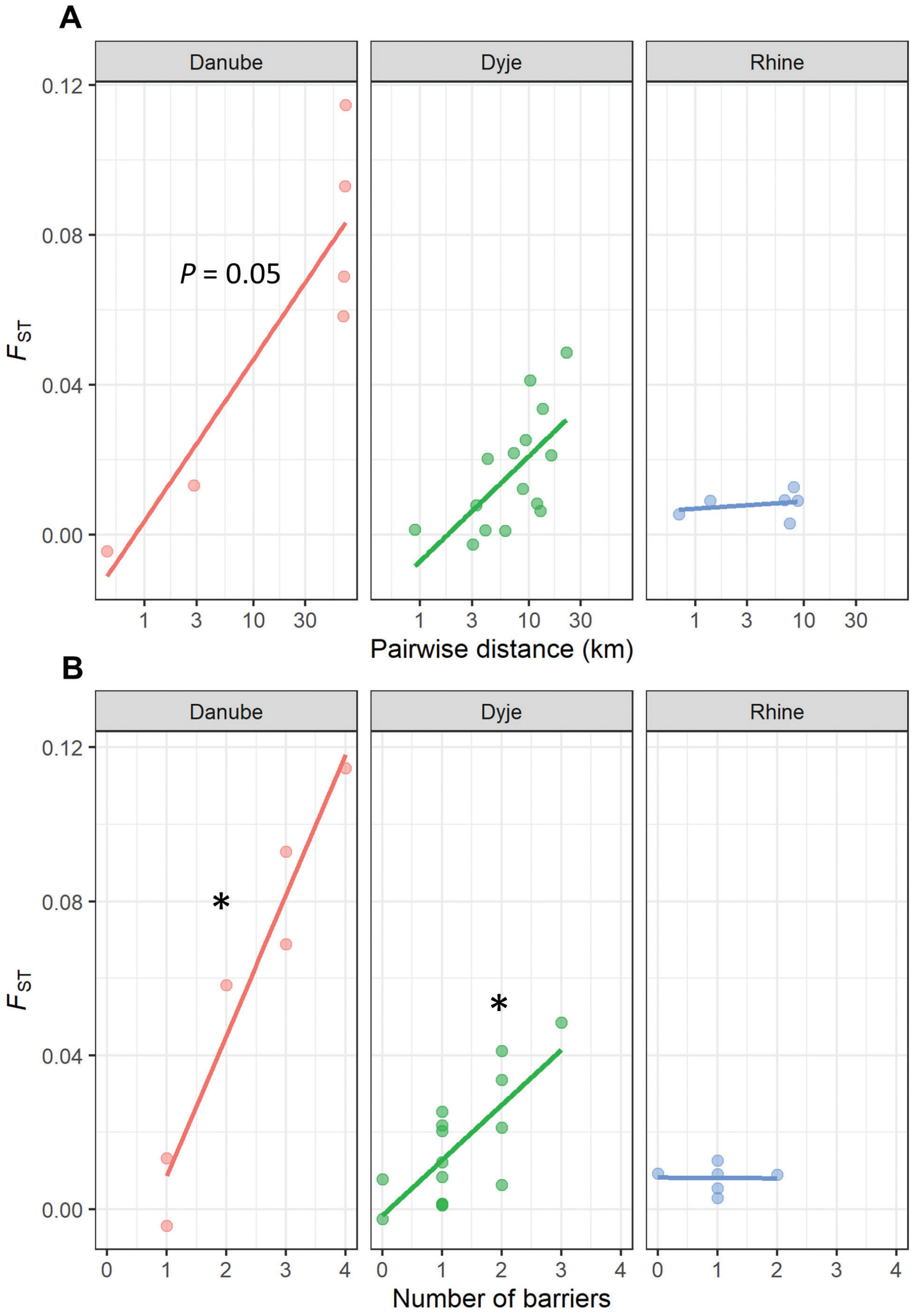

Figure 2. Pairwise F_{ST} values plotted against **A** distance between sampling sites, or **B** number of river barriers between sampling sites, paneled by river system. * shows a significant ($P < 0.05$) result from the MRM models. Slopes derived from a linear regression to aid in visualizing the MRM model results, coloured data points show raw values. Note, the Morava river was not included in the MRM analyses because only two sites were sampled.

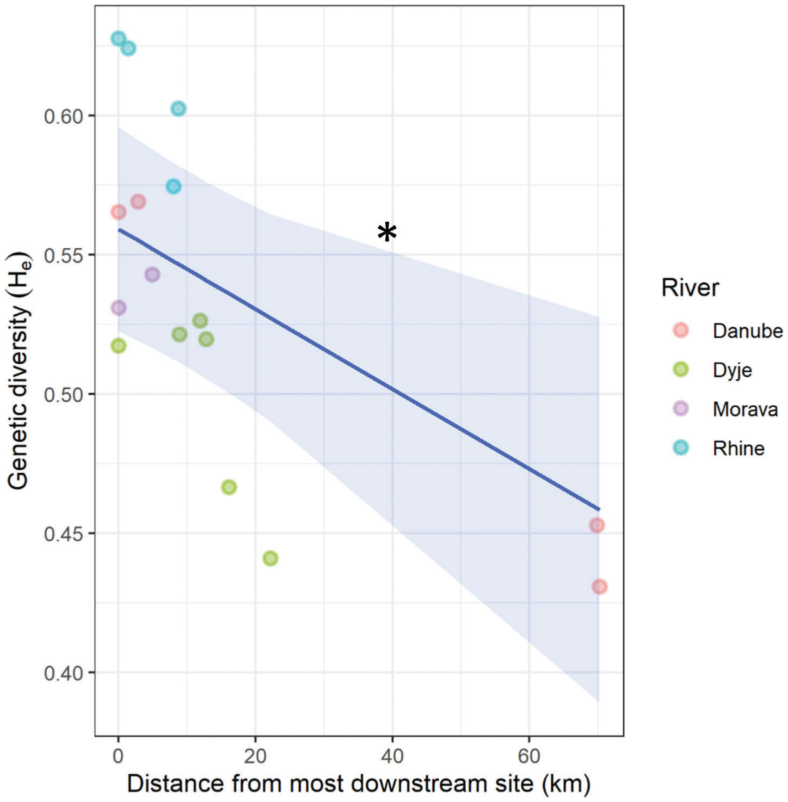

Figure 3. Model prediction (fitted line with 95% confidence interval) showing how the expected heterozygosity (H_e) of round goby populations decreases as sampling progressed upriver and approached the various rivers' invasion fronts. * shows a significant ($P < 0.05$) result for geographic separation from the LMM. Coloured data points show raw H_e values.

Within-population genetic diversity represented by H_e declined as we sampled further upstream (LMM; est. ± std. error = –0.0014 ± 0.0005, z = –2.84, *P* = 0.0046), but was not clearly associated with the number of barriers between the sampling site and the most downstream site (est. ± std. error = –0.010 ± 0.0086, z = –1.18, *P* = 0.24, Fig. 3). When we remove the two far right data points, the effect of distance is still significant (est. ± std. error = –0.0059 ± 0.0025, z = –2.34, *P* = 0.019, Suppl. material 2: fig. S1). The modified Garza-Williamson indices suggested that all populations had experienced population size bottlenecks (M ranged from 0.19–0.23 in the Rhine, 021–0.28 in the Danube, 0.19–0.20 in the Dyje, and 0.20–0.21 in the Morava Rivers).

Discussion

We investigated whether river barriers slowed the dispersal of invasive round goby using a spatial population genetics approach, sampling round goby populations on either side of barriers in four different rivers in their invasive range in Europe. We found that populations were significantly differentiated over increasing distances and increasing numbers of barriers, depending on the river system. Starting broadly, round goby population differentiation was marginally associated with distance and significantly associated with barrier number in the Danube River (Germany); with increasing number of barriers, but not with distance in the Dyje River (Czech Republic); and with neither distance nor number of barriers in the Rhine River (Switzerland). Our findings that populations differentiate with distance (albeit marginally after accounting for barrier number) are in line with previous work at similar spatial scales. For instance Bronnenhuber et al. (2011) showed genetic differentiation in round goby populations as they invaded up river tributaries in the North American Great Lakes, with populations differentiating after approximately 50 km in one river. Similarly, Björklund and Almqvist (2010) found that round goby populations in the Baltic Sea differentiated over distances of only 30 km (though the authors used a different miscrosatellite marker set as Bronnenhuber et al. (2011)). Brandner et al. (2018) documented the progression of the round goby population spreading up the Danube River in Germany, and recorded a 30 km movement of the invasion front upstream over the course of four years (between 2010 and 2014). Overall, our findings suggest that with increasing numbers of river barriers, upstream round goby dispersal becomes progressively more difficult, a pattern that is likely to be mirrored in other fish species navigating these rivers.

River barriers are highly diverse, ranging in size and structure and differing in their implementation of fish passage solutions (Table 1). However, even with fish passage solutions in place, upstream fish passage efficiency is still low for non-salmonid fishes (averages ranging from 18–51%, Sun et al. 2023), and how efficient fish are at passing each barrier depends on characteristics the passage type (e.g., attraction, design, slope, flow) and of the migratory behaviour and swimming performance of the species (Noonan et al. 2012; Sun et al. 2023). Fish with migratory life-histories like salmonids generally pass more efficiently than non-migratory species (Noonan et al. 2012; Sun et al. 2023). Besides upstream migration of the invasion front, many fish have pelagic larval phases that may facilitate downstream dispersal in river systems. Although round goby are benthic and have no strictly pelagic larval phase, round goby larvae have been sampled nocturnally in the pelagic zones in lakes (Hensler and Jude 2007; Hayden and Miner 2009) and sampled in drift nets in European rivers (Janáč et al. 2013; Borcherding et al. 2016). However, no downstream drift distances have been reported yet for this species, which is partly because round goby can display plasticity in spawning times and reproductive output across different habitats making it challenging to get such estimates (Klarl et al. 2024). Furthermore, it is also unknown how well round goby larvae would pass river barriers with different fish passage solutions while passively drifting downstream.

We observed significant upstream-downstream differentiation across three individual barriers, one within each of the three river systems. The rivers and barriers studied here were highly diverse, reflecting the diversity of barrier types that have been installed in European river systems. Therefore, we explored our data to investigate whether there were any consistent patterns of differentiation associated with one barrier type. However, given the low level of replication within barrier types in our dataset, this investigation should be treated as preliminary. Overall, however, we saw no clear indiciation that any particular river barrier type (e.g., weir, dam – with or without fish passage solutions) was related to round goby population differentiation. In two of the cases where we observed significant cross-barrier differentiation, it occurred at small spatial scales with less than 2 km between the upstream and downstream sites. The Poikam dam in the Danube has no fish passage solution (e.g., fish ladders), which may hinder round goby dispersal across the barrier. However, at the same time there is a large lock system allowing ships to pass by the dam, which fish can presumably use as well. Meanwhile, the Birsfelden dam in the Rhine also showed genetic differentiation and has a vertical slot fish passage. Vertical slot passages may be more challenging for round goby to pass if the flow is high, the passage is steep, or the passage contains no bottom structure on which the goby can anchor itself (Pennuto and Rupprecht 2016; Wiegleb et al. 2023). However, the Birsfelden dam also has a boat lock, and gobies are regularly observed at high densities in the lock (pers. obs. PB). Birsfelden in Basel, Switzerland, is also located between two commercial harbours, which thereby increases the chances at this site that round goby from different geographic and genetic backgrounds have been introduced on either side of this barrier in particular (Adrian-Kalchhauser et al. 2016). The final barrier with significant differentiation was at the Břeclav weir in the Dyje river, which was equipped with a boulder ramp-style fish passage. This type of passage might be more easily passable for round goby, but it should be noted that the up- and downstream sampling sites were also separate by a comparatively larger spatial distance than at the Poikam or Birsfelden dams (~22 km).

It should be noted that several mechanisms can contribute to our observed population structures, including stochasticity in the genetic makeup of the founder population, gene flow, genetic drift (particularly in small populations), mutations, and different selective regimes across populations. Our measures of population differentiation, however, ought to be most strongly influenced by founder effects, gene flow (or hinderances to gene flow) and somewhat less by genetic drift, as our study populations are extremely young (founded within the past 8–15 years) (Sefc et al. 2007). Indeed, we found that the Garza-Williamson indices calculated for each sampling site suggested that the populations had all gone through recent population size declines. These findings are consistent with the round goby populations in these rivers all having been recently founded.

We also found that round goby showed less genetic variation in the furthest upstream—and therefore presumably most recently invaded—sampling sites relative to the downstream sites within the same river system. Lower genetic diversity at the invasion front can occur in newly invaded areas and diversity can increase later as alleles accumulate over time (Roman and Darling 2007). Newly invaded areas can therefore show evidence of founder effects, i.e. a reduced representation of genetic diversity in the novel population compared to the source population combined with further loss of diversity due to strong genetic drift while the novel population is small. Recently, Green et al. (2023) showed that round goby populations were less genetically diverse with increasing inland invasion off the Baltic Sea. However, in contrast, Bronnenhuber et al. (2011) found that genetic diversity was similar and relatively high among lake and recently colonized river populations in North America. It is important to note that we did not sample any round goby source populations or older, established populations within the European range, as this was not the primary focus of our current work. Population differentiation estimates between novel and source populations are likewise affected by the stochastic representation of alleles by the founders and the subsequent genetic drift in the novel population. Following upstream range expansion, substantial downstream gene flow (via pelagic larvae) may even carry the genetic founder effects taking place at the expansion front, and bring these effects back to the source populations altering their genetic make-up and opposing genetic differentiation. Given the observed patterns in genetic diversity and differentiation, the range expansion of round goby in the investigated river systems seems to proceed in the face of contraints to gene flow and these are apparently insufficient to prevent the spread of the species.

In conclusion, we have used a spatial genetics sampling approach to test the hypothesis that river barriers would slow round goby dispersal as indicated by population differentiation. This work was motivated by an increasing recognition of the benefits of river connectivity, which may also come with the cost of increased spread of invasive species, like the round goby (Mclaughlin et al. 2013; Thieme et al. 2023). In the Baltic Sea, round goby continue to expand northward, and they have recently been documented in inland freshwater tributaries (Verliin et al. 2017). There is concern that round goby will negatively affect native fish populations in these freshwater habitats via competition and egg predation, and this is of special concern for rivers hosting spawning grounds for species with eggs left to develop unguarded, such as salmonids (Verliin et al. 2017; Wallin Kihlberg et al. 2024) or other non-caregiving fish (e.g., Common barbel, *Barbus barbus* Linnaeus, 1758). Our work suggests that barriers, such as hydropower dams or weirs, appear to have an additive effect slowing the spread of round goby. Whilst restoration of river systems including their connectivity should stay a key priority (Geist and Hawkins 2016), understanding the mechanisms of how barriers affect dispersal of undesired invasive species is useful for their management. Since round goby ultimately benefit from artificial rip-rap structures and other anthropogenic modifications of river systems (Brandner et al. 2018; Roche et al. 2021), conservation or restoration of functionally intact river habitats is key in limiting their population densities and impacts on native fauna (Pander et al. 2016; Ramler and Keckeis 2020). Although mitigation options are presently quite limited for most established invasive species, future work assessing a greater number of barriers and detailed descriptions of their features (e.g., flow, incline, substrate) will add to a growing body of literature optimizing the design of barriers and fish passage solutions that potentially limit round goby (and other invasive fish) passage.

Authors' Contributions

ESM: Conceptualization, Funding acquisition, Investigation, Writing original draft, Writing – review & editing Visualization; KMS: Formal analysis, Writing - review & editing; PB: Investigation, Resources, Writing – review & editing; TB: Resources; KB: Investigation, Resources, Writing – review & editing; AF: Funding acquisition, Writing – review & editing; JG: Investigation, Resources, Validation, Writing – review & editing; MJ: Investigation, Resources, Validation, Writing – review & editing; PJ: Investigation, Resources, Validation, Writing – review & editing; JP: Investigation, Resources, Validation, Writing – review & editing; JMM: Investigation, Validation, Writing – review & editing; APHB: Conceptualization, Funding acquisition, Formal Analysis, Investigation, Writing original draft, Writing – review & editing, Visualization.

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Ethics and permits

We sampled round goby from four European rivers in three countries, and all field collections were carried out under the appropriate licenses issued within each country (Czech Republic: JMK 153264/2020; Switzerland: authorizations for special fishing catches 01/02/2022 by Authority for Environment and Energy; Germany: electrofishing activities carried out under the license number 31-7562 issued by the district office of Freising, Bavaria, Germany).

Data availability

Species georeferenced records are available at the European Alien Species Information Network: https://easin.jrc.ec.europa.eu/easin/RJD/Download/5c243fea-47d6-489d-8d37-129fb35ff91b.

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Besides the first, second, and last authors, the remaining authors are ordered alphabetically and contributions should be considered relatively equal. We would like to thank the anonymous reviewers for the feedback on this publication.

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Supplementary material 1

Supplementary data

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Data type: xlsx

Explanation note: Supplementary data file containing the data and the Arelquin analysis output that were used in the analyses of the data presented here.

Link: https://doi.org/10.3391/ai.2025.20.3.152950.suppl1

Supplementary material 2

Supplementary figure and table

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Data type: docx

Explanation note: Word document containing a supplementary figure and table. figure S1 shows the model prediction of how the expected heterozygosity (He) of round goby populations decreases as sampling progresses upstream and approaches various invasion fronts. table S1 shows the marker polymorphism of 10 microsatellites used in this study for each of the 16 populations samples.

Link: https://doi.org/10.3391/ai.2025.20.3.152950.suppl2