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Title/Titill: The tale of the founder founder: assessing the origin of a recently established fsh in Icelandic waters

Year/Útgáfuár: 2025

Version/Útgáfa: Author's manuscript (pre-print)

Please cite the original version: Henke, T., Pálsson, S., Hemmer-Hansen, J. *et al.* The tale of the founder flounder: assessing the origin of a recently established fish in Icelandic waters. *Hydrobiologia* (2025). https://doi.org/10.1007/s10750-025-05838-w

Vinsamlega vísið til útgefnu greinarinnar:

Title: The tale of the founder flounder – Assessing the origin of an alien fish in Icelandic waters

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Abstract:

The geographic origin of an alien species is an important aspect of a species' invasion history used for classification and management plans but can additionally deliver information on introduction pathways that require the attention of managers and scientists. The invasion history of European flounder (*Platichthys flesus*), an alien flatfish species first documented in Icelandic waters in 1999, has not been fully elucidated. There have been different hypotheses on the European flounder's origin and introduction pathway, suggesting either a potentially natural arrival from the Faroe Islands or an introduction via ballast water from the coasts of northwestern Europe. To clarify the geographic origin of European flounder found in Iceland, we used microsatellite analysis of purposefully collected samples from 14 sites around Iceland and reference data stemming from previously published research of flounder population structure in the Faroese and other European populations. Our results indicate that the Faroese population is the most likely source of the European flounder found in Iceland. There was weak IBD between Icelandic samples and the Faroese population. Additionally, we identified 9 hybrids between alien European flounder and native European plaice (*Pleuronectes platessa*) and further documented signatures of introgression between the species. European flounder x European plaice hybrids are commonly found throughout the overlapping native range of both species but has previously not been documented in Iceland.

Keywords: biological invasions, origin, European flounder, microsatellite analysis, hybridization

Statements and Declarations:

Conflict of interest: The authors declare no conflict of interest.

Data availability: Data will be made public when the manuscript is accepted for publication.

Acknowledgements: We thank all the landowners and managers that provided recommendations and allowed us access to rivers and estuaries in order to collect European flounder samples. We furthermore would like to thank Michelle Valliant, Anja Nickel, Martin Jonés, Adéla Kelnerová, Norwenn Guilllerm, Karólina Mist Stefánsdóttir, and Kolbeinn Sæmundur Hrólfsson for helping out during the fieldwork. Theresa Henke was funded by Rannsóknasjóður (the Icelandic Research Fund, Grant number 239953-051).

Introduction:

As biological invasions continuously increase (IPBES 2023; Seebens et al. 2017), developing effective management approaches to prevent species' introductions becomes of paramount importance. Once a species arrives in a new environment and establishes a population that supplies further spread, the chances of eradication from the ecosystem become negligible for most taxa (IPBES 2023). The geographic origin of an alien species is a crucial part of a species' invasion history as alien species are, per definition, species that are introduced to new regions beyond the barriers of their native ranges under anthropogenic influence (IPBES 2023). While the geographic origin might not be the sole deciding factor for management, integrating knowledge on a species´ invasion history in management decisions will strengthen the outcome (Buckley et al. 2016). Critically, an understanding of the geographical origin of alien species can draw attention to potential pathways that require management to prevent future introductions (Carlton and Ruiz 2005; Chapman et al. 2015; Pyšek et al. 2013) and deliver potential insights into the level of invasiveness and impact of an alien species on native ecosystems (Paolucci et al. 2013; Pyšek et al. 2013). Population genetics has provided valuable information about biological invasions, including the identification of geographic origin of an alien species and the invasion dynamics within the invaded range (Lawson Handley et al. 2011; Sakai et al. 2001). For instance, using cytochrome (COI) sequencing to define the origin and the invasion route of the coffee berry borer (*Hypothenemus hampei*, Ferrari 1867) that is invading Hawaii (Chapman et al. 2015), or applying microsatellite analysis to investigate the invasion history of Carpobrotus, a wide-spread invasive genus of succulent plants (Novoa et al. 2023).

It is well established that biological invasions can have profound impacts on the recipient environment, most often affecting native species by inducing changes in the ecosystem as well as through competition and predation (IPBES 2023). Molecular tools have been used to study various genetic impacts of alien species (Lawson Handley et al. 2011). Alien species can impact native species via interspecific hybridization and subsequent introgression, introducing maladaptive genes or breaking down adaptive genotype combinations, especially in the case of rare species (Kelly et al. 2010; Kovach et al. 2015). Rapid spread and introgression of aliens can also contribute to undesired global homogenization (Olden et al. 2004). For example, Cutthroat trout (*Oncorhynchus clarkia*, Richardson 1836), a species that throughout its native range is threatened by the widespread invasive species rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) as hybridization and introgression is frequently observed (Kovach et al. 2015). Introgression between these two species is marked by changes in two traits, size at spawning and age of emigration as juveniles, producing larger cutthroat trout individuals that leave rivers at a younger age (Kovach et al. 2015). Despite documented selection against this rainbow trout introgression, fertile hybrids are produced which may eventually lead to an introgressed population and loss of adaptive cutthroat trout genetic variation (Allendorf and Leary 1988; Kovach et al. 2015).

European flounder (*Platichthys flesus*, Linnaeus 1758), a flatfish species native to the coastal regions of western Europe (Wilson and Veneranta 2019), was first documented in Iceland in 1999 (Jónsson et al. 2001) and is currently classified as potentially invasive (Thorarinsdóttir et al. 2014). The first documented sighting stemmed from the southwest of Iceland (Jónsson et al. 2001), and in 2000 catches were reported near the town of Höfn in southeast Iceland (Astthorsson and Palsson 2006). In the following years, a rapid spread of the species was documented clockwise along the southern and western coast to northern Iceland (Astthorsson and Palsson 2006; Gunnarsson et al. 2015; NA 2017), as well as counterclockwise from the southeast corner along the eastern coast, albeit at a slower speed (Henke et al. 2024; NA 2017). The arrival of the European flounder in Icelandic waters has sparked two opposing hypotheses on its source population. The initial hypothesis stated that the European flounder arrived from the Faroe Islands potentially via natural dispersal with currents (Jónsson et al. 2001; Thorarinsdóttir et al. 2014). European flounder in the Faroe Islands are considered native (Joensen and Tåning 1970) and represent the geographically closest population to Iceland. A later hypothesis suggested arrival of European flounder via ballast water from central Europe (Gunnarsson et al. 2015; Thorarinsdóttir et al. 2014). European flounder is previously known to have been introduced to the Great Lakes in North America via ballast water but failed to establish a population (Cudmore-Vokey and Crossman 2000; Ricciardi and MacIsaac 2000).

Despite a scarcity of studies evaluating the impact of European flounder on the Icelandic ecosystem, an overlapping niche use and potential competition with native fish species have been highlighted (Henke et al. 2020; Hlinason 2013; O´Farrell 2012). On nursery grounds in Icelandic waters, European flounder largely overlaps with juveniles of the native flatfish European plaice (*Pleuronectes platessa*, Linnaeus 1758) (Henke et al. 2020). Hybridization between European flounder and European plaice are known to occur both naturally and artificially and hybrids have been observed for instance at the western boundary of the Baltic Sea (He et al. 2020) and the Bornholm basin (Kijewska et al. 2009). *European flounder x European plaice* hybrids are considered to have an interspecies status as they show intermediate coloration (He et al. 2020). Based on morphological characteristics, hybrids are more similar to European plaice, although they can be differentiated by vertebrae counts and scale thorn patterns (He et al. 2020). It has been suggested that morphological classification could result in misidentification and in turn lead to errors in stock assessments and management (He et al. 2020; Kijewska et al. 2009).

Based on the premise of previously documented genetic difference between the Faroese populations and other European flounder populations, as well as low genetic divergence of other populations throughout Europe (Hemmer-Hansen et al. 2007), we aimed to 1) identify which of the two hypotheses was more likely: whether Icelandic European flounder is originating from the Faroe Islands, or from elsewhere in Europe possibly via ballast water by utilizing microsatellite analysis, 2) to analyze the species' spread dynamics within Iceland by exploring potential genetic structure of European flounder within Iceland, and 3) to assess whether there were any indications that introgression between European flounder and native European plaice had occurred in Iceland.

Methods

Sampling

Catches and reports of European flounder in Iceland indicated that it is most frequent and catchable in estuaries (Henke et al. 2024). The sampling plan for the current study was therefore based on reported sightings by recreational anglers (Henke et al. 2024), choosing 3 sites in each quarter of Iceland for the initial sampling conducted in 2020 (Table 1, Figure 1). For two sites, Önundarfjörður (n=29) and Borgarfjörður (n=19), samples were complemented by previously available individuals caught in the summer of 2017 (Table 1). In 2021, 4 sites Hvesta and Bjarnarfjarðará in northwest as well as Hörgá and Eyjafjarðará in north Iceland were sampled (Figure 1). In total, 16 sites around Iceland were sampled, and European flounder were caught in all of them apart from Hofsá in northeast Iceland (Figure 1). All sampling was done using a beach seine (1.5 x 10 m, mesh size = 6mm) aiming for a sample size of 20-30 individuals per locality.

Due to low sample sizes, the sites “Svarfaðardalsá”, “Hörgá” and “Eyjafjarðará” in Eyjafjörður (NE Iceland), were merged to the group “Eyjafjörður” (n = 9) (Table 1, Figure 1). The remaining samples ranged in size from 14 (Vatnsdalsá) to 35 individuals (Breiðdalsá and Varmá) (Table 1). For the European reference samples, we used microsatellite data for 224 European flounder individuals from four European sites, namely the Faroe Islands, the Irish Sea, Trondheim (Norway) and Thyborøn (Denmark), previously published in Hemmer-Hansen et al., (2007). We also used previously unpublished microsatellite data from 42 Icelandic European flounder collected in southwest Iceland in 2007 (Table 1).

As hybridisation of European flounder and European plaice is common (He et al. 2020; Kijewska et al. 2009), we also collected 114 European plaice from northwest, northeast, southeast and southwest Iceland with 27 – 30 individuals collected across 3-4 stations per region (Figure 1; Table 1). This sampling was conducted during the annual flatfish survey conducted by the MFRI in August 2021 and intended to identify potential hybrids in our samples of European flounder and to assess potential introgression between the species. During the same survey six European flounder were caught at three sampling stations in the southwest of Iceland (Figure 1; Table 1), these were pooled to one group and included in the analysis.

We created maps displaying the sampling locations for the Icelandic European flounder and European plaice using the *sf* package (Pebesma 2018; Pebesma and Bivand 2023) in R (R Development Core Team 2023). The underlying geospatial data for these maps was retrieved from the National Land Survey of Iceland (Iceland 2020).

Genotyping/microsatellite analysis

DNA was extracted using DNeasy extraction kits (QIAGEN) as well as Chelex (Estoup et al. 1996). Following the methodology of Hemmer-Hansen et al. (2007), all samples were scored for the nine microsatellites LIST 1001 (Watts et al. 1999) GenBank Accession no. AF149831), PL142 and PL167 (Hoarau et al. 2002) Accession nos. AF406750 and AF406751) and StPf1001, StPf1002, StPf1004, StPf1005, StPf1015 and StPf1022 (T.J. Dixon, J.B. Taggart, S. G. George, unpublished data, accession nos: AJ315970, AJ315975, AJ315973, AJ315974, AJ538212, AJ538320) on a SeqStudio Genetic Analyzer. All microsatellites were developed specifically for European flounder or successfully adapted from applications for European plaice (Hemmer-

Hansen et al. 2007). We included individuals originally genotyped in Hemmer-Hansen et al. (2007) in each run to ensure consistent and calibrated genotyping across studies.

Data preparation and statistics

We conducted initial data exploration and transformation for further analysis using the software GenAleX (version 6.503; (Peakall and Smouse 2006; Peakall and Smouse 2012). As high numbers of null alleles in the data can bias the analysis (Carlsson 2008), we determined the amount of null alleles using the *poppr* package (Kamvar et al. 2015; Kamvar et al. 2014) and excluded the locus PL142 due to a high amount of null alleles (19.76%, supplementary Table 1). As European plaice samples were not scored for StPf1015, this locus was omitted from analyses that concerned both species (i.e. analyses for hybridisation) but included in the analyses solely concerning the population structure of European flounder.

Due to the potential of hybridisation between European flounder and European plaice we analysed our samples for indications of hybridisation and introgression applying the Bayesian clustering approach implemented in the software STRUCTURE (version 2.3.4, Pritchard et al. (2000)). We grouped the Icelandic samples consisting of European flounder (excluding the reference samples collected in 2007, Table 1) and European plaice in three groups: 1) Icelandic European plaice (all adults); 2) pooled adult European flounder samples with an arbitrary size minimum set at 20 cm under the assumption that defining morphological characteristics will be clearly visible at this size; and 3) all remaining European flounder samples. We performed a STRUCTURE analysis using K = 2 and running 10 iterations with MIGPRIOR set to 0.05. We ran the analysis with prior population information (Popflag) for groups 1 and 2 and under the assumption of admixture for individuals without population information. The burn-in consisted of 100.000 runs followed by 200.000 Markov Chain Monte Carlo (MCMC) iterations. We additionally ran the analysis without MIGPRIOR and prior population information, but as this returned similar results, we based the identification of hybrids on the results of the initial analysis. We classified all samples with a q-value of less than 0.9 as hybrids. All hybrids as well as all cases of European flounder samples classified as “true” European plaice (q-value < 0.1 for European flounder cluster) (Supplementary Table 2) were excluded from all further analysis concerning the population structure of European flounder.

To explore the genetic diversity of the Icelandic samples, both European flounder and European plaice, as well as the reference populations, we calculated the number of alleles per group (A), allelic richness, expected heterozygosity (H_E), observed heterozygosity (H_O), and the inbreeding coefficient (F_{IS}) using the R-packages *hierfstat* (Goudet and Jombart 2022) and *pegas* (Paradis 2010) as well as *stats* and *base* (R Development Core Team 2023). We further analysed the genetic population structure of the European flounder by estimating genetic distance between sampling groups and reference populations as pairwise F_{ST} values and estimated the corresponding p-values using the *hierfstat* package (Goudet and Jombart 2022). As sequential Bonferroni correction using *hierfstat* package (Goudet and Jombart 2022) indicated no significant pairwise F_{ST} values between Icelandic sampling locations, we pooled all Icelandic samples together to examine the genetic distance between Iceland and the Faroe Islands as well as a pooled group consisting of the remaining three reference populations, i.e. Irish Sea, Thyborøn, and Trondheim (ITT group). To visualise the patterns in genetic distances between all sample groups, we generated a multidimensional scale (MDS) plot.

To identify potential clusters of individual European flounder, we ran a discriminant analysis of Principal Components (DAPC) with the number of retained principal components identified applying crossvalidation using the *adegenet* package (Jombart 2008; Jombart and Ahmed 2011). To further investigate the population structure of European flounder and infer the most likely number of populations (K) present in the data, we performed further Bayesian clustering analyses with STRUCTURE (version 2.3.4, Pritchard et al. (2000)). Under the assumption of admixture and correlated allele frequency, we ran these analyses for K = 1-6 with 10 iterations per K, and a burn-in of 100.000 runs followed by 200.000 MCMC iterations. Using the software STRUCTURE harvester (Earl and vonHoldt 2011), we identified the most likely K using the method of Evanno et al. (2005).

To explore the population structure within Iceland and to identify potential points of introductions we conducted an isolation by distance analysis by plotting the genetic distance between sampling groups against the geographic distance. We calculated the genetic distance as $F_{ST} /(1- F_{ST})$ according to Rousset (1997) and approximated the geographic distance between populations using a measuring tool available at www.map.is. We conducted these measurements within the known distribution range of European flounder along the southern and western coasts of Iceland as well as large parts of the northern and eastern coasts (Henke et al. 2024). As the spread of the European flounder to the northeast corner of Iceland has only recently been confirmed in 2023 (Henke et al. 2024), we conducted the distance measurements excluding this area under the assumption that spread between samples has not occurred through here, i.e. the furthest distance was between Eyjafjörður in the north along the western and southern coasts to Norðfjarðará in the east, following the Icelandic coastline to mirror the habitat utilization of European flounder of staying within coastal waters. We tested for significance by applying the Mantel test using the *Vegan* package (Oksanen et al. 2022). To examine whether the genetic distance between Icelandic populations and the source population (i.e. Faroese population) increases with geographic distance to the source population in the Faroe Islands, we calculated linear regression between pairwise genetic and geographic distances between sampling locations using the *stats* package (R Development Core Team 2023). We furthermore calculated linear regression between the average allelic richness across all eight loci in each sample and the according geographic distance to the Faroese population, as well as the expected heterozygosity and the geographic distance, to examine for potential population bottlenecks within Iceland.

Results

In total, 328 European flounder samples and 114 European plaice samples were collected in Iceland for this study (Table 1) and scored for the nine loci. These samples were complemented by the data of 266 European flounder samples, scored for the same nine loci, representing populations from the Faroe Islands, Thyborøn (Denmark), the Irish Sea, Trondheim (Norway) (Hemmer-Hansen et al. 2007), and Iceland (collected in 2007). Locus PL142 showed a high number of null alleles (19.76 %) with more than 45% of data missing for the sites Borgarfjörður, Breiðdalsá, Ölfusá, and Önundarfjörður (Supplementary Table 1).

European plaice and European flounder hybridisation

The Bayesian clustering analysis run on the three groups of Icelandic European plaice (group 1) and European flounder (group 2 – adults) and European flounder (group 3 – juveniles) using prior population information revealed 23 individuals that had been morphologically classified as European flounder (Vatnsdalsá = 19; Norðfjarðará = 3, Eyjafjörður = 1) but scored a q-value of less than 0.1 and were consequentially classified as European plaice (Figure 2, supplementary Table 2). Furthermore, 7 individuals previously classified as European flounder (MFRI = 1; Norðfjarðará = 1; Varmá = 1, Breiðdalsá = 1; Vatnsdalsá = 2; Eyjafjörður = 1) as well as 2 individuals previously classified as European plaice were identified as likely *European flounder x European plaice* hybrids ($0.1 < q < 0.9$) (Figure 2; supplementary Table 2). That represents 1.8 and 2.0 percent for European plaice and European flounder, respectively, or a similar frequency of hybrids identified for both species. We subsequently omitted all 32 individuals that were identified as European plaice or hybrids from analysis specific to European flounder population structure (Figure 1).

European flounder descriptive statistics

For European flounder in Iceland, the average number of alleles per sampling group ranged from 3.0 to 5.1 and H_E ranged from 0.454 to 0.546. The F_{IS} coefficient only significantly deviated from 0 for the Ölfusá samples, where we recorded a heterozygote deficiency (Supplementary Table 1). The average number of alleles across loci was higher in the reference populations than in Iceland, ranging from 6.3 for the Faroese population to >10 for the remaining three populations with Thyborøn showing the highest number of alleles with 10.9 (Supplementary Table 3). Expected heterozygosity (H_E) ranged from 0.583 to 0.71 and there were two instances of heterozygote deficiency detected for the Faroese and Irish Sea population but no significant F_{IS} values within the reference samples (Supplementary Table 1).

Genetic structure

The pairwise F_{ST} values ranged from 0 to 0.126 (Figure 3), but no values were significant after sequential Bonferroni correction. When pooling the samples into three groups "Iceland", "Faroe Islands", and "ITT" the pairwise F_{ST} values between groups were Iceland – Faroe Islands = 0.039 ($p = 0.01$), Iceland – ITT = 0.114 ($p = 0.01$), and Faroe Islands – ITT = 0.056 ($p = 0.01$) (Supplementary Table 4) and was significant after sequential Bonferroni correction (all 0.03, supplementary table 4). The genetic distances reflected the geographic distances of the Faroese and Icelandic populations from the mainland of Europe, as summarized by the MDS plot, where the Faroe Islands were placed between the clusters of Icelandic samples and the ITT populations (Figure 4). The results of the DAPC revealed a similar pattern but with a closer connection between the Icelandic samples and the Faroese population than of the Faroese to the ITT samples (supplementary Figure 1). STRUCTURE harvester (Earl and von Holdt 2011) results suggested a most likely number of groups as 2. Examining $K = 2$, there was a clear distinction between Iceland and the ITT sites (Figure 5). While Icelandic individuals were predominantly assigned to group 1, the ITT individuals were overwhelmingly assigned to group 2. The Faroese population placed in-between as an admixed population with both clusters present (Figure 5). When increasing K to 3, Icelandic individuals show admixture of group 1 and 2, while the Faroese mainly assigned to group 1 and ITT to group 3 (Figure 5).

Genetic variation within Iceland

Considering all Icelandic European flounder groups, the isolation by distance analysis returned no significant result (Mantel test: $R^2 = 0.1414$, $p = 0.123$). When excluding the groups MFRI and Eyjafjörður from the analysis, due to being strong outliers in the MDS, the results remained non-significant (Mantel test: $R^2 = 0.209$, $r = 0.055$) (Figure 6 A). However, linear regression between geographic (i.e. measured distance in km between each Icelandic sample and the Faroese population) and genetic (i.e. pairwise F_{ST} values for each Icelandic sample with the Faroese population) distance between Icelandic samples and the Faroese population showed that genetic distance significantly increased with geographic distance ($R^2 = 0.34$, $p = 0.0207$; Figure 6B). Conversely, allelic richness in Icelandic groups was not significantly correlated with the geographic distance to the Faroese population ($p = 0.7045$), neither was the expected heterozygosity ($p = 0.664$).

Discussion

In this study we explored the geographic origin of European flounder found in Icelandic waters based on the two hypotheses of natural colonisation or introduction with ballast water (Jónsson et al. 2001; Gunnarsson et al. 2015). We have shown that the native Faroese population of European flounder represents the most likely source population. There was limited population structure within Iceland indicative of a broad and rapid spread whereas significant IBD between Icelandic samples and the Faroese population suggests stepwise colonization. Moreover, there were clear signs of hybridisation between alien European flounder and native European plaice as well as indications of introgression between the species.

Geographic origin of Icelandic European flounder

Contrasting Icelandic samples against reference data stemming from four European populations within the species' native range (Hemmer-Hansen et al. 2007) highlighted that European flounder found in Icelandic waters most likely originated from the Faroe Islands. Since the first official documentation of the species in 1999 (Jónsson et al. 2001) two different hypotheses had been raised addressing where it arrived from. While it had been suggested that European flounder arrived from the Faroe Islands (Jónsson et al. 2001; Thorarinsdóttir et al. 2014), potential arrival via ballast water from western Europe has also been discussed (Gunnarsson et al. 2015; Thorarinsdóttir et al. 2014). The study of Hemmer-Hansen et al.

(2007) indicating a genetic difference between the Faroese and other European populations offered a valuable opportunity to address the origin of this alien species using established methodology. The current analyses show that Icelandic populations are genetically more closely related to the Faroese population than to any other population in Europe.

In the Faroe Islands, European flounder is a native species (Joensen and Tåning 1970). The Faroese population shows lower allelic richness in comparison to other European populations and Hemmer-Hansen et al. (2007) argued that this reduced allelic richness was the result of a founding event after the last glaciation and is upheld by restricted geneflow due to oceanographic and bathymetric barriers. The comparatively lower allelic richness found in Icelandic European flounder suggests that another founding event took place upon the species' arrival in Iceland. Reduced genetic diversity can be a limiting factor for the successful establishment of an alien species (Lawson Handley et al. 2011). However this has not been the case for , European flounder in Iceland that has successfully established, a population that has rapidly spread to most regions of the country (Henke et al. 2024). Throughout its native range, European flounder is known to show high plasticity in the utilization of different aquatic habitats along salinity gradients, including full saline, estuary and freshwater habitats (Daverat et al. 2012; Le Pichon et al. 2014). This plasticity could have facilitated the establishment of European flounder in Iceland.

Invasion dynamics of European flounder in Iceland

Our results show isolation by distance based on samples from Iceland and the likely origin of the Faroe Islands, but no population structure within Iceland. Investigating the population structure of an alien species within the invaded range can provide insights into the invasion dynamics, that is, inferences on the invasion routes of alien species have traditionally been made by using two types of methods, direct methods based on current and historical species' observations and indirect methods using genetic patterns based on molecular analyses (Lawson Handley et al. 2011). For example, Rato et al. (2023) used microsatellites to explore the colonization routes of an alien gecko in the Mediterranean. Their study revealed that while the Iberian Peninsula has historically been the main source for individuals spreading, most introductions that occur currently stem from mainland Italy and are likely driven by the plant nursery industry (Rato et al. 2023).

The current analysis shows a significant relationship between genetic and geographic distances when comparing Icelandic to Faroese sampling locations and a weak (although non-significant) isolation by distance within Iceland. These results suggest that a stepwise process could have partially contributed to the spread of flounder within Iceland and/or effects from contemporary gene flow from the Faroese source population. Stepwise colonization processes have been documented for other introduced species such as the invasive yellow fever mosquito (*Aedes aegypti*) in North America (Pless et al. 2022). Understanding where in Iceland European flounder first arrived would provide additional information that could contribute to assessing the most likely introduction pathway. Although, the flounder source population most likely originated from the Faroe Islands they could have arrived in Iceland either by introduction via ballast water or by natural dispersion. However, any genetic analyses could further be complemented by combining them with direct methods in an interdisciplinary approach. Henke et al. (2024) has shown the benefits of combining local ecological knowledge with traditional scientific sampling when reconstructing the current spread of European flounder in Iceland. By building upon the methodology applied in Henke et al. (2024) with particular focus on the temporal factor of species' observations, the resulting distribution data could be incorporated with more detailed information on the invasion dynamics suggested by further genetic analyses for a better understanding how the European flounder spread throughout Iceland.

Future classification of European flounder in Iceland

The remaining uncertainty on how flounder arrived in Iceland, by natural dispersion or introduction via ballast water, has implications on the classification of European flounder in Iceland. Given the common definition of alien species as "a species whose presence in a region is attributable to human activities that have enabled it to overcome the barriers that define its natural range" (IPBES, 2023), European flounder would no longer be considered an alien species in the case of natural dispersal. When the dynamics of an alien species' introduction remain unclear, but one or more pathways of introduction are known to be possible for this species, it is considered polyvectic (Carlton and Ruiz 2005). We argue that neither potential introduction pathway can be confidently excluded, and therefore propose to preserve the current classification as "potentially invasive" (Thorarinsdóttir et al. 2014) with the addition of the term "polyvectic".

Interspecific hybridisation between European flounder and European plaice

We identified 9 hybrids among the Icelandic samples, of which two were initially morphologically identified as European plaice and 7 as European flounder as well as signatures of introgression beyond first generation hybrids in all 9 individuals. In Iceland, interspecific hybridisation between fish species has been observed between European eel (*Anguilla Anguilla*) and American eel (*Anguilla rostrata*) (Pujolar et al. 2014) and has frequently been discussed in the context of escaped farmed salmonids caught in streams alongside native salmonids (Guðmundsson et al. 2017; Guðmundsson et al. 2023). Identifying European flounder x European plaice hybrids among the samples is in line with the literature, as this is a well-known phenomenon throughout the species' mutual native range (He et al. 2020; Kijewska et al. 2009). The geographical distribution of the identified hybrids currently does not appear to be linked to a specific pattern. Adult hybrids, initially morphologically identified as either European flounder or European plaice, were found in samples along Iceland's east and west coast. Juvenile hybrids were identified in northern and eastern Iceland. No samples provided more than one hybrid, except for Eyjafjörður in northern Iceland where two juvenile hybrids were detected (despite a low total sample size of 9). However, the current sampling includes considerable variation in European flounder size as well as both freshwater and marine environments, complicating inferences on these dynamics. Future studies should include specifically designed sampling to assess patterns in the hybridization between European flounder and European plaice in more detail.

Interspecific hybridisation and introgression can cause severe negative impacts in the context of biological invasions (Huxel 1999). Concerning the hybridisation between European flounder and European plaice, it has been suggested that potential misidentifications of pure specimens when solely morphologically analysed, could lead to errors in stock assessments and the development of management strategies as hybrids are more likely to resemble European plaice (He et al. 2020; Kijewska et al. 2009). Potential consequences of the observed hybridization and introgression among the two species in Iceland will have to be assessed in future studies.

Conclusion

Population genetic studies addressing biological invasion can greatly expand our knowledge on different aspects of an alien species' invasion history. In this study we showed that the native Faroese population of European flounder was a likely source for alien European flounder found in Iceland. However, our results only allowed for limited inferences on the invasion dynamics of this species within Iceland and should be further investigated using higher resolution genetic methods, and sampling of different life-stages, in order to gain additional insights on where and potentially how European flounder first arrived in Iceland. Moreover, hybrids of European flounder x European plaice were identified as well as signs of introgression between the species.

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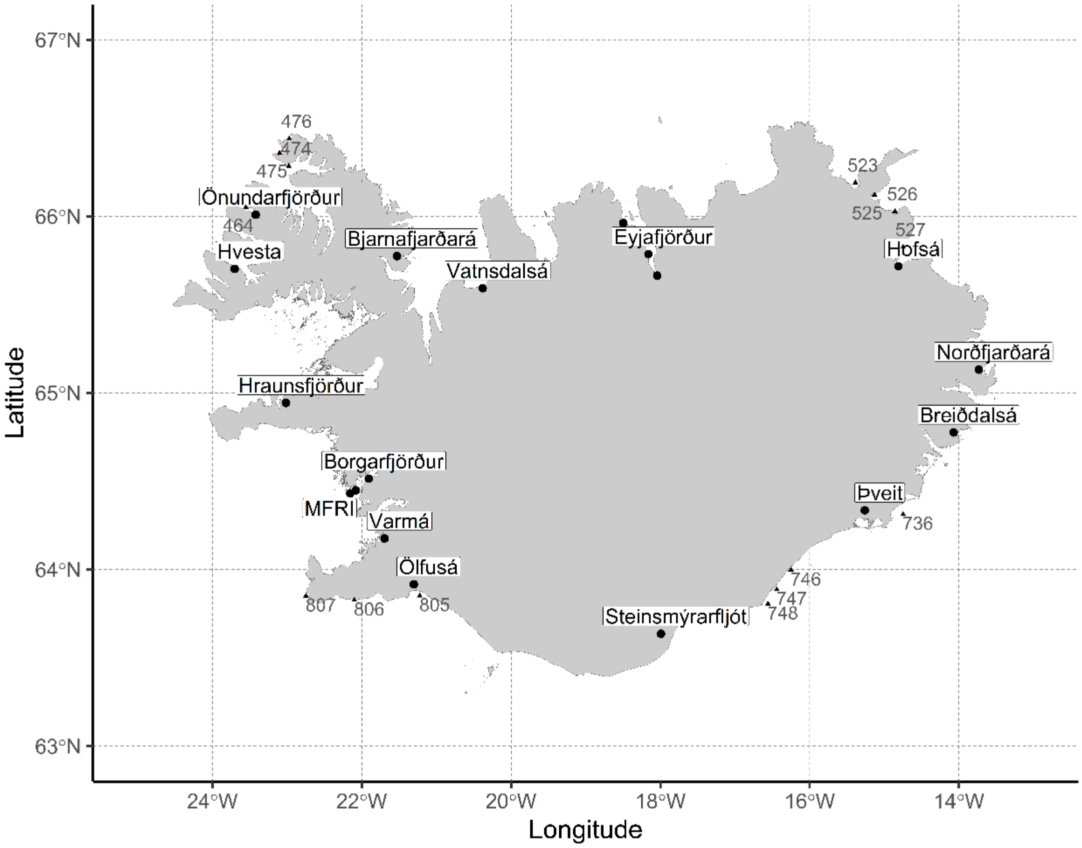


Figure 1 Sampling locations in Iceland, consisting of sampling sites for European flounder marked in black site names as well as sampling sites for European plaice marked in grey numbers representing trawling stations of the MFRI flatfish survey.

Table 1: Sampling information for each site including date, coordinates, sample size (n) and, where available, salinity, as well as size range and mean size of individuals per sample. Information on the numbers of putative hybrids of European flounder samples identified as European plaice is presented in supplementary Table 2.

Sampling site	*Region*	*Date*	*Coordinates (Lat/Lon).*	*n*	*Salinity*	*Size (cm) Range (Mean)*
Icelandic *P. flesus*						
Ölfusá	SW Iceland	05.07.2020	63.9172, -21.3041	**34**	0	2 – 21.9 (6.7)
Varmá	SW Iceland	06.09.2020	64.1766, -21.6955	**34**	4.8	2 – 21.3 (4.2)
Borgarfjörður	SW Iceland	05.07.2020	64.5154, -21.9063	**11(19)**	23	6.6 – 24.4 (10.7)
MRFI samples	SW Iceland	2021		**6**		25 – 46 (35.25)

Station 845		25.08.2021	64.4487, -22.0817	2		25 – 32 (28.5)
Station 846		26.08.2021	64.4323, -22.1555	3		30 – 46 (40.5)
Station 849		26.08.2021	64.7972, -23.5590	1		41
Hraunsfjörður	NW Iceland	06.07.2020	64.9446, -23.0154	**26**	1.8	5.4 – 25.9 (10.8)
Hvesta	NW Iceland	17.08.2021	65.7029, -23.7036	**30**	0	2.9 – 5.5 (4.3)
Önundarfjörður	NW Iceland	2017	66.0102, -23.4218	**(29)**		2.6 – 11.4 (6.3)
Bjarnarfjörður	NW Iceland	20.08.2021	65.7755, -21.5281	**28**	0	3.5 – 11.2 (5.9)
Vatnsdalsá	NW Iceland	09.07.2020	65.5932, -20.3819	**14**	0.6	5.4 – 31.6 (24.4)
Eyjafjörður (pool)	NE Iceland			**9**	0	3.5 – 35.5 (13.1)
Svarfaðardalsá		17.08.2020	65.9624, -18.4967	1	0.06/0.6	
Hörgá		12./13.08.2020	65.7863, -18.1604	3	9	
Eyjafjarðará		12.08.2021	65.6641, -18.0425	5	9	
Nordfjarðará	NE Iceland	19.08.2020	65.1328, -13.7351	**25**	17	2.8 – 29.1 (21.9)
Breiðdalsá	SE Iceland	20.08.2020	64.7771, -14.0715	**35**	10.6	6 – 29.4 (21.6)
Þveit	SE Iceland	20.08.2020	64.3362, -15.2617	**19**	0	5 – 26.2 (13.6)
Steinsmýrarfljót	SE Iceland	21.08.2020	63.6368, -17.9919	**29**	0	6.6 – 11.8 (9.3)
Icelandic *P. platessa*						
SW *P. platessa*	SW Iceland	2021		**30**		
Station 805			63.8522, -21.2233	10		
Station 806			63.828, -22.1003	10		
Station 807			63.8512, -22.747	10		
NW *P. platessa*	NW Iceland	2021		**29**		
Station 464			66.0505, -23.5523	9		
Station 474			66.2852, -22.9787	4		
Station 475			66.3582, -23.1023	10		
Station 476			66.44, -22.9722	6		
NE *P. platessa*	NE Iceland	2021		**27**		
Station 523			66.1907, -15.3877	3		
Station 524			66.1213, -15.1323	4		
Station 526			66.0278, -14.8578	10		
Station 527			65.826, -14.7488	10		
SE *P. platessa*	SE Iceland	2021		**28**		
Station 736			64.3135, -14.7493	10		
Station 746			63.9983, -16.2503	2		
Station 747			63.8873, -16.4415	8		
Station 748			63.8062, -16.5598	8		
Reference *P. flesus*						
Iceland 2007	Iceland		-	**42**		
Faroe Islands	Faroe Island		62, -6.45	**78**		
Thyborøn	Denmark		57, 8	**48**		
Irish Sea	Ireland		54, -4	**49**		
Trondheim	Norway		64.6, 11	**49**		

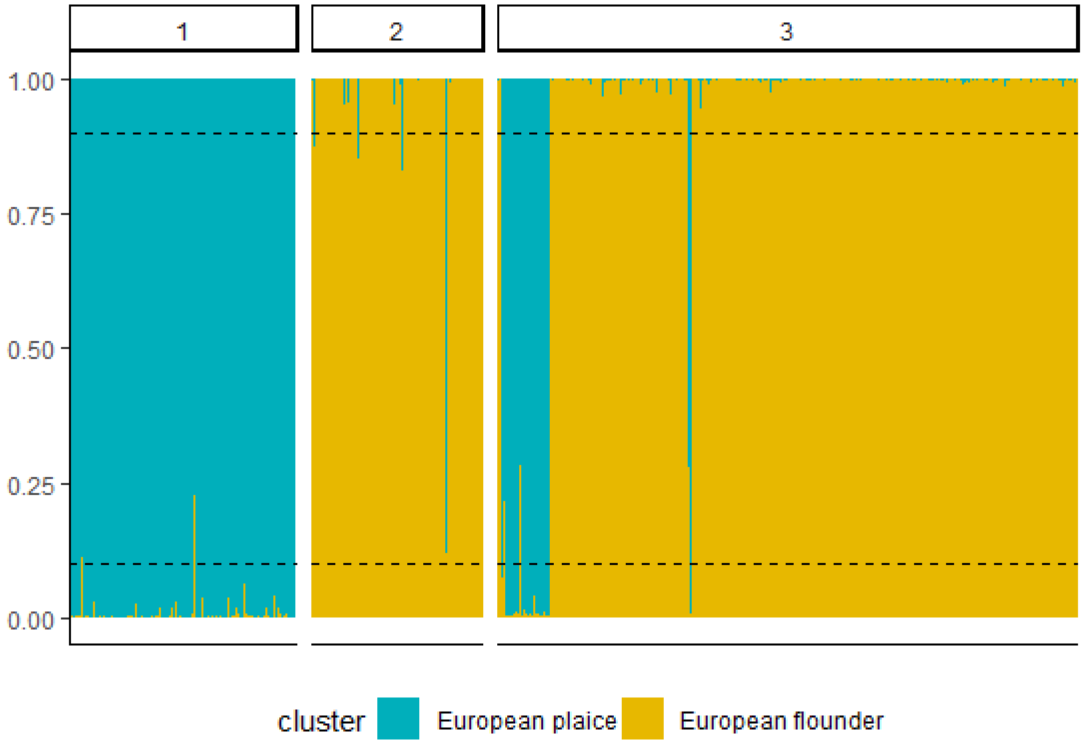
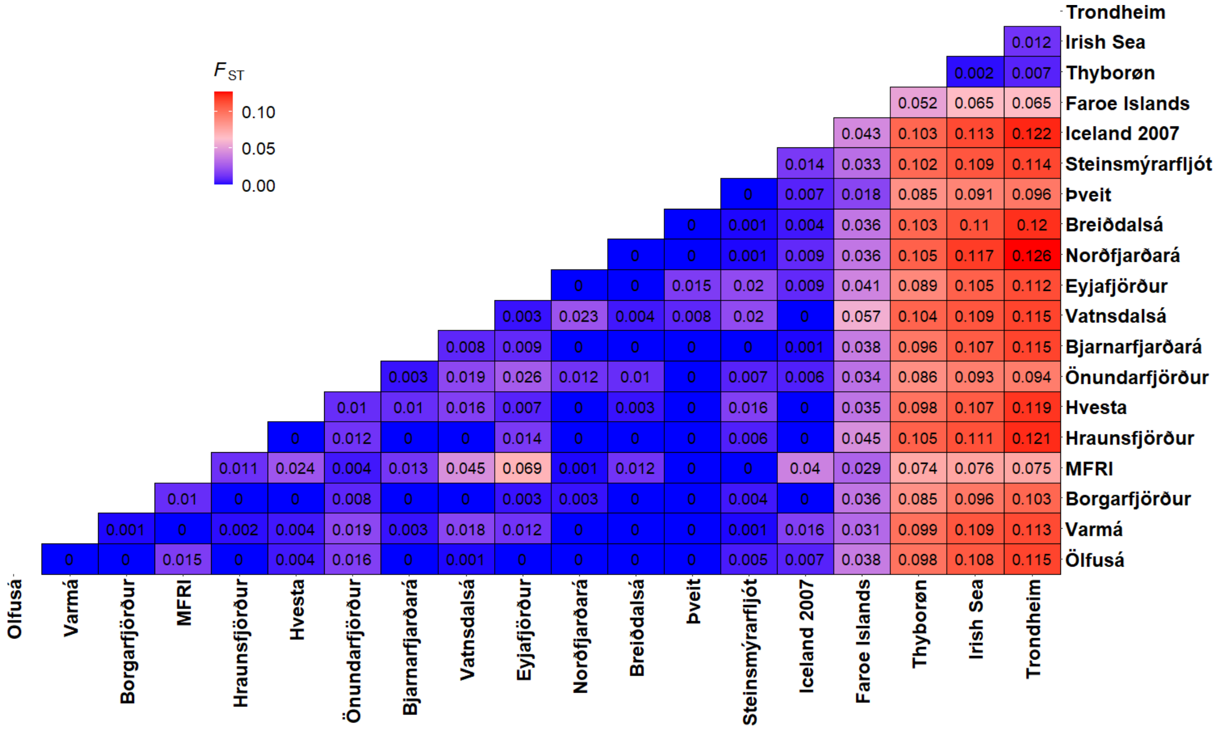


Figure 2. Barplots of genetic assignments of Icelandic European flounders and European plaice individuals to two clusters (K) based on microsatellite variation, using a Bayesian assignment test with prior population information. Pooled groups are indicated by numbers with 1 = adult European plaice; 2 = adult European flounder (> 20 cm); and 3 = remaining European flounder. Dashed lines set at 0.1 and 0.9 indicate the threshold q-values used to classify an individual as a hybrid.



	Olfusá	Varmá	Borgarfjörður	MFRI	Hraunsfjörður	Hvesta	Önundarfjörður	Bjarnarfjarðará	Vatnsdalsá	Eyjafjörður	Norðfjarðará	Breiðdalsá	Þveit	Steinsmýrarfljót	Iceland 2007	Faroe Islands	Thyborøn	Irish Sea	Trondheim
Trondheim																			
Irish Sea																			0.012
Thyborøn																		0.002	0.007
Faroe Islands																	0.052	0.065	0.065
Iceland 2007																0.043	0.103	0.113	0.122
Steinsmýrarfljót															0.014	0.033	0.102	0.109	0.114
Þveit														0	0.007	0.018	0.085	0.091	0.096
Breiðdalsá													0	0.001	0.004	0.036	0.103	0.11	0.12
Norðfjarðará												0	0	0.001	0.009	0.036	0.105	0.117	0.126
Eyjafjörður											0	0	0.015	0.02	0.009	0.041	0.089	0.105	0.112
Vatnsdalsá										0.003	0.023	0.004	0.008	0.02	0	0.057	0.104	0.109	0.115
Bjarnarfjarðará									0.008	0.009	0	0	0	0	0.001	0.038	0.096	0.107	0.115
Önundarfjörður								0.003	0.019	0.026	0.012	0.01	0	0.007	0.006	0.034	0.086	0.093	0.094
Hvesta							0.01	0.01	0.016	0.007	0	0.003	0	0.016	0	0.035	0.098	0.107	0.119
Hraunsfjörður						0	0.012	0	0	0.014	0	0	0	0.006	0	0.045	0.105	0.111	0.121
MFRI					0.011	0.024	0.004	0.013	0.045	0.069	0.001	0.012	0	0	0.04	0.029	0.074	0.076	0.075
Borgarfjörður				0.01	0	0	0.008	0	0	0.003	0.003	0	0	0.004	0	0.036	0.085	0.096	0.103
Varmá			0.001	0	0.002	0.004	0.019	0.003	0.018	0.012	0	0	0	0.001	0.016	0.031	0.099	0.109	0.113
Ölfusá		0	0	0.015	0	0.004	0.016	0	0.001	0	0	0	0	0.005	0.007	0.038	0.098	0.108	0.115

Figure 3 Heatmap indicating the pairwise F_{ST} -values between Icelandic and reference populations. Pairwise F_{ST} -values are shown in different colors ranging from blue representing low values to red representing high values. None of the associated p-values remained significant after applying sequential Bonferroni correction

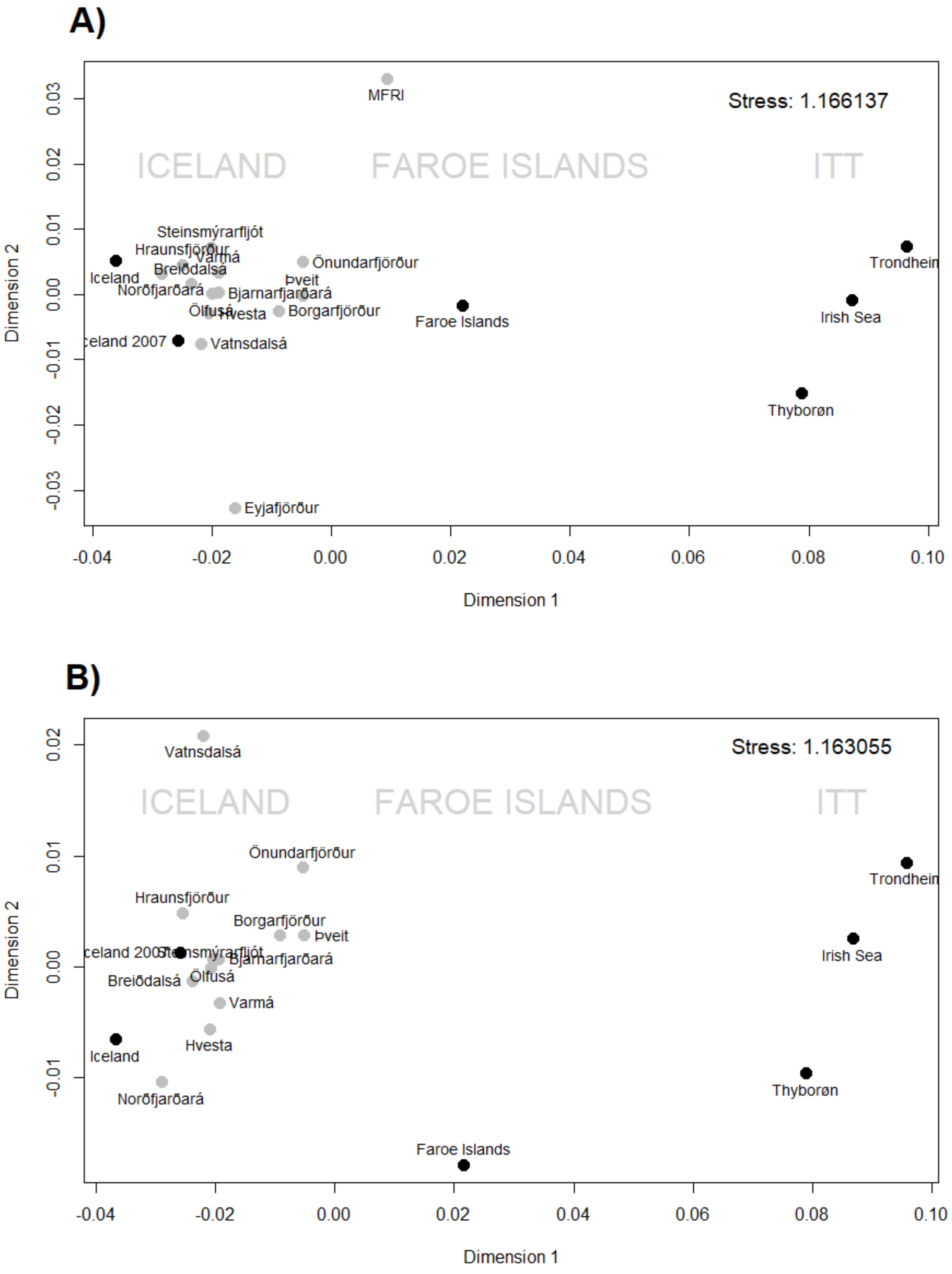


Figure 4. A) MDS plot presenting the genetic distance between Icelandic, Faroese and ITT populations. B) In order to display the genetic distances between Icelandic populations in more detail, we excluded the two clear outliers (MFRI and Eyjafjörður) from A. For both plots the stress value is displayed within the graphs. Plotting dimension 3 did not provide additional insights and was therefore not included.

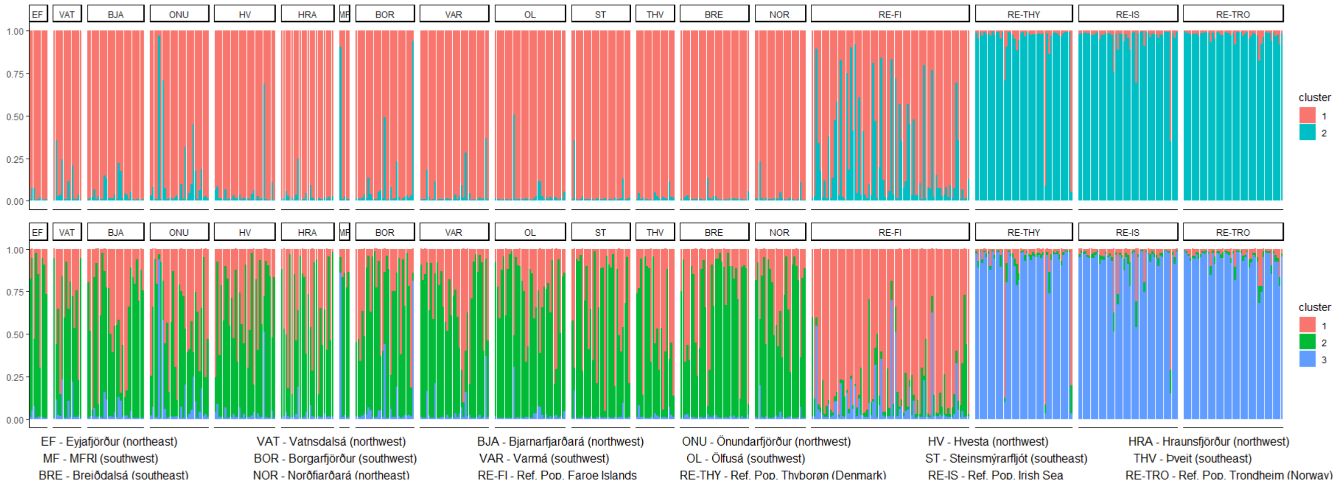


Figure 5. Barplots of the admixture proportions for each individual indicating the genetic structure of European flounder in the 14 samples from Iceland as well as the reference populations.

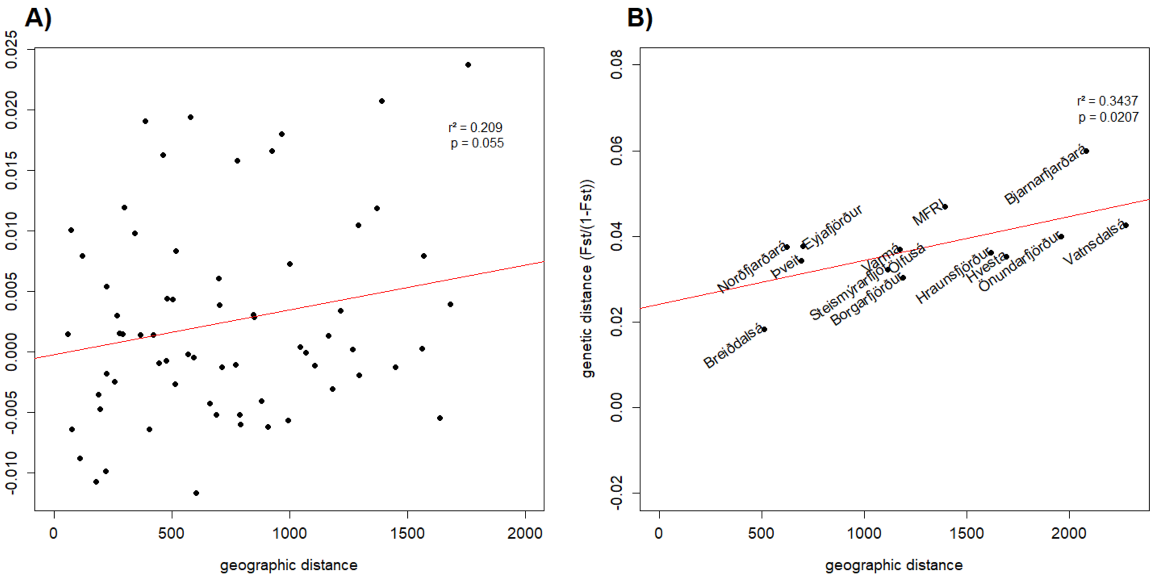


Figure 6: The plots illustrate the results of the Isolation by distance. (A) Genetic distances between Icelandic samples (F_{ST} /(1- F_{ST}) according to (Rousset 1997) are plotted against the geographic distance (obtained as described in methods) for all Icelandic populations excluding the previous outliers Eyjafjörður and MFRI. (B) We specifically display the genetic distance between each Icelandic sample and the source population in the Faroe Islands in relation to the according geographic distance.