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Canadian Science Publishing

ARTICLE

Population genetics reveal patterns of natural colonisation of an ecologically and commercially important invasive fish

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Abstract: Although historical records of introductions that trigger successful biological invasions are common, subsequent patterns of dispersal and colonisation routes are unclear. We use microsatellites to examine genetic population structuring of established invasive brown trout (*Salmo trutta*) populations in Newfoundland, Canada, for evidence of "natural" dispersal, human-mediated introductions, and colonisation routes. We also explored ancestry of contemporary populations relative to presumed progenitors. Results analysed using STRUCTURE, DAPC, a NJ tree and F_{ST} comparisons support records of historical introductions; current Newfoundland populations are largely descended from Scottish stock, with St. John's the primary introduction site. Subsequent dispersal of these trout was facilitated principally by anadromy, largely consistent with a classic stepping-stone model, with significant isolation-by-distance. With one exception, dispersal along the north and south coasts of the Avalon peninsula appears to be natural and independent, involving stochastic processes resulting in unique outcomes for population composition. This study is a good example of dispersal patterns during a contemporary invasion underscoring the potential for non-anadromous founders to re-express anadromy, facilitating colonization of distant sites.

Résumé : Bien que les cas documentés d'introductions qui déclenchent des envahissements biologiques durables soient répandus, les motifs de dispersion et les voies de colonisation subséquents ne sont pas bien établis. Nous utilisons les microsatellites pour examiner la structure génétique de populations de truites brunes (*Salmo trutta*) envahissantes établies à Terre-Neuve (Canada) afin de chercher des indices de dispersion « naturelle » et d'introductions médiées par l'humain et de l'information sur d'éventuelles voies de colonisation. Nous explorons également l'ascendance de populations actuelles par rapport à des progéniteurs présumés. Les résultats analysés avec STRUCTURE, DAPC, un arbre phylogénétique et la comparaison de FST appuient les cas documentés passés d'introductions; les populations actuelles à Terre-Neuve descendent en bonne partie du stock écossais, St. John's en constituant le principal lieu d'introduction. La dispersion subséquente de ces truites a principalement été facilitée par l'anadromie, ce qui concorde assez bien avec un modèle classique d'étapes intermédiaires, l'isolement par la distance étant aussi importante. À une exception près, les dispersions le long des côtes nord et sud de la péninsule d'Avalon semblent être naturelles et indépendantes, mues par des processus stochastiques qui ont produit des résultats uniques sur le plan de la composition des populations. L'étude fournit un bon exemple de motifs de dispersion durant un envahissement moderne, soulignant la possibilité pour des fondateurs non anadromes de réexprimer une anadromie, facilitant ainsi la colonisation de sites distaux. [Traduit par la Rédaction]

Introduction

The distribution of species through time and space is the result of the complex interplay between evolutionary history and ever-changing ecological factors. Identifying the colonisation pathways and mechanisms involved in these changes, and the consequences for population genetic diversity, are important both for understanding present day species and population distributions, and for predicting potential future responses to anthropogenic climate change (Elton 1958; Lockwood et al. 2013). Even for the most recent major global species distribution changes, after the retreat of glaciers at the end of the Pleistocene, a significant amount of evolutionary information will have been lost. Hundreds, or even thousands, of generations will have passed since the events of interest and, hence, many relevant evolutionary processes that would have been active in that time will not be easily detected. Recent species range expansions, whether natural or human-induced, provide useful contemporary analogues for studying the evolutionary trajectory of such events. Contemporary patterns of genetic variability, in the case of invasive

Received 9 July 2020. Accepted 27 March 2021.

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species, can offer useful insights into these processes, which may have major relevance as species respond to contemporary environmental change.

Here, we investigate the genetics of introduction, subsequent patterns of dispersal and colonisation routes of brown trout *Salmo trutta* on the island of Newfoundland, eastern Canada, as a model of species range expansion. *Salmo trutta* is an excellent species for such an investigation since it has been successfully introduced worldwide from its native western Palaearctic distribution, chiefly for sports angling, and usually with good written records (Jonsson and Jonsson 2011; Ferguson et al. 2019*b*; Lobón-Cerviá et al. 2019). The species has also been extensively studied genetically, both within and outside its native range (see Lobón-Cerviá and Sanz 2018 for a recent review). These, and many other studies, have demonstrated high genetic variability within its native range relative to other salmonid species and its remarkable capacity to adapt to changing environments (Prodöhl et al. 2019). Importantly, individuals can either remain largely resident within their natal streams or lake (lake or river resident), undertake migrations to larger rivers (fluvial-adfluvial), lakes (lacustrine-adfluvial), or estuaries (semi-anadromy) or to the sea (anadromy) (Ferguson 2006; Ferguson et al. 2019*a*). Non-anadromous populations are confined to individual catchments, as dispersing among catchments would require moving via sea-migration, and so lack interconnections unless there is human intervention, such as in stocking. In contrast, the anadromous forms can colonise other catchments following the marine phase. Where they occur in sympatry, alternative life-history forms are typically not reproductively isolated, and the expression of alternative migratory tactics is mediated by complex gene-environment interactions that may involve a mix of a few major-effect and many minor-effect genetic loci (Dodson et al. 2013; Ferguson et al. 2019*a*). While within the species' native range, current evidence suggests very little effective gene flow between populations because of geographical barriers, strong natal homing and local adaptation (Quéméré et al. 2016; Prodöhl et al. 2017, 2019); the situation may be different during initial colonisation of virgin habitats. Recent evidence in Pacific salmonids has revealed density-dependent rates of straying (natal dispersal) consistent with collective navigation resulting from social interactions (Berdahl et al. 2016).

Detailed records exist of the introduction of *S. trutta* to Newfoundland more than a century ago, during an approximately 25-year period, to sites near the capital St. John's (Fig. 1) and of the putative subsequent natural dispersal of the species along the north and south coasts of the Avalon Peninsula (Westley and Fleming 2011). According to records, nearly all these introductions were from the Howietoun hatchery in Scotland, with much of the founding broodstock coming from Lough Leven in eastern Scotland (but see Westley et al. 2013). This extensive historical record provides a powerful background against which to assess present-day patterns of genetic variability in the colonised range: contemporary samples can be collected from the founding populations (Howietoun and Loch Leven) and primary Newfoundland introduction site (St. John's), followed by sampling regularly spaced catchments outwards along the likely expansion routes.

In the present study, we conducted such a sampling programme, genotyped the sampled fish at 16 putatively neutral microsatellite loci, and assessed the relative levels of genetic variability and divergence within and between sampled sites. We then tested for correlations between geographic and genetic distances among sites. Two major colonisation models, the stepping stone model and the island model have been reviewed by Allendorf and Luikart (2007). The stepping stone model, which is characterised by higher levels of genetic similarity between neighbouring populations along the colonisation route, and also by a reduction in genetic diversity in more recently colonised sites due to bottleneck effects, is regarded as indicative of natural colonisation from a single focus, whereas the island model; where all populations are derived independently from a single point of introduction, would be indicative of human intervention (in this case by stocking). Launey et al. (2010); in an extensive study of *S. trutta* colonising rivers in the Kerguelen Islands from three foci of introduction, described dispersals involving anadromous fish straying with no reported stocking, consistent with the stepping stone model. Westley and Fleming (2011); in an ecological based study, postulated the same method of colonisation for *S. trutta* in Newfoundland.

The two aims of the current study were to (1) use genetic evidence to test the validity of the reported European origin of the introduction to Newfoundland and (2) to investigate the dispersal patterns and colonisation routes of *S. trutta* in Newfoundland aiming to distinguish between natural and human-mediated colonisation, and to draw general inferences on the population genetics of invasive anadromous salmonid fish species.

Materials and methods

Study system

The island of Newfoundland (108 860 km^2; Fig. 1) is located on the northeast coast of North America. The island was completely covered by ice during the last glacial maximum (McPhail and Lindsey 1970); and today has numerous lakes and short, swift-flowing rivers that tend to be of low primary productivity (Bell and Liverman 2008). Native (defined here as natural post-glacial recolonisation) salmonid species are restricted to brook trout *Salvelinus fontinalis*, Atlantic salmon, *Salmo salar* and Arctic char *Salvelinus alpinus*. Brown trout *S. trutta* were introduced to the St. John's area, on the east side of the island's Avalon Peninsula (Fig. 1), between 1883 and 1906, to enhance the fisheries of the region (Westley and Fleming 2011), with their subsequent dispersal as described above.

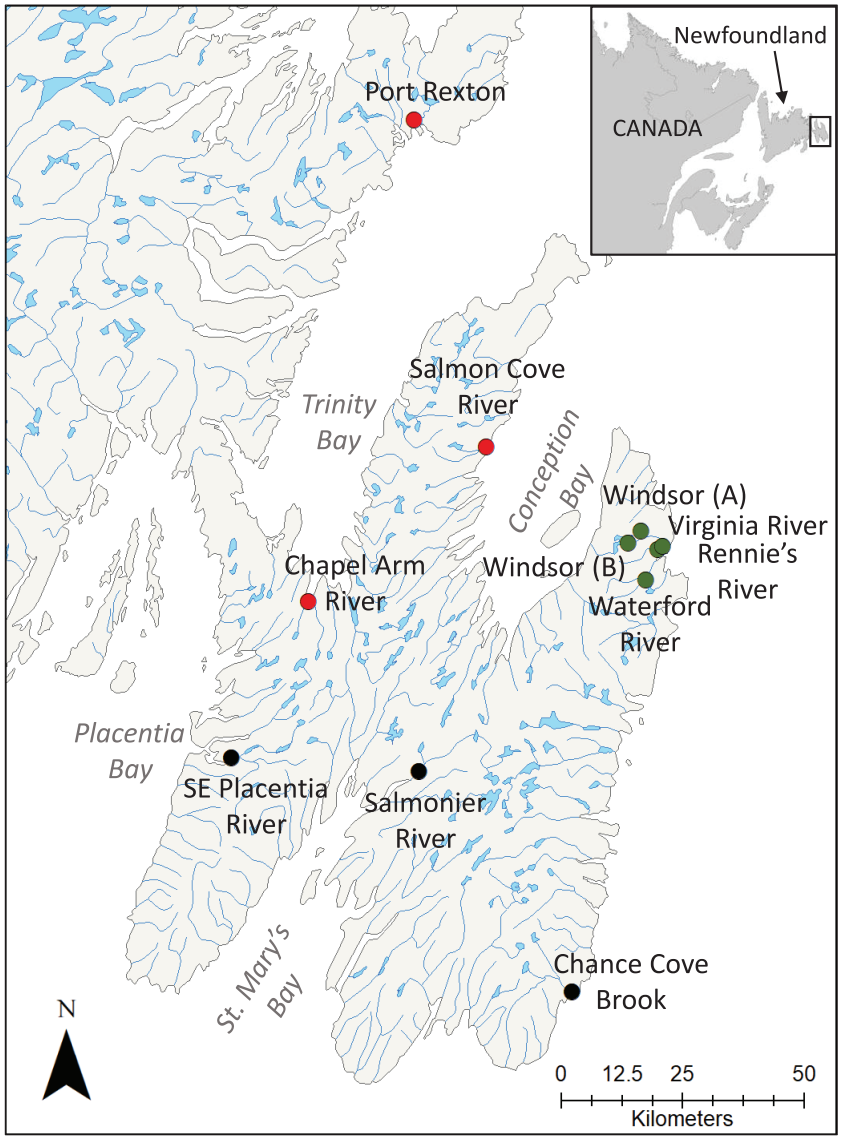
Sample collection

Juvenile *S. trutta* were sampled from 11 sites in Newfoundland during the summers of 2008 and 2010 (Table 1; Fig. 1) using a single-pass electrofishing method (Smith Root LR-24 electrofisher). Where possible at riverine sites, 300–400 m of river was sampled to reduce the risk of collecting fish from a small number of families. At each site, the aim was to sample 50 fish representing each of one or two cohorts (0+ and 1+) in each summer. Age classes were determined in the field by body length and later validated by scale reading. Where too small a number of trout were present in the 1+ year class, 0+ fry were collected in two summers (see Table 1). Sites were selected based on the known presence of *S. trutta* (Westley and Fleming 2011) and on spatial distance along the coast from the site of first introduction (St. John's).

We assumed that the sites furthest from St. John's represented the most recent colonisations, an assumption supported by Fisheries and Oceans Canada (DFO) survey data (DFO 2010) reviewed by Westley and Fleming (2011). In St. John's itself, we sampled: two streams entering the landlocked lake (Windsor Lake) to which *S. trutta* were reported to have been first introduced in 1883; two rivers within the St. John's catchment that were the focal points of the main introductions between 1883 to 1906 (Rennie's and Virginia rivers); and from a neighbouring catchment draining into St. John's harbour (Waterford River). Outside St. John's, three additional sites each to the north and to the south of the Avalon Peninsula (six in total) were sampled at progressively greater geographic distances from St. John's (Fig. 1; Table 1).

A proportion of the sampled fish were euthanised with an overdose of anaesthetic (clove oil) and biopsy samples of muscle or either caudal or adipose fin were preserved in molecular grade (99%) ethanol in individually labelled 1.5 mL Eppendorf tubes and stored at −20 °C for genetic analysis. Others were fin clipped and released back to the river (Westley et al. 2012). To provide a

Fig. 1. Sampling locations of *Salmo trutta* in Newfoundland (green = St. John's; red = North Avalon and black = South Avalon). Composite map was created using ArcGIS and PowerPoint, with basemaps from public domain shapefiles.



contemporary genetic sample representing the putative source originally used to stock Newfoundland's *S. trutta*, we obtained tissue samples from juveniles from the Howietoun hatchery, Stirling (2011 0+ and 2+ hatchery cohorts), as well as a sample of 38 scale sets collected by anglers from the Loch Leven fishery (wild source of the Howietoun Hatchery broodstock) during June and July 2001.

Molecular analysis

Genomic DNA was extracted from tissue samples using the Puregene procedure (Qiagen Ltd). Extracted DNA was checked for quality by comparison against *Hind*III digested λ DNA on 0.8% 0.5X TBE ethidium bromide-stained agarose gels. DNA was quantified using a Nanodrop ND-1000 and diluted to a working concentration of 2–5 ng·μL^{-1}.

Samples were genotyped for 16 microsatellite loci in two multiplex reactions: Panel 1: *Ssa*416UoS, *Cocl-Lav*-4, *One*9μASC, *CA*048828, *Ssa*85, *One*102-a, *One*102-b, *Ssa*406UoS, *CA*054565, *Str*2QUB, Panel 2: *Str*3QUB, *CA*060177, *Ssa*197, *Ssa*D71, *SaSa*TAP2A and *Ssa*410UoS. These markers were chosen from 38 salmonid derived loci evaluated by Keenan et al. (2013*a*) for *S. trutta* genetic research. Further information about primer sources, polymerase chain reaction (PCR) conditions and genotyping are given in Keenan et al. (2013*a*). Resulting PCR products were resolved on an ABI3730XL 96 capillary DNA analyser (Thermofisher) and scored using Genemarker v1.6 (Softgenetics). Allele scoring was double-checked by independent users, before assembly of genotypes into an Excel database for subsequent analyses.

Statistical analysis

Only individuals that could be unambiguously scored ≥13/16 loci (i.e., >80% multilocus genotype information) were used in downstream analyses. We used COLONY v2.0.5.0 (Jones and Wang 2010) to identify putative full-sibs among the samples. In instances where full-sibs were identified, we followed the recommendations of Hansen and Jensen (2005) and capped any full sib families to a maximum of three randomly selected individuals. We are conscious that aggressive sib removal can reduce performance of certain population genetic methods, but the impact of reducing large sibships to random subsets of this size is

Table 1. Details of the sampling locations used in this study showing year of collection, age class and number of individual *S. trutta* analysed (after removal of sibships and individuals with less than 13 loci genotyped).

Region	Sampling site	Latitude	Longitude	Sample (year, age)	*N*	Total
Scotland	Loch Leven	56°11′49.0″N	3°22′31.62″W	1990s	38	38
	Howietoun	56°04′20.03″N	3°57′04.20″W	2010 0+	36	68
				2010 2+	32	
St. John's	Windsor-Parkers Brook (A)$^{+}$	47°36′6.10″N	52°46′46.01″W	2008 0+	44	90
				2010 0+	46	
	Windsor-Middle Rocky Brook (B)$^{+}$	47°35′32.07″N	52°47′48.16″W	2010 0+	49	95
				2010 1+	46	
	Rennie's River	47°34′40.46″N	52°42′57.34″W	2008 0+	46	96
				2010 0+	50	
	Virginia River	47°35′18.66″N	52°41′26.92″W	2008 1+	47	86
				2010 0+	39	
	Waterford River	47°31′30.63″N	52°44′58.95″W	2008 1+	47	96
				2010 0+	49	
North Avalon	Salmon Cove River	47°46′54.47″N	53°10′11.60″W	2008 0+	49	99
				2010 0+	50	
	Chapel Arm River	47°30′45.99″N	53°40′43.64″W	2010 0 +	47	98
				2010 1+	51	
	Port Rexton	48°23′48.41″N	53°19′43.62″W	2008 0+	40	88
				2010 0+	48	
South Avalon	Chance Cove Brook	46°46′18.90″N	53°0′59.04″W	2008 0+	49	98
				2010 0+	49	
	Salmonier River	47°10′25.87″N	53°39′46.92″W	2010 0+	45	92
				2010 1+	47	
	SE Placentia River	47°13′20.70″N	53°53′38.43″W	2008 0+	49	95
				2010 0+	46	

Note: See Fig. 1 for map of locations.

minimal (Waples and Anderson 2017). Furthermore, a certain amount of sibling exclusion is essential to avoid potential STRUCTURE analyses biases.

Two independent approaches were employed to establish the source of *S. trutta* introduction in Newfoundland and to examine the patterns and levels of contemporary population structuring. We first employed the programme STRUCTURE v2.4 (Pritchard et al. 2000) that uses a Bayesian framework to cluster multi-locus genotypes into the minimum number of genetically distinct groups/populations that best explain the data, maximising within-group Hardy–Weinberg Equilibrium (HWE) and minimising within-group linkage disequilibrium (LD). Importantly, this approach does not require populations to be defined before analysis.

STRUCTURE was first run with Canadian and Scottish samples in a single analysis to provide an assessment of the likelihood of Howietoun/Loch Leven *S. trutta* as the historical source of introduction to Newfoundland. The Scottish samples were excluded from subsequent STRUCTURE analysis where the aim was to examine contemporary population structuring.

STRUCTURE runs, using only the Newfoundland samples, were carried out using a hierarchical approach design, which minimises potential biases related to the use of this programme (e.g., Janes et al. 2017); and which has been found to be particularly useful to describe dispersal patterns and colonisation routes. This hierarchical strategy aimed to identify major genetic groupings within the data (i.e., potentially related by common ancestry), and further refine these down to the population level. We repeated this procedure until no further significant population partitioning could be identified in the sample set (i.e., $K = 1$ in the focal data subset). At each level of the hierarchy, and on each subsequent data subset, we ran STRUCTURE 20 times for each *K* value (i.e., in the range 1–20) using the following parameters: length of burn-in period = 100 000; number of Markov chain Monte Carlo reps after burning = 100 000; admixture model, allele frequencies correlated. No location priors were used (i.e., analyses were entirely based on genetic data). Results from multiple STRUCTURE runs for each *K* value (outputs) were collated using the Perl script STRUCTURE HARVESTER (Earl and vonHoldt 2012); which implements the "ad hoc" approach proposed by Evanno et al. (2005) to identify the approximate optimum number of genetic clusters explaining the data. STRUCTURE HARVESTER was also used to produce the input files for CLUMPP (Jakobsson and Rosenberg 2007), which was employed to summarise results from multiple STRUCTURE runs for the best *K* value. For the CLUMPP runs we used the Greedy search method with option 2 and random input orders set to 20 000.

The second independent approach, which also does not require prior knowledge of population, was based on the Discriminant Analysis of Principal Components (DAPC) framework of Jombart et al. (2010). Like STRUCTURE, this non-parametric multivariate approach identifies groups of more genetically similar individuals (i.e., populations) within a data set comprising individual multi-locus genotypes. In the absence of information about the origin of individuals, DAPC uses a combination of sequential *K*-means with an increasing number of clusters (*K*). For each *K* value, a statistical measure of goodness of fit (BIC — Bayesian information criterion — lower values imply better fit) is estimated. The pattern of change in BIC estimated between increasing *K* values is useful to select the best number of "populations" explaining the data. The approach also provides a useful graphical assessment of between/among population genetic differences (i.e., principal components plots). DAPC analysis was carried out using the Adegenet v1.4-2 package (Jombart 2008) following the authors guidelines. Using the same rationale as per previous

Fig. 2. STRUCTURE plot illustrating close genetic relationship between present-day Scottish *Salmo trutta* (Loch Leven and Howietoun) and those from the introduced St. John's area.

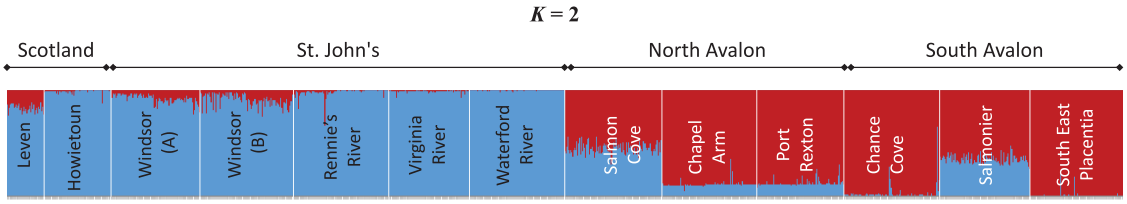
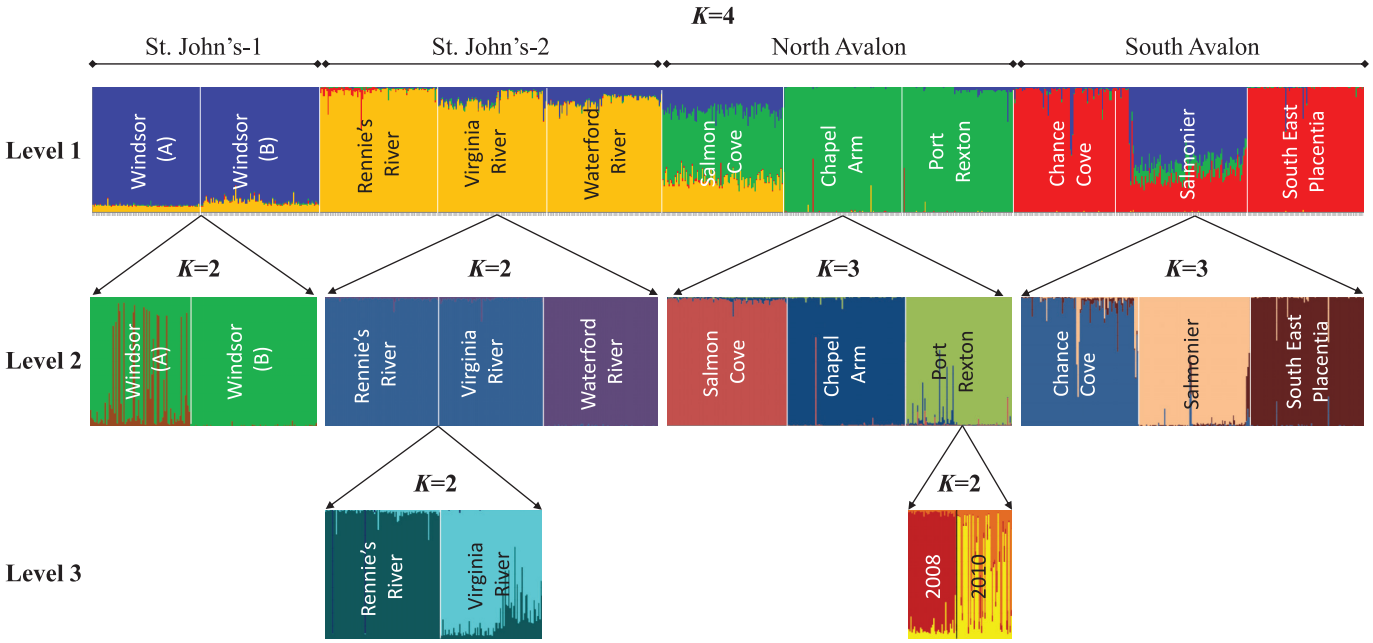


Fig. 3. Hierarchical STRUCTURE plots of Newfoundland samples determining contemporary population structuring and illustrated with reference to partial knowledge of historical population expansion.



STRUCTURE analysis, DAPC was initially run using the whole data set (including Scottish samples) and, to further explore patterns of population substructuring, dispersal patterns and colonisation routes, the analysis was re-run with sub-data sets corresponding to broader groups identified in previous run (i.e., mimicking a "hierarchical" approach) without the inclusion of the Scottish samples.

Following these analyses, basic summary statistics for the inferred populations (e.g., allelic richness, observed (H_o) and expected (H_e) heterozygosity) were calculated using divBasic function as implemented in diveRsity v1.9.89 (Keenan et al. 2013*b*). Tests for departure from linkage disequilibria and conformance with Hardy–Weinberg equilibrium (HWE), as post hoc within-population marker quality control, were carried out in GENEPOP (Rousset 2008), using an exact test with the following parameters: 10 000 dememorisations, 100 batches, 1000 iterations per batch.

Weir and Cockerham's (1984) equivalent (θ) of Wright's *F*-statistics were estimated both for the whole data set and for pairwise population samples derived from STRUCTURE/DAPC analyses. We similarly calculated the D_{EST} metric of genetic differentiation (Jost 2008); which is less biased by the high heterozygosity of microsatellites but is subject to its own limitations (Wang 2012). Finally, we estimated Nei's D_A genetic distance between all inferred population pairs and used these to build an unrooted neighbour-joining (NJ) tree (Nei et al. 1983) using the programme POPTREE2 (Takezaki et al. 2010). We used bootstrapping (10 000) to assess the confidence for the tree nodes.

To test for isolation-by-distance, we compared a matrix of geographic distance to a matrix of D_A using a Mantel test (10 000 null permutations), implemented in the Isolation by Distance Web Service (IBDWS; Jensen et al. 2005). Geographic distance was measured as least cost distance for migration by sea (Westley and Fleming 2011).

Results

Following the removal of sibships and individuals for which full genotypic data was not available for at least 13 microsatellite loci (80% of typed markers), 1139 *S. trutta* were available for analysis (Table 1). Sibship analysis, carried out using COLONY, identified only ten instances where more than three full-sibs were present (Windsor Lake A = 6, Salmon Cove = 1, Chapel Arm River = 1, Port Rexton = 1 and South East Placentia = 1).

The initial STRUCTURE analysis, incorporating contemporary samples from Howietoun and Loch Leven, returned $K = 2$ as the most likely number of clusters explaining the data: the two Scottish samples clustered with the St. John's samples. The second cluster comprised samples from the north and south Avalon Peninsula (Fig. 2). Targeted hierarchical STRUCTURE analyses restricted to samples from Newfoundland suggested a pattern of genetic structuring consistent with a geographical spread from St. Johns (Fig. 3). The first level (Level 1) of the analysis identified four clusters: (1) St. John's-1 represented by the landlocked Windsor Lake samples (Parker's Brook and Middle Rocky Brook; subsequently referred to as Windsor Lake A & B, respectively) where,

as stated above, the first introductions occurred; (2) St. John's-2 represented by the samples from Rennie's and Virginia rivers (part of the same catchment, and also the site of subsequent large introductions), and the Waterford River (a neighbouring catchment of the first two rivers); (3) the north Avalon cluster; (4) the south Avalon cluster. At the Level 2, these groups further resolved into two (Windsor Lake A & B), two (Rennie's River/Virginia River and Waterford River), three (Salmon Cove, Chapel Arm and Port Rexton) and three (Chance Cove, Salmonier and South East Placentia) clusters, respectively. At Level 3, two instances of further group partitioning were observed. The first separated Rennie's River and Virginia River, while the second separated the temporally spaced samples in Port Rexton. No further sub-structuring was evident in the data. Port Rexton was the only site with significant temporal heterogeneity.

With minor exceptions, the results of the DAPC analyses (Fig. 4) were remarkably similar to that of the STRUCTURE analyses. However, no differences were observed between temporal samples within sites (including Port Rexton). The samples from St. John's, north and south Avalon aggregate together into these larger regions (Fig. 4*b*) but, on closer examination (subsequent DAPC analyses involving samples within regional groups), are also distinct from each other (Figs. 4*c*, 4*d*, 4*e*, 4*f*, and 4*g*). Also, the Scottish samples (Loch Leven and Howietoun) clearly group with the St. John's cluster (Fig. 4*a*). Both STRUCTURE and DAPC analyses support the presence of 12 independent genetic clusters or inferred populations (excluding samples from Scotland), which coincided with the original sampling sites. All subsequent analyses were focused on these inferred populations.

Overall, for all inferred populations, the number of alleles per locus ranged from 3 (*Ssa*416) to 67 (*Ssa*410UoS) with an average of 21.3. Allelic richness (*ar*), H_O, H_E, and HWE *P*-values for the 12 Newfoundland inferred populations are presented in Appendix A, Table A1. There was no evidence of linkage disequilibrium between loci. With one exception, there were no consistent major deviations from HWE for any of the populations after Bonferroni correction (Rice 1989). For the *Ssa*410UoS locus, however, seven out of 12 populations were found to deviate from HWE expectations. This microsatellite was also the most polymorphic among all the other markers examined. The average *ar* for *Ssa*410UoS was 16.5 alleles per population. For the other marker loci *ar* ranged from 1.89 (*Ssa*416) to 13.8 (*Ssa*406UoS) per population (average = 6.23). Further inspection of allele segregation at this locus suggests the presence of low frequency null alleles that are likely to explain the observed deviation from HWE. Since the exclusion of this marker locus did not affect the outcome of the analyses, we elected not to remove it from subsequent analyses.

Overall F_{ST} and D_{EST} were 0.096 (95% CI 0.093–0.10) and 0.192 (95% CI 0.185–0.199), respectively. Population pairwise F_{ST} and D_{EST} estimates are displayed in Table 2. While, not surprisingly, F_{ST} values were of lower magnitude in comparison to D_{EST}, they were highly correlated. Pairwise F_{ST} values ranged from 0.007 (95% CI 0.003–0.013) between Windsor A and Windsor B, to 0.181 (95% CI 0.165–0.199) between SE Placentia River and Port Rexton 2008 (Table 2*a*). Pairwise D_{EST} values ranged from 0.009 (95% CI 0.001–0.022) between Port Rexton 2008 and Port Rexton 2010 to 0.264 (95% CI 0.241–0.290) between Port Rexton 2008 and Waterford (Table 2*b*). While all pairwise comparisons were found to be significant, estimates for both F_{ST} and D_{EST} were lower for within geographical clusters comparisons (St. John's, North Avalon and South Avalon) relative to those involving samples from different clusters.

The unrooted Nei's D_A NJ tree confirms the broader geographical groups (St. John's, North and South Avalon), the association of the Scottish samples with the St. John's group, and sample individuality in each case (Fig. 5). Re-running this analysis using the Howietoun sample as the outgroup provides further support for St. John's as the original point of introduction of *S. trutta* into Newfoundland (data not shown).

In addition to confirming the source and location of the initial introductions of *S. trutta* in Newfoundland and given insights into contemporary patterns of population genetic structuring, these previous analyses also provided useful information as to the likely mode of dispersal and colonisation routes. Thus, results seem to suggest independent colonization routes from the St. John's region to the north and south of the Avalon peninsula, respectively. Both the discreetness of the northern and southern colonisation routes is evident from Fig. 6, where STRUCTURE Level 1 groups are superimposed on the map of SE Newfoundland. With one exception, this figure suggests a clear geographical stepping stone model of colonisation, which seems to be consistent with straying of anadromous individuals. The Salmonier River population is the exception to this pattern. While the population is clearly related to the southern group, the genetic pattern is not totally characteristic of the classical stepping stone model of isolation by distance observed among the other populations. This inconsistency is also evident from the DAPC analysis (Fig. 4), where the Salmonier River population, while linked to south Avalon, is "genetically" closer to the St. John's group, thus deviating from the expected geographical pattern observed in the north Avalon group (i.e., Salmon Cove to Port Rexton to Chapel Arm). The unrooted Nei's D_A NJ tree (Fig. 5) also agrees with these results, with the Salmonier River population genetically more related to the St. John's group, again not being consistent with the expected geographic pattern.

Examination of allelic diversity statistics (*ar* values) in St. John's populations and along the northern Avalon and southern Avalon previously assumed colonisation routes, suggest declining values, which are consistent with a natural stepping stone pattern of colonisation by anadromous individuals. Thus, the average *ar* value in St. John's is 8.28 (Appendix A, Table A1). Along northern Avalon Peninsula *ar* estimates are 7.45, 5.33 and 5.19 for Salmon Cove, Chapel Arm, and Port Rexton, respectively. Along southern Avalon *ar* values are 6.36, 6.85 and 5.02 for Chance Cove, Salmonier River and South East Placentia, respectively.

The stepping stone model of colonisation is further supported by the pairwise F_{ST} and D_{EST} estimates (Tables 2*a* and 2*b*), with adjacent sites along the northern and southern putative colonisation routes exhibiting lower divergence in comparison to the more distant ones. We also observed a strong positive correlation between geographical distance (km) and genetic differentiation (Nei's D_A) among the Newfoundland sampling sites (Mantel $r = 0.623$, $p = 0.001$; Fig. 7), again providing good support for an isolation-by-distance model.

Discussion

Our results are complementary to previous work on *S. trutta* invasions, building upon those studies' methodologies by using a larger panel of microsatellite loci, and by applying a broader set of analytical methods. Notwithstanding the different assumptions of these three analytical methods, results are largely similar in that they support our inferences about this invasion, including on those instances where the historical data might be incomplete. Genetic data from the two sampled Scottish populations confirms the close relationship between the Howietoun hatchery and the wild Loch Leven population, in agreement with hatchery records over the last century (Iain Semple, Howietoun Hatchery manager, personal communication). The genetic similarity of these two samples to that of the St. John's samples (Windsor Lake, Rennie's and Virginia rivers, and the Waterford River), in turn, supports the historical records suggesting that the brown trout that were introduced to St. John's, via the Howietoun hatchery, were themselves composed mainly of Loch Leven genetic stock. This is an important point in understanding post-invasion genetic

Fig. 4. (*a*, *b*, *c*, *d*, *f*, and *g*) Scatterplots of Newfoundland *S. trutta* samples on the two principal components of DAPC following hierarchical approach as detailed in text. Individuals are represented as dots and the groups as eclipses. Eigenvalues of the analysis are also shown in each case. (*e*) Density plot for the first (and only since there are only two groups) discriminant function.

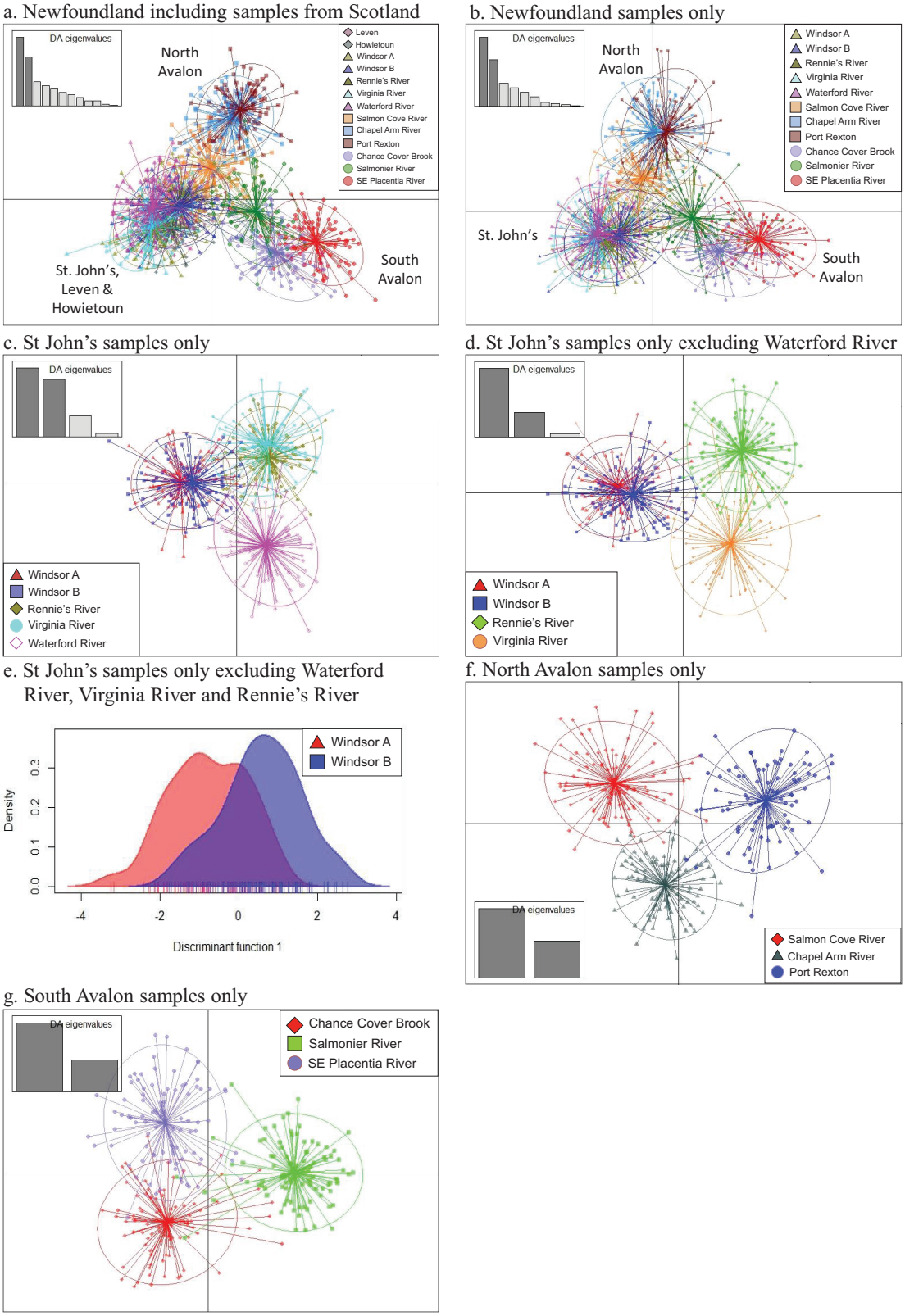
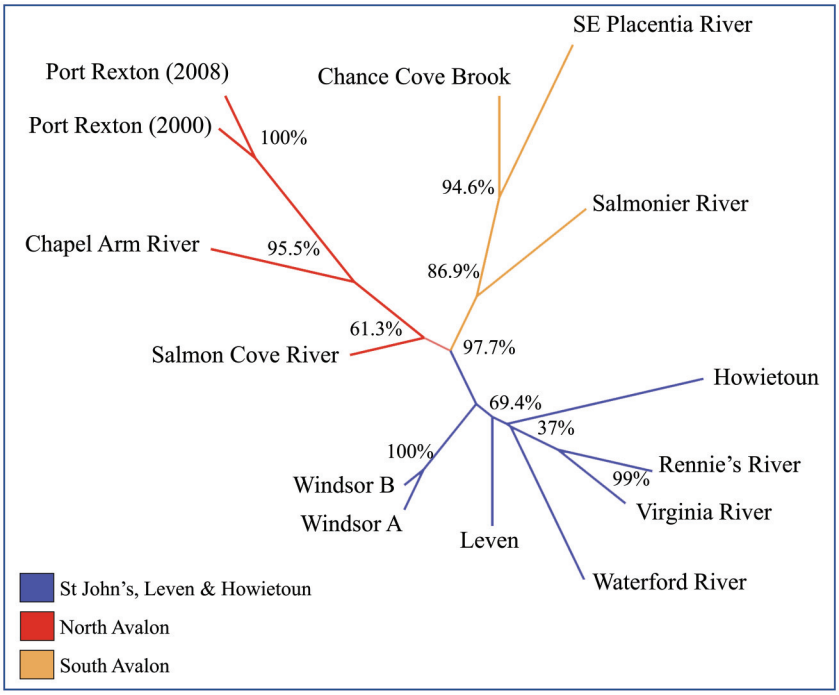


Table 2. Pairwise Weir and Cockerham F_{ST} (A) and D_{EST} (B) estimates (below diagonal) and associated 95% C.I. (above diagonal) between inferred populations.

Inferred population	01	02	03	04	05	06	07	08	09	10	11	12
***(a)* F_{ST} estimates**												
01- Windsor (A)	—	(0.003–0.013)	(0.045–0.06)	(0.046–0.062)	(0.058–0.078)	(0.061–0.079)	(0.096–0.12)	(0.095–0.123)	(0.104–0.138)	(0.07–0.091)	(0.064–0.087)	(0.104–0.13)
02- Windsor (B)	0.007	—	(0.04–0.055)	(0.037–0.053)	(0.049–0.068)	(0.058–0.075)	(0.099–0.121)	(0.108–0.136)	(0.112–0.147)	(0.067–0.087)	(0.071–0.092)	(0.105–0.128)
03- Rennie's River	0.051	0.047	—	(0.026–0.041)	(0.045–0.061)	(0.057–0.073)	(0.106–0.126)	(0.106–0.133)	(0.119–0.15)	(0.081–0.101)	(0.085–48)	(0.097–0.118)
04- Virginia River	0.054	0.045	0.034	—	(0.052–0.071)	(0.058–0.077)	(0.11–0.132)	(0.119–0.148)	(0.122–0.155)	(0.097–0.121)	(0.088–0.107)	(0.119–0.142)
05- Waterford River	0.068	0.058	0.053	0.061	—	(0.058–0.076)	(0.104–0.126)	(0.13–0.164)	(0.15–0.182)	(0.101–0.125)	(0.077–0.095)	(0.121–0.146)
06- Salmon Cove River	0.070	0.066	0.064	0.067	0.067	—	(0.06–0.079)	(0.099–0.128)	(0.091–0.118)	(0.088–0.111)	(0.067–0.088)	(0.115–0.141)
07- Chapel Arm River	0.108	0.110	0.116	0.120	0.115	0.069	—	(0.093–0.131)	(0.099–0.126)	(0.126–0.153)	(0.105–0.126)	(0.136–0.16)
08- Port Rexton 2010	0.109	0.122	0.118	0.133	0.146	0.113	0.111	—	(0.019–0.049)	(0.139–0.172)	(0.1–0.133)	(0.137–0.168)
09- Port Rexton 2008	0.120	0.129	0.134	0.138	0.165	0.104	0.111	0.033	—	(0.162–0.196)	(0.106–0.137)	(0.165–0.199)
10- Chance Cove Brook	0.080	0.077	0.091	0.109	0.113	0.099	0.139	0.155	0.179	—	(0.099–0.128)	(0.062–0.085)
11- Salmonier River	0.075	0.081	0.095	0.097	0.086	0.077	0.116	0.116	0.121	0.113	—	(0.094–0.122)
12- SE Placentia River	0.117	0.117	0.107	0.130	0.133	0.128	0.147	0.152	0.181	0.073	0.108	—
***(b)* D_{EST} estimates**												
01- Windsor (A)	—	(0.003–0.024)	(0.107–0.146)	(0.111–0.156)	(0.132–0.177)	(0.084–0.122)	(0.139–0.181)	(0.149–0.193)	(0.155–0.202)	(0.127–0.165)	(0.114–0.162)	(0.18–0.232)
02- Windsor (B)	0.012	—	(0.091–0.128)	(0.081–0.12)	(0.11–0.154)	(0.099–0.132)	(0.163–0.202)	(0.188–0.235)	(0.186–0.241)	(0.12–0.158)	(0.123–0.167)	(0.177–0.225)
03- Rennie's River	0.125	0.109	—	(0.057–0.095)	(0.106–0.146)	(0.115–0.149)	(0.197–0.239)	(0.198–0.25)	(0.217–0.267)	(0.155–0.201)	(0.178–48)	(0.157–0.2)
04- Virginia River	0.131	0.100	0.075	—	(0.11–0.154)	(0.1–0.136)	(0.168–0.204)	(0.213–0.273)	(0.209–0.269)	(0.175–0.228)	(0.146–0.189)	(0.209–0.258)
05- Waterford River	0.153	0.131	0.125	0.131	—	(0.111–0.145)	(0.167–0.208)	(0.21–0.273)	(0.241–0.29)	(0.183–0.229)	(0.119–0.154)	(0.212–0.259)
06- Salmon Cove River	0.103	0.114	0.132	0.118	0.126	—	(0.08–0.117)	(0.112–0.162)	(0.089–0.128)	(0.131–0.174)	(0.103–0.143)	(0.156–0.207)
07- Chapel Arm River	0.160	0.183	0.218	0.185	0.187	0.097	—	(0.096–0.149)	(0.116–0.153)	(0.169–0.208)	(0.128–0.157)	(0.165–0.206)
08- Port Rexton 2010	0.171	0.211	0.223	0.242	0.240	0.135	0.121	—	(0.001–0.022)	(0.208–0.256)	(0.133–0.185)	(0.179–0.23)
09- Port Rexton 2008	0.178	0.213	0.241	0.239	0.264	0.108	0.135	0.009	—	(0.228–0.272)	(0.129–0.18)	(0.221–0.269)
10- Chance Cove Brook	0.146	0.138	0.177	0.201	0.206	0.153	0.188	0.233	0.251	—	(0.158–0.207)	(0.062–0.1)
11- Salmonier River	0.137	0.144	0.204	0.167	0.136	0.123	0.142	0.158	0.154	0.183	—	(0.128–0.172)
12- SE Placentia River	0.205	0.201	0.178	0.232	0.235	0.180	0.186	0.206	0.245	0.079	0.150	—

Note: Colour pattern (heatmap) indicates comparative levels of divergence between comparisons with "red" meaning higher levels of between population divergence in comparison to "blue". All pair-wise population comparisons were found to be significant (see text for additional details). Solid lines within matrix identify populations belonging to St. Johns (Windsor(A), Windsor(B), Rennie's and Virginia rivers and Waterford River), North Avalon (Salmon Cove River, Chapel Arm River and Port Rexton 2008 and 2010) and South Avalon (Chance Cover, Salmonier River and South East Placentia River), respectively.

Fig. 5. Unrooted NJ tree based Nei's D_A genetic distance. Bootstrap support for nodes is given as percentage values. Broad geographical locations of inferred populations are represented by different colours. Scottish samples are included for comparison.



diversity patterns in this species, as the historical records compiled by Westley and Fleming (2011) show that, although the vast majority of recorded trout brought to Newfoundland were of Loch Leven heritage, there were also smaller numbers of fish of German origin (von Behr strain), in addition to unknown numbers of "English" (precise source unknown) fish. We consider it unlikely that these latter introductions made a lasting genetic contribution to the invasion of the Avalon Peninsula, as we did not find evidence in our analyses that might indicate founders of a substantially different lineage (e.g., deviations from HWE).

From present-day patterns of genetic diversity in Newfoundland, the introduced stock appears then to have dispersed out from St. John's, moving naturally north and south along the Avalon Peninsula, via anadromous migrants, following a stepping-stone pattern (Beebee and Rowe 2004; Allendorf and Luikart 2007). While we acknowledge that it is technically possible for stepping-stone human-mediated stocking to have produced these patterns, we consider natural dispersal by anadromous trout to be the more parsimonious explanation, particularly given the remoteness and lack of viable transportation among areas during this period. The samples from land-locked Windsor Lake brown trout do not fit this pattern, potentially indicating a different demographic history for these populations, including a separate foundation event and (or) population bottlenecks, in the almost century since initial introduction (Hustins 2007; Westley and Fleming 2011).

In our three north Avalon samples, F_{ST} values and IBD results seem to confirm the hypothesis of Westley and Fleming (2011) that expansion was natural and mediated by anadromous "wandering". Lower levels of genetic diversity further from the "source", and high inter nearest-neighbour levels or population divergence (F_{ST} and Nei's D_A) are suggestive of founder effects. Similarly, the first and last samples of south Avalon series show the same trends as in the north Avalon, suggesting the same mode of colonisation. The Salmonier River sample is a clear outlier. The evidence seems to support the occurrence of unrecorded direct stocking, probably from the landlocked Windsor Lake, in addition to natural anadromous colonisation. A lesser degree of unrecorded stocking may have taken place in Salmon Cove, again from Windsor Lake (see Fig. 7). In his book, Hustins (2007) reports that the Whitbourne River, which drains into Rocky River near Salmonier was stocked via the roadway, and that Salmon Cove was stocked with "German browns". However, there are no records of the numbers involved or better documentation of those stockings. Both the similarities among results from different statistics in the north and south Avalon expansions and the rates of independent colonisation routes are striking. As indicated by Launey et al. (2010), coastal terrain can influence rate of marine mediated dispersal, which in our case seems similar North and South of the Avalon peninsula (Ian Fleming, personal communication).

Inter neighbour pairwise F_{ST} population values observed in Newfoundland were similar to wild populations within the native range. The existence of genetic structuring between rivers in Newfoundland is similar to that observed in the species native range (Ferguson 2006; Cauwelier et al. 2011; Prodöhl et al. 2019); with pairwise F_{ST} ranging from 0.09 in neighbouring invasive populations to 0.173 in more geographically distant samples. These values are similar to levels of differentiation seen in other colonising populations of brown trout (Valiente et al. 2007; pairwise F_{ST} = 0.072 to 0.175 in Patagonian rivers, Launey et al. 2010; pairwise F_{ST} = 0 to 0.173 in rivers in the Kerguelen Islands).

The patterns we describe agree with those of Launey et al. (2010) on *S. trutta* introduced to the Kerguelen Islands. That study examined three independently founded invasive populations that were all derived from the same French hatchery strain. In all three cases, F_{ST} increased and genetic variability decreased with linear, assumed-natural, anadromous dispersal. These independent linear patterns are consistent with the independent linear patterns that we detected along the Avalon Peninsula relative to St. John's. Thus, their findings are similar and complementary to ours, and so suggest a more general mode of dispersal (expansion of distribution over time). It also suggests a classic linear stepping stone model of migration, with pairwise F_{ST} values increasing with increasing geographic distance from the

Fig. 6. Level 1 STRUCTURE plot (excluding samples from Scotland) superimposed on area of study. Colour code follows that of Fig. 3 – Level 1. For each pie, colour proportions represent the average cluster membership for all individuals within inferred populations. Composite map was created using ArcGIS and PowerPoint, with basemaps from public domain shapefiles.

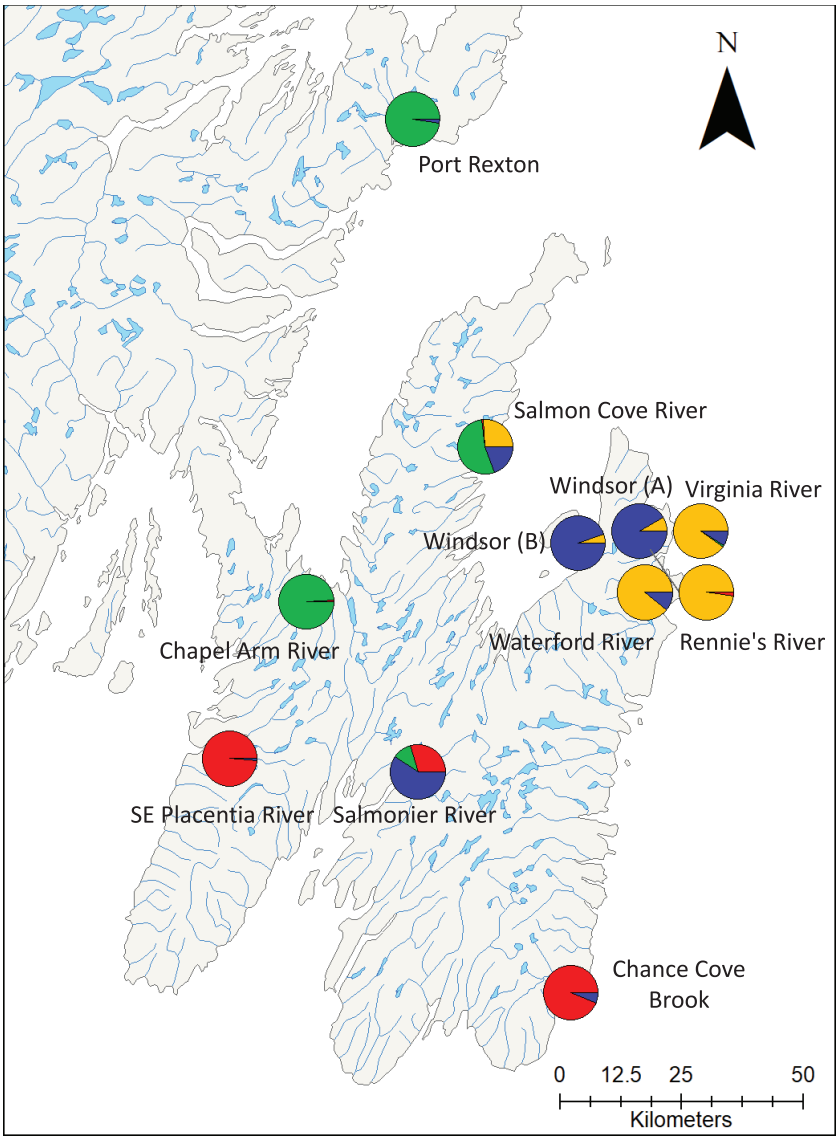
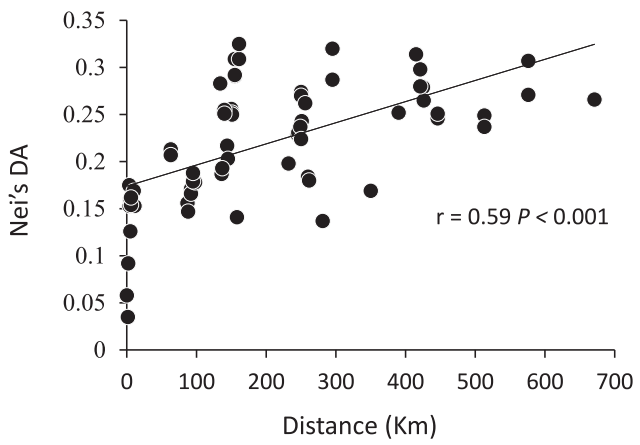


Fig. 7. Mantel test illustrating the positive correlation between geographic (km — measured as least cost distance) and genetic distance (Nei's D_A) for Newfoundland sampling sites ($r = 0.59$, $p < 0.001$).



initial source of the invasion in both the Kerguelen Islands and in Newfoundland.

An aspect of the founder effects inherent in such dispersal relates to the resultant reduction in genetic variability. It has been suggested that admixture provided by genetic input from various sources is important for successful introductions (Allendorf and Luikart 2007); although this concept does not factor in possible effects of extrinsic outbreeding depression. A lack of multi-stock admixture does not appear to have hindered the establishment and further dispersal of *S. trutta* in Newfoundland, where contemporary populations appear to originate from a single donor population. Whether this is a general property of salmonid invasions is arguable. As well as the Kerguelen example given above (Launey et al. 2010), research on introduced salmonids in Patagonia has concluded that loss of genetic diversity by founder effects may not be posing a major evolutionary restraint (Valiente et al. 2007, 2010). Instead, it may be the case that salmonids are in line with studies of other species that showed that bottlenecks can reduce variability at neutral loci without affecting, or having only a small effect on, quantitative trait variation (Dlugosch and

Parker 2008; Purcell et al. 2012). A formal test of this hypothesis would require analogous genetic studies of the founders of unsuccessful salmonid invasions (Koskinen et al. 2002). Adaptive phenotypic plasticity is another mechanism that may have enhanced population persistence during early colonisation stages (i.e., Baldwin effects), even if additive genetic variation for fitness-related traits was initially low, thereby facilitating subsequent spread (Yeh and Price 2004).

Brown trout are well known among biologists and anglers for their facultative anadromy (Ferguson 2006; Ferguson et al. 2019*a*). While some fish will spend their whole lives in the streams in which they were spawned, others may migrate to lakes or large rivers before returning to spawn (adfluvial), and others may be fully anadromous, "smoltifying" and going to sea in a manner similar to Atlantic salmon (*Salmo salar*). While some *S. trutta* populations produce exclusively "sea trout" and others no sea trout, many populations produce both, to varying degrees. No consistent set of genetic traits have yet been found to predict individual-level anadromy in *S. trutta*, but environmental factors such as food supply and temperature are likely to play proximate roles in migratory decisions (Ferguson et al. 2019*a*; Archer et al. 2019). The present study provides a valuable piece of context to facultative anadromy. While Loch Leven used to produce sea trout up to the 1830s, these anadromous trout disappeared in the 1830s as a consequence of damming and canalisation work in the area (Maitland 1887). By the late 1800s and early 1900s, all Loch Leven trout were essentially non-anadromous. The pattern of dispersal along the Avalon Peninsula, however, suggests that the introduced Loch Leven trout had retained (A) a capacity for anadromy, and (B) a capacity to use anadromy to colonise new sites. Launey et al. (2010) found a similar shift to anadromy in Kerguelen Islands; their source fish having originated from streams on the north slopes of the Pyrenees. Experimental laboratory work with Irish brown trout also shows that anadromy can re-emerge in populations that are naturally non-anadromous in the wild, under conditions of reduced food supply (Archer et al. 2019).

Anadromous female *S. trutta*, in general, have both more and larger eggs than freshwater residents due to their much larger size at age (Poole et al. 2006). Thus, a new population could hypothetically be founded by very low numbers of individuals. However, new stable populations would not be expected to be established by anadromous straying fish until a certain level of population density is reached in the donor catchment (see Ulvan et al. 2018). This could be part of the reason why the brown trout's rate of spread in Newfoundland (approx. 4 km·year^{-1}; Westley and Fleming 2011) and other similar island habitats (i.e., the Kerguelan Islands; Ayllon et al. 2004) is relatively slow, especially when compared with other studied invasions of salmonids (e.g., Chinook salmon (*Oncorhynchus tshawytscha*) in South America: 54 km·year^{-1}; Correa and Gross 2008; Chinook salmon in New Zealand: 13 km·year^{-1}; Kinnison et al. 2008). It is likely that the marine ecology of *S. trutta*, coastal as opposed to oceanic, is an important determinant of the speed of species spread, the brown trout having primarily an inshore migration.

Within their native range, it has been recorded that anadromous *S. trutta* will recruit into vacant habitats, as has been observed by the recolonisation of sea trout into the Tyne system in Britain (Milner et al. 2004). Atlantic salmon have also, in recent times, in Britain and in other areas, been recorded as beginning to recolonise rehabilitated habitats that were previously unsuitable due to factors such as high pollutant levels (Griffiths et al. 2011; Ikediashi et al. 2012). This suggests that straying in salmonid species is an effective method of colonising new or rehabilitated habitats, both in existing and novel environments (Milner et al. 2000).

We have presented evidence of the expansion pattern and genetic differences between established colonised populations of brown trout on Newfoundland, showing how populations can diverge from a common source over a short time period (ca. 130 years) and that colonization can occur from the re-expression of life histories that facilitate dispersal. The present study focused on differences between neutral genetic markers. These differences may not correlate with traits relating to fitness, such as reproductive success, survival, or phenotypic adaptations influenced by adaptive loci (Westley et al. 2013). However, neutral markers have been utilised frequently to demonstrate differences between population groupings for management and conservation purposes (Beebee and Rowe 2004; Allendorf and Luikart 2007) and have an important role in addressing these issues.

What do our results tell us about invasions of facultatively anadromous salmonid species in general? As judged from successful introductions worldwide (MacCrimmon and Marshall 1968), *S. trutta* is a successful coloniser and invader and is likely to be a successful competitor with other freshwater and anadromous fish species. In fact, the brown trout is considered one of the top 100 most successful invasive species worldwide (Lowe et al. 2000). Our results here from a relatively recent and well recorded introduction outside the native range, may give further support to the mechanism involved in such a process being dominated by profound founder events with associated high levels of genetic drift, at least in functionally anadromous fish species like *S. trutta*. They may also shed light on the development of population structure in such species (being established at founding and maintained by local adaptation). Here Westley et al. (2012) specifically suggests evolving local adaptation.

Acknowledgements

We acknowledge Iain Semple, Manager, Howietoun Hatchery, University of Stirling for assistance with Howietoun biopsy tissue samples, in addition to samples generously provided by Lough Leven Anglers and Professor Eric Verspoor University of the Highlands and Islands. CO'T, PMcG, TC, CB, PP, and TR were supported by the Beaufort Marine Research Award in Fish Population Genetics funded by the Irish Government under the NDP Sea Change Programme. CO'T received a Third Level (2009) Ireland–Newfoundland Marine Research Grant as part of the Ireland–Newfoundland Partnership, which enabled her to undertake part of her PhD studies at Memorial University. PMcG, PP, KPP were also supported by Science Foundation Ireland, the Marine Institute and the Department for the Economy, Northern Ireland under the Investigators Programme Grant Number SFI/15/IA/3028. PW was supported by funding from the Newfoundland Institute for Biodiversity, Ecosystem Science and Sustainability. The collections in Newfoundland were supported by the Natural Sciences and Engineering Research Council of Canada. Travel to Ireland and Scotland by PW, IF, and CO'T to secure European baseline samples was sponsored by the Newfoundland Ireland Business Partnership fund.

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Appendix A

Appendix Table A1 appear on following pages.

Table A1. Summary statistics of inferred Newfoundland populations.

	*Ssa*416	*Cocl-Lav*-4	*One*9uASC	*CA*048828	*Ssa*85	*One*102-a	*One*102-b	*Ssa*406UoS	*CA*054565	pp*Str2*	pp*Str3*	*CA*060177	*Ssa*197	*Ssa*D71	*SaSa*TAP2A	*Ssa*410UoS	Overall
Windsor-PRK																	
ar	1.96	5.00	4.51	13.25	4.52	2.00	14.97	13.96	2.95	9.53	3.00	5.51	4.54	10.53	5.00	19.64	7.55
size	70	60	75	75	75	74	75	74	72	75	74	74	69	73	75	75	73
obs_het	0.057	0.517	0.707	0.893	0.747	0.189	0.987	0.932	0.014	0.880	0.514	0.824	0.667	0.904	0.653	0.973	0.654
HWE_exact	1.00	0.01	0.20	0.02	0.05	1.00	0.01	0.05	0.00	0.78	0.49	0.29	0.52	0.51	0.58	0.00	
Windsor-MRB																	
ar	1.81	5.00	4.97	13.15	4.00	2.00	14.02	17.32	1.00	8.93	3.00	5.41	4.00	9.11	5.41	19.03	7.39
size	93	94	95	95	95	95	95	95	95	95	95	93	94	94	94	93	94
obs_het	0.032	0.511	0.695	0.884	0.716	0.168	0.916	0.916	0.000	0.811	0.453	0.774	0.745	0.872	0.713	0.957	0.635
exp_het	0.032	0.743	0.694	0.884	0.700	0.172	0.907	0.910	0.000	0.828	0.459	0.746	0.672	0.840	0.719	0.919	0.639
HWE_exact	1.00	0.00	0.62	0.02	0.88	0.59	0.27	0.15	—	0.32	0.29	0.55	0.20	0.67	0.34	0.00	
Rennie's River																	
ar	2.99	7.27	7.97	17.46	4.74	2.00	12.83	22.32	3.57	16.96	5.05	8.73	5.60	12.08	5.96	21.37	9.81
size	96	96	95	96	96	95	94	96	95	96	96	94	95	96	96	96	96
obs_het	0.281	0.635	0.779	0.938	0.552	0.232	0.851	0.854	0.074	0.906	0.563	0.691	0.674	0.885	0.760	0.948	0.664
exp_het	0.266	0.727	0.778	0.896	0.589	0.205	0.870	0.893	0.149	0.914	0.591	0.766	0.665	0.859	0.750	0.928	0.678
HWE_exact	0.86	0.02	0.91	0.91	0.39	0.60	0.55	0.04	0.00	0.12	0.57	0.00	0.01	0.37	0.51	0.50	
Virginia River																	
ar	2.99	5.99	6.89	16.55	3.00	2.00	14.20	18.99	1.70	14.91	4.99	7.80	5.11	10.88	6.89	20.49	8.96
size	86	86	86	86	86	86	86	85	86	83	86	81	86	76	73	86	84
obs_het	0.349	0.802	0.837	0.930	0.628	0.326	0.860	0.929	0.023	0.880	0.628	0.790	0.767	0.671	0.658	0.872	0.684
exp_het	0.346	0.772	0.748	0.900	0.625	0.303	0.835	0.893	0.023	0.889	0.616	0.760	0.713	0.734	0.771	0.905	0.677
HWE_exact	0.52	0.05	0.42	0.28	0.86	0.72	0.71	0.88	1.00	0.03	1.00	0.44	0.41	0.11	0.03	0.09	
Waterford River																	
ar	2.92	5.00	5.03	15.08	3.80	2.00	9.84	17.18	1.99	7.88	4.00	6.07	6.15	10.77	5.62	19.74	7.69
size	95	95	96	96	95	92	96	96	96	96	96	92	96	89	96	96	95
obs_het	0.126	0.758	0.719	0.875	0.400	0.196	0.854	0.906	0.000	0.792	0.573	0.533	0.802	0.820	0.563	0.875	0.612
exp_het	0.120	0.762	0.725	0.887	0.486	0.243	0.833	0.919	0.080	0.814	0.605	0.521	0.760	0.852	0.569	0.909	0.630
HWE_exact	1.00	0.01	0.94	0.00	0.00	0.07	0.70	0.21	0.00	0.35	0.11	0.54	0.13	0.31	0.96	0.01	
Salmon Cove River																	
ar	3.00	5.40	5.40	9.36	3.00	2.00	13.49	13.54	1.00	10.78	3.92	6.29	4.00	11.24	5.50	21.25	7.45
size	98	96	98	98	98	98	98	98	98	98	98	96	97	96	98	97	98
obs_het	0.357	0.708	0.561	0.827	0.643	0.296	0.857	0.816	0.000	0.561	0.418	0.635	0.691	0.906	0.531	0.948	0.610
exp_het	0.353	0.739	0.643	0.818	0.646	0.280	0.884	0.859	0.000	0.596	0.431	0.657	0.702	0.874	0.597	0.910	0.624
HWE_exact	0.35	0.55	0.17	0.79	0.55	1.00	0.52	0.31	—	0.05	0.28	0.79	0.78	0.42	0.07	0.00	
Chapel Arm River																	
ar	2.00	4.84	4.79	4.80	3.64	2.00	11.02	6.99	2.80	7.59	3.80	6.08	4.00	6.00	3.05	11.85	5.33
size	97	90	96	97	97	96	96	96	97	97	97	95	95	97	97	97	96
obs_het	0.196	0.478	0.698	0.763	0.598	0.125	0.792	0.688	0.124	0.742	0.567	0.663	0.568	0.711	0.258	0.773	0.546
exp_het	0.209	0.506	0.689	0.718	0.644	0.117	0.845	0.698	0.117	0.715	0.557	0.707	0.632	0.797	0.251	0.799	0.563
HWE_exact	0.62	0.09	0.63	0.25	0.87	1.00	0.05	0.01	1.00	0.40	0.48	0.01	0.21	0.07	0.38	0.00	

Table A1 (*concluded*).

	*Ssa*416	*Cocl-Lav*-4	*One*9uASC	*CA*048828	*Ssa*85	*One*102-a	*One*102-b	*Ssa*406UoS	*CA*054565	pp*Str2*	pp*Str3*	*CA*060177	*Ssa*197	*Ssa*D71	*SaSa*TAP2A	*Ssa*410UoS	Overall
Port Rexton 2010																	
ar	1.00	4.00	3.00	7.00	3.00	1.00	10.00	8.00	2.00	8.00	2.00	5.00	4.00	6.00	4.00	15.00	5.19
size	39	38	38	39	39	35	39	39	39	39	39	38	37	39	39	39	38
obs_het	0.000	0.579	0.500	0.513	0.564	0.000	0.821	0.795	0.154	0.821	0.590	0.711	0.459	0.769	0.205	0.974	0.528
exp_het	0.000	0.703	0.502	0.659	0.534	0.000	0.806	0.716	0.142	0.789	0.479	0.740	0.538	0.679	0.213	0.824	0.520
HWE_exact	—	0.34	1.00	0.05	0.84	—	0.82	0.65	1.00	0.81	0.20	0.15	0.22	0.90	0.41	0.00	
Port Rexton 2008																	
ar	1.00	4.00	3.98	6.94	3.00	1.00	7.78	9.58	2.00	5.00	2.00	4.81	2.97	5.00	4.00	13.52	4.79
size	48	47	45	47	48	48	48	48	48	48	48	47	45	45	48	48	47
obs_het	0.000	0.723	0.622	0.766	0.396	0.000	0.708	0.771	0.271	0.667	0.417	0.660	0.511	0.689	0.354	0.896	0.528
exp_het	0.000	0.724	0.550	0.675	0.438	0.000	0.751	0.758	0.291	0.660	0.413	0.633	0.492	0.654	0.368	0.837	0.515
HWE_exact	—	0.09	0.80	0.68	0.17	—	0.53	0.13	0.62	0.19	1.00	0.00	0.24	0.97	0.03	0.00	
Chance Cove Brook																	
ar	1.00	7.29	6.25	8.82	5.36	2.00	8.58	10.53	3.14	9.40	3.38	5.90	3.91	9.36	2.80	14.10	6.36
size	97	98	95	98	98	98	98	97	98	98	98	96	98	92	98	98	97
obs_het	0.000	0.673	0.642	0.837	0.663	0.122	0.684	0.742	0.102	0.776	0.469	0.552	0.694	0.837	0.327	0.816	0.559
exp_het	0.000	0.765	0.686	0.780	0.637	0.115	0.727	0.807	0.098	0.821	0.440	0.583	0.680	0.865	0.341	0.828	0.573
HWE_exact	—	0.13	0.00	0.31	0.69	1.00	0.02	0.15	1.00	0.00	0.44	0.24	0.47	0.03	0.72	0.00	
Salmonier River																	
ar	1.00	5.29	5.37	10.11	4.23	2.00	9.38	11.13	3.31	9.76	5.11	9.66	6.54	8.51	4.31	13.95	6.85
size	91	93	93	93	93	93	93	93	91	93	93	89	85	87	92	92	92
obs_het	0.000	0.634	0.688	0.871	0.419	0.258	0.720	0.817	0.088	0.892	0.677	0.742	0.624	0.701	0.446	0.837	0.588
exp_het	0.000	0.634	0.721	0.794	0.582	0.350	0.737	0.854	0.126	0.831	0.646	0.767	0.694	0.754	0.489	0.821	0.612
HWE_exact	—	0.46	0.01	0.77	0.00	0.02	0.02	0.15	0.00	0.03	0.49	0.00	0.05	0.01	0.26	0.02	
SE Placentia River																	
ar	1.00	6.59	4.29	5.76	3.83	1.62	7.97	7.73	2.50	7.84	3.66	6.22	5.44	4.94	3.98	6.99	5.02
size	94	92	94	94	94	91	93	94	93	94	94	92	85	92	94	93	93
obs_het	0.000	0.674	0.500	0.681	0.691	0.022	0.817	0.766	0.022	0.840	0.564	0.554	0.741	0.620	0.532	0.645	0.542
exp_het	0.000	0.667	0.653	0.737	0.661	0.022	0.820	0.778	0.042	0.796	0.594	0.619	0.761	0.622	0.562	0.710	0.565
HWE_exact	—	0.32	0.01	0.20	0.26	1.00	0.39	0.65	0.01	0.26	0.35	0.00	0.94	0.48	0.83	0.14	

Note: Significant deviations from HW expectations are highlighted.