MOLECULAR ECOLOGY

Molecular Ecology (2010) **19**, 2025–2037 doi: 10.1111/j.1365-294X.2010.04628.x

Loss of genetic integrity correlates with stocking intensity in brook charr (*Salvelinus fontinalis*)

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Abstract

Supportive breeding and stocking performed with non-native or domesticated fish to support sport fishery industry is a common practice throughout the world. Such practices are likely to modify the genetic integrity of natural populations depending on the extent of genetic differences between domesticated and wild fish and on the intensity of stocking. The purpose of this study is to assess the effects of variable stocking intensities on patterns of genetic diversity and population differentiation among nearly 2000 brook charr (*Salvelinus fontinalis*) from 24 lakes located in two wildlife reserves in Québec, Canada. Our results indicated that the level of genetic diversity was increased in more intensively stocked lakes, mainly due to the introduction of new alleles of domestic origin. As a consequence, the population genetic structure was strongly homogenized by intense stocking. Heavily stocked lakes presented higher admixture levels and lower levels of among lakes genetic differentiation than moderately and un-stocked lakes. Moreover, the number of stocking events explained the observed pattern of population genetic structure as much as hydrographical connections among lakes in each reserve. We discuss the implications for the conservation of exploited fish populations and the management of stocking practices.

Keywords: brook charr, genetic diversity, microsatellites, population structure, salmonids, stocking

Received 25 September 2009; revision received 27 February 2010; accepted 3 March 2010

Introduction

Environmental impacts originating from human activities have caused important decline in wild animal populations throughout the world (International Union for Conservation of Nature 2009). This is especially important in salmonid fishes which have a considerable economical and recreational value (i.e. sport and commercial fishing) and whose stocks have been exhausted by overharvesting, habitat loss and biological invasions (Waples & Hendry 2008). As a result, stocking, performed with non-native or domesticated fish, is now commonly performed in many lakes and rivers (Hindar *et al.* 1991; Aprahamian *et al.* 2003). However, whereas stocking was originally regarded as a measure to counteract population declines, recent studies have suggested that stocking domestic hatchery-reared individuals could instead hinder the recovery of wild populations (Ford 2002; reviewed in Araki *et al.* 2008). Domestic and wild fish often drastically differ in terms of behaviour, morphology and genetics due to the different selection regimes they experience (Hindar *et al.* 1991; Gross 1998; Heath *et al.* 2003; Huntingford 2004). For example, domestic fish have been shown to be more aggressive than wild fish as a result of the higher density under which they are maintained in captivity (Gross 1998; Einum & Fleming 2001; Blanchet *et al.* 2008). As well, domestic fish are typically genetically distinct from wild populations, both in terms of allelic composition and genetic diversity (reviewed in Hutchings & Fraser 2008; Finnegan & Stevens 2008; Shikano

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et al. 2008). At the genomic level, pronounced differences in genome wide levels of gene expression have also been reported between domestic and wild salmon (Roberge *et al.* 2006; Normandeau *et al.* 2009).

Such differences entail possible important consequences of stocking hatchery-reared domestic fish into wild populations. A reduction of the genetic differentiation between populations and a loss of genetic diversity in the recipient populations (Araguas *et al.* 2004; Hansen *et al.* 2006; Eldridge & Naish 2007; Karaiskou *et al.* 2009) might ultimately lead to a loss of local adaptation and a reduction in fitness of wild populations (reviewed in Araki *et al.* 2008; reviewed in Finnegan & Stevens 2008; reviewed in Hansen *et al.* 2009). These impacts might substantially reduce the evolutionary potential of wild populations and affect their chance of persistence (Stockwell *et al.* 2003; Frankham 2005). Yet, few studies have empirically quantified the impacts of stocking on the genetic integrity of wild populations. A recent study by Hansen *et al.* (2009) documented the effects of stocking on several rivers from western Jutland, Denmark. Following a severe decline of the populations of brown trout (*Salmo trutta*), the rivers were subjected to stocking with non local strains. The authors showed that a genetic change occurred between historical and contemporary time periods and that in most cases the genetic differentiation was lower between hatchery strains and contemporary samples than between hatchery strains and historical samples. However, the determinants of the observed levels of population genetic structure remained unclear.

Here we assess the impacts of variable stocking practices on the genetic integrity of population of brook charr (*Salvelinus fontinalis*, Salmonidae) in two wildlife reserves in Québec, Canada. Brook charr is one of the most important fish species from both an economical and recreational point of view in Eastern Canada. For example, six millions specimens are stocked every year in lakes throughout the Province of Québec which represents 50% of all business generated by its aquaculture industry (Ministère des Ressources Naturelles et de la Faune du Québec 2008). Yet, despite the importance of this species, very little is known about the possible genetic outcomes of such stocking practices on charr wild populations. Specifically, we quantitatively compared the effects of different stocking intensities on the resulting genetic diversity within lakes and population genetic differentiation among 24 lakes from two distinct geographic areas with different stocking history. We further assessed the relative importance of stocking intensity and putative ecological determinants of population structure on the observed patterns of genetic differentiation among lakes within each reserve.

Materials and methods

Sampling sites

Sampling was conducted in the Portneuf and Mastigouche wildlife Reserves in Québec, Canada (47°09′N, 72°17′W and 46°40′N, 73°30′W respectively; Fig. 1). We chose lakes in these reserves because of their well documented stocking histories since 1971. Reserves were created then, with the objective of preserving the wildlife resources while allowing various recreational activities such as fishing. Thus, some lakes are subjected to stocking to support recreational fishing activities, to decrease the pressure of fishing on the natural populations or to restore the depleted populations. Consequently, the stocking intensity is more or less important depending on the objectives for the lakes. This enabled us to select lakes from three categories of stocking intensity: non-stocked (NS), moderately stocked (MS) and heavily stocked (HS) (Table 1). A NS lake was a lake where no stocking event was documented from 1971 to 2007. A MS lake underwent stocking in less than 50% of years over the past 15 years (from 1992 to 2007). On average, such type of lake was stocked with 5819 ± 3427 (mean ± SD) and 6604 ± 6715 fish per year respectively for the Portneuf and the Mastigouche Reserve. A HS lake underwent stocking events in more than 50% of the past 15 years (from 1992 to 2007) with on average 14926 ± 12930 and 34425 ± 12641 stocked fish per lake per year respectively for the Portneuf and the Mastigouche Reserve. The origin of stocked fish was also known for each reserve: for the Portneuf Reserve, only domestic fish from a single hatchery were stocked; for the Mastigouche Reserve, domestic–wild hybrids from three other hatcheries were used for stocking, with wild males originating from lakes within the reserve being crossed to domestic females. Brook charr were sampled in 24 lakes (11 in Portneuf and 13 in Mastigouche) using multifilament gillnets (1.8 m deep × 38 m long) in June 2007 and 2008 (Table 1). Tissues were also obtained from hatcheries (56 individuals and 122 individuals respectively for the Portneuf and Mastigouche hatchery sources). Tissue samples (adipose fins) were preserved in 95% ethanol until DNA extraction.

DNA extraction

DNA was extracted from fin clips (5 mm^2) using a slightly modified version of Aljanabi & Martinez (1997) salting out method. Namely, after addition of the saline solution, the mixture was centrifuged for 30 min at 10 000 ***g***. Also, DNA precipitation was achieved by incubation at −20 °C with 600 μL isopropanol for

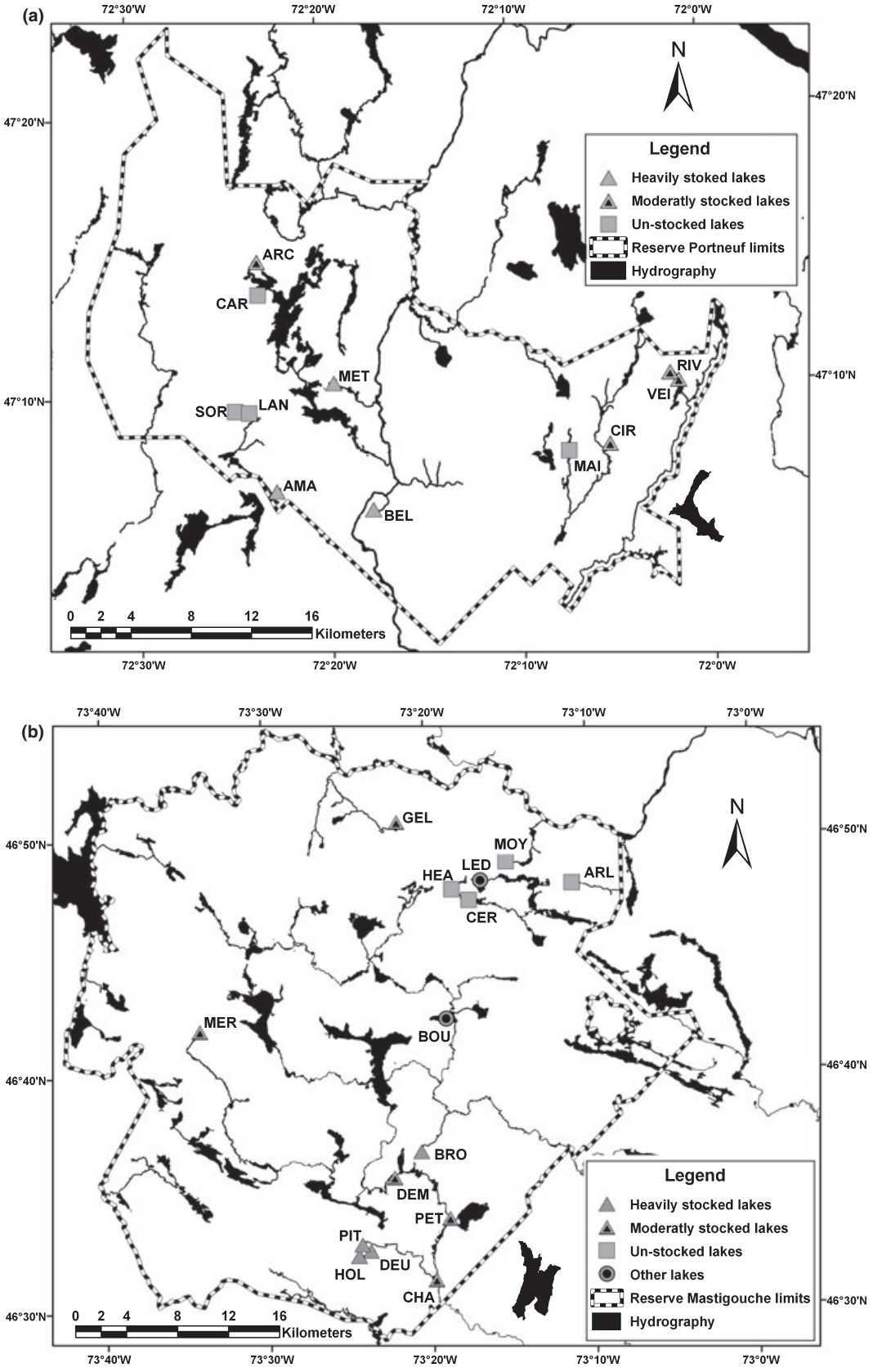

Fig. 1 Geographical locations of lakes within the two wildlife reserves: (a) Portneuf, and (b) Mastigouche, in Québec, Canada. See Table 1 for abbreviations of lake names.

Table 1 Characteristics of the lakes in each reserve including their stocking intensity category (SI), the number of fish sampled (N_s), the number of fish genotyped (N) over 2 years of sampling, allelic richness (A_r), mean observed (H_O) and expected (H_E) heterozygosity and F_{IS} for each lake and the average for all the lakes of a same stocking intensity (with the sum of fish genotyped for a same stocking intensity)

Reserve	Lake	SI	N_s	N	A_r	H_O	H_E	F_{IS}
Portneuf	Caribou (CAR)	NS	79	79	2.966	0.524*	0.555	0.059
	Langoumois (LAN)	NS	55	51	2.787	0.461*	0.568	0.116
	Sorbier (SOR)	NS	92	84	2.959	0.511	0.542	0.065
	Main de Fer (MAI)	NS	129	102	2.487	0.469	0.456	−0.043
	Average	NS	355	316	2.798	0.491	0.530	0.049
	Rivard (RIV)	MS	48	46	3.456	0.646	0.614	−0.035
	Veillette (VEI)	MS	125	101	3.425	0.592*	0.632	0.064
	Arcand (ARC)	MS	57	56	2.339	0.491	0.448	−0.099
	Circulaire (CIR)	MS	80	77	2.825	0.518*	0.503	−0.034
	Average	MS	310	280	3.011	0.562	0.549	−0.026
	Belles de Jour (BEL)	HS	81	81	4.160	0.683*	0.738	0.082
	Amanites (AMA)	HS	91	88	3.570	0.572*	0.665	0.157
	Méthot (MET)	HS	102	87	4.210	0.736*	0.753	0.028
	Average	HS	274	256	*3.947*	0.664	0.719	0.089
Mastigouche	Head (HEA)	NS	80	76	3.316	0.606	0.669	0.002
	Cerné (CER)	NS	110	103	3.130	0.538*	0.662	0.041
	Moyen (MOY)	NS	102	97	2.114	0.431*	0.428	−0.032
	Arlequin (ARL)	NS	85	81	2.227	0.368	0.380	0.035
	Average	NS	*377*	357	*2.697*	0.485	0.535	0.011
	Chamberlain (CHA)	MS	110	103	4.285	0.795	0.769	−0.035
	Demarest (DEM)	MS	149	137	3.040	0.570*	0.657	0.029
	Petit St-Bernard (PET)	MS	59	54	3.941	0.655*	0.705	0.082
	Mercure (MER)	MS	90	84	4.067	0.728*	0.722	0.005
	Gélinotte (GEL)	MS	76	72	3.846	0.826*	0.667	−0.153
	Average	MS	484	450	3.836	0.715	0.704	−0.014
	Brochard (BRO)	HS	71	67	3.837	0.752*	0.725	−0.069
	Deux étapes (DEU)	HS	90	85	3.919	0.806*	0.714	−0.151
	Hollis (HOL)	HS	82	82	3.867	0.838*	0.730	−0.163
	Pitou (PIT)	HS	101	100	3.924	0.819*	0.731	−0.132
	Average	HS	344	334	*3.887*	0.803	0.725	−0.129

NS: non-stocked lakes; MS: moderately stocked lakes; HS: heavily stocked lakes.
*Hardy–Weinberg disequilibrium ($P < 0.05$).

30 min. Then, after washing out the pellet with ethanol, we centrifuged at 10 000 *g* for 10 min.

Microsatellites analyses

A total of 852 (Portneuf) and 1141 (Mastigouche) brook charr as well as all individuals from hatcheries were genotyped with ten microsatellite loci (see Table S1, Supporting Information). Microsatellite loci amplification was performed using a GeneAmp PCR 9700 thermocycler (Applied Biosystems) with the following 10 μL reaction mixture: 16 mM Tris–HCl pH 8.3; 16 mM KCl; 16 mM $(NH_4)_2SO_4$; 2.5 mM $MgCl_2$ (sfoC129), 3 mM $MgCl_2$ (sfoC113, sfoC88, SCO216, sfoB52, sfoD75), 3.5 mM $MgCl_2$ (sfoC24, sfoC86, SCO218, sfoD100); 0.2 mM of each dNTPs; 0.4 mg/mL BSA; 200 mM forward primer (sfoC129), 400 mM forward primer (sfoC113, sfoC88, SCO216, sfoB52, sfoD75, sfoC24, sfoC86, SCO218, sfoD100); 400 mM reverse primer (sfoC129, sfoC113, sfoC88, SCO216, sfoB52, sfoD75, sfoC24, sfoC86, SCO218, sfoD100); 1 U Taq; 5 ng DNA template. We combined 0.15 μL of 600 Liz (Applied Biosystems) internal size standard and 8.85 μL of Formamide Hi–Di (Applied Biosystems) to 1 μL of pooled PCR products (first pool included sfoC113, sfoC88, sfoD75 and sfoD100, and another pool included SCO216, sfoB52, sfoC24, sfoC86, SCO218 and sfoC129). PCR products were visualized using an AB 3130 capillary DNA sequencer (Applied Biosystems) and allele size was established using the software Genemapper version 4.0 (Applied Biosystems).

Genetic diversity

Deviation from Hardy–Weinberg equilibrium was tested using Genepop (version 4.0; Rousset 2008). Linkage

disequilibrium, the presence of null alleles for each locus, expected and observed heterozygosity (H_E and H_O respectively) were investigated using Cervus (version 3.0.3; Marshall *et al.* 1998; Kalinowski *et al.* 2007). Allelic richness (A_r) and F_{IS} were estimated using FSTAT (version 2.9.3.2; Goudet 1995).

Indexes of genetic diversity (H_O, A_r and F_{IS}) were assessed for each lake in each year, and then averaged for all lakes, in each year and in each reserve, belonging to the same category of stocking intensity. We also estimated genetic diversity for the individuals of hatcheries for the Portneuf and the Mastigouche Reserve respectively. We combined the individuals of the three hatcheries for the Mastigouche Reserve since these were stocked in unknown level of admixture. We used linear mixed models to assess the effects of stocking intensity, reserve of origin, and their interaction on each index of genetic diversity. Lakes were included as a random effect in the model, if significant following a likelihood ratio test. The significance of fixed effects was assessed from their Wald statistic. Analyses were performed with GenStat (version 12; VSN intl.).

Private alleles, defined as alleles for a given locus present in only one of the category of stocking intensity, were estimated for each reserve, both including and removing individuals obtained from hatcheries in order to assess their specific contribution, in terms of private alleles, to each lake, using Convert (version 1.31; Glaubitz 2004). We used a generalized linear model and a Poisson distribution with GenStat to assess the effects of reserve, category of stocking intensity and microsatellites loci on the number of private alleles observed both with and without individuals from hatcheries. Significance was established using the change in deviance resulting from dropping one of the factors from the full model and tested against a chi-square distribution with appropriate change in degrees of freedom.

Population genetic structure

We first evaluated the extent of spatio-temporal variability in the population genetic structure using an analysis of molecular variance (AMOVA) implemented in Arlequin (version 3.11; Excoffier *et al.* 2005). Specifically, for each stocking intensity we assessed the amount of variance attributable to (i) years, (ii) lakes within years and, (iii) individuals within lakes. Given there were no significant differences among years of sampling (results not shown), we pooled data from both years in all remaining analyses.

The extent of genetic differentiation among each pair of lakes, for each stocking intensities category and for each reserve, was assessed using pairwise F_{ST} estimates (Weir & Cockerman 1984) obtained in FSTAT. We tested if there were differences in genetic differentiation between reserves and different levels of stocking intensity using the comparison among groups of samples with FSTAT. We also assessed the significance of the genetic differentiation observed (i) among each type of lakes and (ii) between hatchery fish and fish from each category of lake using Mann–Whitney tests implemented in GenStat. Each F_{ST} estimates between wild and hatchery populations was based on fish sampled in a given lake vs. hatchery fish which were of one source for the Portneuf Reserve and three different hatchery sources for the Mastigouche Reserve.

We performed Mantel tests (Mantel 1967; implemented in FSTAT) to test for isolation by distance (IBD) patterns for each stocking intensity within each reserve. IBD is present if a significant relationship is found between the geographic distance matrix and the pairwise genetic differentiation matrix (using linearized $F_{ST}/(1 - F_{ST})$; Rousset 1997). Geographical distances between lakes for each reserve were estimated linearly using ArcGis program (version 9.2; ESRI Corporation, Redlands, California). Each IBD test was based on 10 000 randomizations.

Finally, we performed Partial Mantel tests (using FSTAT) to assess the relative importance of stocking intensity and ecological determinants on the observed patterns of genetic differentiation among lakes within each reserve. More specifically, we assessed the partial correlations between the extent of genetic differentiation among two lakes and (i) the geographic distance between them, (ii) their level of hydrographical connections and (iii) the average number of stocking events in both lakes. The hydrographical connections refer to the lakes of our study which are connected between them. If a lake has one or more hydrographic connections with another lake, the value 1 was given, if not it was the value 0. The hydrographical network was obtained using ArcGis program (version 9.2; ESRI Corporation, Redlands, CA, USA). The average number of stocking events for a pair of lakes was assessed by averaging their number of stocking events occurring between 1971 and 2007.

Admixture analyses

We estimated the individual admixture proportions (q-values) for each individual in each stocked lake (MS and HS) using STRUCTURE v.2.2. (Pritchard *et al.* 2000). Each analysis included fish sampled in a given lake as well as hatchery fish (fish from a single hatchery or three hatcheries respectively for the Portneuf and the Mastigouche Reserve). In the case of Mastigouche, since the proportions of each putative hatchery source could not be quantified, we pooled fish of all three hatcheries

to constitute a single 'domestic gene pool'. We assumed that fish should be assigned to two distinct genetic groups ($K = 2$; i.e. wild and domestic individuals). An admixture model with correlated allele frequency was used for each analysis with 250 000 steps of the Markov-Chain preceded by a burnin-period of 100 000 steps. We then assessed the relationships between the average individual admixture proportions (q-values) and the number of stocking events for each lake using a non-parametric Spearman correlation.

Results

All loci were highly polymorphic, ranging from nine to 19 alleles, with an average of 13 alleles at each locus. The proportion of null alleles within our data (pooled loci) was 5.8% (range 2.8–11.3%). The locus SCO216 showed the highest proportion of null alleles and was removed from our remaining analyses. Significant deviations from Hardy–Weinberg equilibrium were found in 16 lakes out of 24, nine of which were stocked lakes (Table 1).

Genetic diversity

Name, level of stocking, sample size and measures of genetic diversity are summarized for each lake within each reserve in Table 1. Results of the linear mixed model analysis showed that allelic richness increased significantly with stocking intensity. The differences were 1.19 ± 0.28 and 1.22 ± 0.30 alleles ($P < 0.001$) between MS and NS lakes, and HS and NS lakes respectively. While there was no difference between reserve in terms of allelic richness ($P = 0.156$), there was a significant interaction between reserve and stocking intensity ($P = 0.050$), mainly due to the difference in allelic richness observed for MS lakes between reserves (3.01 ± 0.18 in Portneuf and 3.93 ± 0.15 in Mastigouche) (Table 2). Accordingly, hatchery fish showed higher levels of allelic richness than the wild populations with values of 6.16 and 4.60 for fish stocked in the Portneuf and the Mastigouche Reserves, respectively.

Analyses of differences in observed heterozygosity using linear mixed model showed that the mean observed heterozygosity significantly increased with stocking intensity ($P < 0.001$). The differences were 0.24 ± 0.05 and 0.32 ± 0.06 respectively between MS and NS lakes, and HS and NS lakes (Table 2). Moreover, the mean observed heterozygosity was significantly different between reserves, being higher in the Mastigouche than in the Portneuf reserve (0.56 ± 0.02 in Portneuf and 0.68 ± 0.03 in Mastigouche, $P < 0.001$; Table 2). Again, this was in line with value found in hatchery fish that showed value of observed heterozygosity close to those found in HS lakes (0.72 and 0.77 for the Portneuf and the Mastigouche Reserve respectively).

Table 2 Effects of reserve of origin, stocking intensity and their interaction on allelic richness (A), observed heterozygosity (B) and F_{IS} (C)

(A) Variable	Wald statistic	d.f.	*P-value*
Reserve	2.19	1	0.156
Stocking intensity	29.79	2	<0.001
Reserve × Stocking intensity	7.12	2	0.050
	Estimate	SE	
Lakes'	0.150	0.060	
Residual	0.042	0.014	
(B) Variable	Wald statistic	d.f.	*P-value*
Reserve	9.08	1	0.008
Stocking intensity	40.71	2	<0.001
Reserve × Stocking intensity	5.67	2	0.086
	Estimate	SE	
Lakes	0.005	0.002	
Residual	0.001	0.000	
(C) Variable	Wald statistic	d.f.	*P-value*
Reserve	12.04	1	0.001
Stocking intensity	4.87	2	0.101
Reserve × Stocking intensity	23.29	2	<0.001
	Estimate	SE	
Residual	0.005	0.001	

Full model results from linear mixed models are presented with corresponding Wald statistic for each term when fitted last in model. Significant random effects for each model are also presented with their standard error.

Stocking intensity alone did not affect F_{IS} values (Table 2). However, F_{IS} were significantly different among reserves ($P = 0.001$), being positive in Portneuf and negative in Mastigouche (0.03 ± 0.02 in Portneuf and −0.05 ± 0.02 in Mastigouche; Table 2). The interaction between reserve and stocking intensity was also significant ($P < 0.001$), partly due to the difference between HS lakes of each reserve. Thus F_{IS} values were significantly positive for HS lakes in the Portneuf reserve (mean $F_{IS} = 0.09$, $P = 0.031$) but negative (mean $F_{IS} = -0.13$, $P = 0.008$) for the Mastigouche reserve (Table 1). Once again, F_{IS} values of hatchery individuals were in line with those found in each reserve, being positive in Portneuf (0.022) and negative in Mastigouche (−0.006).

We found a marginally non-significant effect of stocking intensity on the number of private alleles detected ($\chi^2_{(2)} = 4.90$, $P = 0.086$) when running the analyses without hatchery fish. This was due to a greater number of

private alleles being found in HS lakes (eight and seven respectively for the Portneuf and Mastigouche Reserves) than in NS lakes (five and one respectively) ($\chi^2_{(1)} = 3.99$, $P = 0.046$). The number of private alleles for the MS lakes was likely closer to that found in NS lakes for the Portneuf reserve (two and five respectively), while in Mastigouche it was closer to that found in HS lakes (five and seven respectively). Yet, there was no effect of reserve ($\chi^2_{(1)} = 0.14$, $P = 0.71$) or loci ($\chi^2_{(8)} = 4.54$, $P = 0.81$) on the number of private alleles observed. The analyses including the hatchery fish revealed that the contribution of these fish to the number of private alleles observed in stocked lakes was important. Indeed, the difference among stocking intensity was no longer significant when removing alleles from stocked lakes that were similar to hatchery alleles ($\chi^2_{(2)} = 1.39$, $P = 0.50$). Specifically, HS lakes shared four and one alleles with hatchery individuals respectively for the Portneuf and the Mastigouche Reserve. For the MS lakes, the results revealed the sharing of one private allele with hatchery individuals for the Mastigouche Reserve.

Population genetic structure

AMOVA results showed that there was no significant difference in genetic variation between years for any of the stocking intensity and in both reserve (percentage of variation from −8.04% to 2.06%; all $P > 0.14$). Among lakes structure within reserve was responsible for 24.8% and 24.3% ($P < 0.001$ for each reserve) of the variation for NS lakes for the Portneuf and Mastigouche Reserves respectively, whereas HS lakes had much lower values at 4.64% and 1.5% respectively ($P < 0.001$ for each reserve). Moderately stocked lakes were intermediate with the structure among lakes being responsible of 18.4% and 9.2% ($P < 0.001$ for each reserve) of the variation respectively for the Portneuf and Mastigouche Reserves. Within individuals level was responsible for the remaining variation (all values above 74% and all $P < 0.05$ except for HS lakes of the Mastigouche reserve $P > 0.05$). Overall, the AMOVA analyses provided a first indication that the level of genetic differentiation among lakes was impacted by the intensity of stocking whereby an increase in stocking intensity resulted in a weaker genetic structuring among lakes.

Analyses of pairwise F_{ST} among lakes revealed no differences in the mean level of genetic differentiation among reserves ($P = 0.697$). However, there was a significant difference in F_{ST} among stocking intensities within reserves ($P < 0.001$), which indicated that the level of genetic differentiation among lakes decreased as a function of stocking intensity (Fig. 2). Post-hoc Mann–Whitney tests indeed revealed significant differences between each stocking intensity comparison (NS > MS: $U = 45.0$, $P = 0.017$; NS > HS: $U = 12.0$, $P = 0.002$; MS > HS: $U = 24.0$, $P = 0.005$). More specifically, in the Portneuf reserve, F_{ST} among lakes was 0.199 ± 0.045 on average for NS lakes but only 0.050 ± 0.013 for HS lakes (Fig. 2; Table S2, Supporting Information). Similarly, in the Mastigouche reserve values were 0.268 ± 0.055 for NS lakes but reduced to 0.013 ± 0.005 for HS lakes (Fig. 2; Table S2, Supporting Information). Moderately stocked lakes were intermediate in both cases (see Table S1, Supporting Information). Moreover, Mann–Whitney tests on F_{ST} between hatchery fish and each lake category showed, for the Portneuf Reserve, marginally significant greater difference between comparisons involving NS lakes than HS lakes (NS > HS, U = 0, $P = 0.057$) as well as MS lakes than HS lakes (MS > HS, $U = 0$, $P = 0.057$), while no significant differences were found in comparisons of NS lakes and MS lakes ($P = 0.886$). For the Mastigouche Reserve, significant difference was shown among MS lakes and HS lakes comparisons to the hatchery fish (MS > HS, $U = 0$, $P = 0.029$) and marginally significant differences appeared among NS lakes and MS lakes (NS > MS, $U = 2$, $P = 0.063$) and among MS lakes and HS lakes (MS > HS, $U = 2$, $P = 0.063$). More specifically, the F_{ST} among lakes and hatchery fish were on average of 0.220 ± 0.032 and 0.183 ± 0.066 respectively for the Portneuf and the Mastigouche Reserve for NS lakes, 0.215 ± 0.042 and 0.071 ± 0.061 for the same comparisons involving MS lakes and 0.054 ± 0.026 and 0.023 ± 0.004 on average for HS lakes-hatchery fish differentiation.

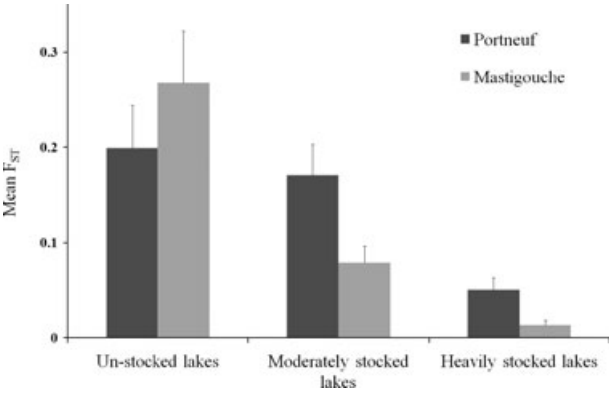

Fig. 2 Average pairwise genetic differentiation (F_{ST}) between lakes for each intensity of stocking of the Portneuf and Mastigouche Reserves. Standard error bars represent the 95% CI on the mean.

Results of the Mantel test of IBD showed that linear geographic distance explained 82.7% of the genetic differentiation among the un-stocked lakes of the Portneuf Reserve (slope = 0.009, $P = 0.03$). This relationship was

Table 3 Partial Mantel tests results with overall r^2 of the model, partial correlation coefficients, slope and associated *P*-values

Reserve	Explanatory variable	Partial correlation coefficient (%)	Slope (β)	*P*-value
Portneuf (r^2 = 0.571)	Geographical distance	−26.1	−0.0003	0.79
	Hydrographical connections	−42.4	−0.2040	<0.001
	Number of stocking events	−56.9	−0.0077	<0.001
Mastigouche (r^2 = 0.442)	Geographical distance	−13.2	−0.0010	0.40
	Hydrographical connections	−37.6	−0.0843	0.031
	Number of stocking events	−53.2	−0.0103	<0.001

marginally non-significant among the un-stocked lakes of the Mastigouche reserve (76.0%, slope = 0.036, P = 0.069). On the other hand, IBD patterns were absent among the heavily and moderately stocked lakes of each reserve (results not shown).

The Partial Mantel tests indicated that overall, geographic distance did not significantly impact the extent of genetic differences among populations within both reserves. In contrast, the test showed that the genetic differentiation between lakes decreased with the average number of stocking events performed and with the strength of hydrographical connections among lakes (Table 3; Fig. 3).

Admixture

Results of admixture analyses are summarized in Table 4. In brief, in both reserves the admixture levels (mean *q*-values) were higher for the HS lakes than for the MS lakes (see Table 4). However, the difference was more pronounced in Portneuf reserve (0.023 ± 0.046 for the MS lakes and 0.451 ± 0.414 for the HS lakes) than in Mastigouche (0.428 ± 0.290 for the MS lakes and 0.449 ± 0.238 for the HS lakes). Spearman correlations also showed that average individual admixture increased significantly with the number of stocking events in both reserves (Portneuf: r = 0.65; t = 2.53; d.f. = 9; P = 0.032; Mastigouche: r = 0.68; t = 2.92; d.f. = 10; P = 0.015).

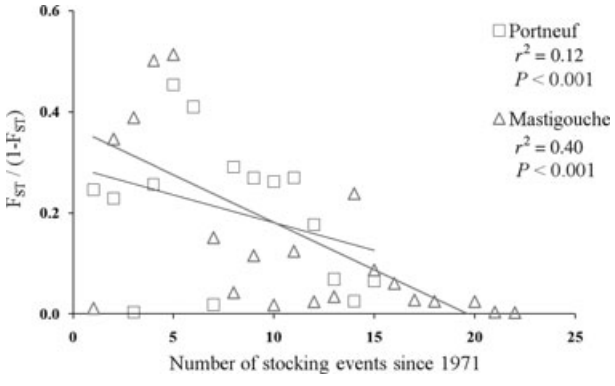

Fig. 3 Relationships (resulting from the Partial Mantel test) between the average value of pairwise genetic differentiation (F_{ST}) values of lakes in both lakes and the average number of stocking events occurred since 1971 in both lakes for the Portneuf and Mastigouche Reserve. The r^2 and the *P*-value (obtained from the Partial Mantel test) are indicated for each reserve.

Discussion

Our main objective was to assess the impact of stocking intensity on the level of genetic diversity and the resulting population genetic structure in brook charr. Our results showed that stocking domesticated brook charr in wild populations greatly affected their genetic integrity by modifying the nature of genetic diversity within populations and by reducing the level of population genetic differentiation among lakes.

Our findings of higher genetic diversity in stocked lakes contrast with the usual perception that domestic fish should harbor lower genetic diversity (Hutchings & Fraser 2008; Shikano *et al.* 2008; Karaiskou *et al.* 2009). For example, Blanchet *et al.* (2008) showed that captive bred Atlantic salmon (*Salmo salar*) have lower allelic richness and heterozygosity than their wild counterparts. Also, Hansen *et al.* (2006) found that hatchery-reared catla (*Catla catla*) populations exhibited a lower genetic variation, in terms of allelic richness and heterozygosity, than wild populations. Other studies, however, found no difference between the genetic diversity of hatchery-reared populations and wild populations. For example, Small *et al.* (2009) showed that in general, supplemented populations of chum salmon (*Oncorhynchus keta*) had the same level of genetic diversity and similar effective population size as unsupplemented populations in the Puget Sound area (Washington, USA).

The apparent increase in genetic diversity with stocking intensity we report here has to be taken with caution. Indeed, our results revealed that such diversity is partly due to the contribution of new private alleles of domestic origin as indicated by the sharing of private

Table 4 Mean individual admixture (q-value) ± SD for each stocked lake and the average for all lakes belonging to a given stocking intensity (SI)

Reserve	Lake	SI	Mean individual admixture (*q*-value) ± SD
Portneuf	Rivard (RIV)	MS	0.024 ± 0.035
	Veillette (VEI)	MS	0.012 ± 0.018
	Arcand (ARC)	MS	0.023 ± 0.066
	Circulaire (CIR)	MS	0.034 ± 0.066
	Average	MS	0.023 ± 0.046
	Belles de Jour (BEL)	HS	0.519 ± 0.453
	Amanites (AMA)	HS	0.520 ± 0.463
	Méthot (MET)	HS	0.315 ± 0.325
	Average	HS	0.451 ± 0.414
Mastigouche	Chamberlain (CHA)	MS	0.385 ± 0.236
	Petit St-Bernard (PET)	MS	0.184 ± 0.295
	Mercure (MER)	MS	0.367 ± 0.401
	Gélinotte (GEL)	MS	0.777 ± 0.227
	Average	MS	0.428 ± 0.290
	Brochard (BRO)	HS	0.738 ± 0.219
	Deux étapes (DEU)	HS	0.350 ± 0.272
	Hollis (HOL)	HS	0.323 ± 0.235
	Pitou (PIT)	HS	0.383 ± 0.226
	Average	HS	0.449 ± 0.238

MS: moderately stocked lakes; HS: heavily stocked lakes.

alleles between individuals of hatcheries and stocked lakes. In fact, additional analyses revealed that the correlation between the most common alleles of hatchery fish (having a frequency of 0.10 or higher) and those found in HS lakes was higher (0.71) than those in MS lakes (0.25) and NS lakes (0.42). Partial correlations (taking into account the similarity between each sample) showed a positive value (0.69) between hatchery and HS lakes, while MS lakes and NS lakes showed negative relationships with hatchery alleles (−0.30 and −0.10 respectively). This further supports our suggestion that genetic diversity in stocked lakes has been modified largely due to stocking activities. We suggest that such results are likely explained by a combination of factors, including differential effect of genetic drift and by differences in origins between domestic and wild populations.

It has been suggested that alleles of domestic origin, favoured in captivity, may be deleterious and recessive when fish are returned in the natural environment (Frankham 2008). As a result, several studies reported that hatchery fish have lower fitness in natural environment than wild fish (reviewed in Araki *et al.* 2008; reviewed in Hansen *et al.* 2009). Araki *et al.* (2008) showed for example that when selection (in captivity and in the wild) and heritability are strong, and if selection acts on a single trait (e.g. growth rate), a rapid fitness decline can be observed. Others suggested, using a meta-analysis approach, that domestic fish lose almost 20% of their fitness per generation (reviewed in Araki *et al.* 2007). As for brook charr, few studies specifically aimed at assessing the differences in genetic diversity or fitness among groups of different origins. Fraser (1981) showed that domestic brook charr were caught only in the first year following stocking whereas recoveries of hybrids and wild strains were spread over the 3–4 following years, suggesting a lower survival of domestic brook charr in natural environment. Lower rate of survival could be explained in parts by intraspecific and interspecific competition. Indeed, the absence of competitors during hatchery rearing relaxes natural selection for competitive abilities. For example, the recovery of stocked brook charr was four times higher in lakes with no competitors than in lakes containing both white sucker (*Catostomus commersoni*) and native brook charr (Lachance & Magnan 1990). Unfortunately, we have no data on survival of brook charr depending on stocking intensity in the present study system. The assessment of differential survival in the current context would surely represent a promising avenue for future research.

Our results showed that genetic differentiation among heavily stocked lakes is three to 16 times lower than in un-stocked lakes (F_{ST} = 0.050 vs. 0.199 and 0.013 vs. 0.268 for the Portneuf and the Mastigouche reserve respectively). The effect of stocking intensity on the genetic structure of wild fish populations has been assessed in a few other studies (Araguas *et al.* 2004; Karaiskou *et al.* 2009). For example, on the west coast of Denmark, after the drastic decline of brown trout (*Salmo trutta*) wild populations in the 1960–1970s, intensive stocking of domesticated brown trout took place

until 1996. However, according to Hansen (2006), these stocking continue with the permanent brood stock of introgressed Skjern River trout. By comparing old scale samples (1945–1956) from the wild populations prior to stocking to contemporary samples, Hansen (2002) showed a strong genetic contribution from domesticated brown trout, with a few remaining presumed non introgressed wild trout in the Skjern River. More specifically, Hansen *et al.* (2006) showed that the level of genetic differentiation between contemporary samples (introgressed populations) (mean F_{ST} = 0.028, SD 0.014) was about half as strong as between historical samples (wild populations) (mean F_{ST} = 0.045, SD 0.016), thus also pointing to a relative homogenization of the population structure due to stocking. Similarly, a study on the supplementation of lake trout (*Salvelinus namaycush*) populations in Southern Ontario (Canada), with exogenous individuals from hatchery strains of the Great Lakes region, showed a homogenization of the population structure of some stocked lakes (Halbinsen & Wilson 2009). More specifically, lakes with evidence of introgression exhibited a homogenization of the genetic structure among populations (Halbinsen & Wilson 2009). Another study conducted on coho salmon (*Oncorhynchus kisutch*) in Puget Sound (Washington, USA) showed a decrease of the level of genetic differentiation decreased with the number of fish hatchery released within rivers (Eldridge & Naish 2007).

The level of genetic differentiation among un-stocked lakes (F_{ST} = 0.199 and 0.268 for the Portneuf and the Mastigouche reserve respectively) were consistent with reported level of differentiation for natural population of brook charr (F_{ST} = 0.159–0.201) from the nearby La Mauricie National Park (Québec, Canada) (Angers & Bernatchez 1998). IBD analyses confirmed that such patterns of differentiation were present only in un-stocked lakes of both reserves reflecting drift and possible local adaptation of these populations. IBD patterns disappeared when including all lakes in the analyses, in which case hydrographical connections and the number of stocking events better explained the observed differentiation patterns in each reserve (Table 3), thus reemphasizing the importance of stocking in modifying the genetic population structure in this system. A previous study on brook charr populations from Gros Morne National Park in Newfoundland, Canada showed no relationships between genetic divergence of populations and contemporary waterway distance (Poissant *et al.* 2005). Instead, the authors showed that the level of genetic divergence among populations in their system was strongly correlated with inferred historical landscape features (Poissant *et al.* 2005). The authors suggested that the patterns of genetic diversity found within these populations were the results of small distance migrations during colonization and differentiation through drift. In other freshwater fish species, geographical and hydrographic connections have been shown to be main determinants of population structure. For example, Crispo *et al.* (2006) showed among natural populations of Trinidadian guppies (*Poecilia reticulata*) that geographical distance, associated with waterfalls, increased genetic divergence and reduced dispersal and long-term gene flow. As well, Olsen *et al.* (2008) showed in different populations of western Alaska chum salmon (*Oncorhynchus keta*) that gene flow was reduced with the increasing complexity of hydrographic connections.

Overall, our admixture analyses revealed that the mean individual admixture level increased significantly with the number of stocking events performed at a given location. This corroborates the results of Hansen & Mensberg (2009) who showed that two rivers with different stocking intensities presented different levels of introgression. In details, the Skjern River (945 000 stocked individuals) supported a large number of admixed individuals (71%), whereas the majority of individuals of the Storaa River (around 100 000 individuals) were non-admixed (79%). In contrast, a study by Taylor *et al.* (2007), on the effects of stocking non-native rainbow trout (*Oncorhynchus mykiss*) into the Athabasca River (Alberta, Canada) revealed little detectable genetic introgression between hatchery and wild fish.

While this study did not aim to specifically address this issue, our results also suggested that the outcome of stocking might be slightly variable depending on the number of hatcheries and on the origin of fish used. For example, the heterozygosity values for the moderately and heavily stocked lakes were greater in the Mastigouche Reserve than the Portneuf Reserve and F_{IS} values of the heavily stocked lakes of the Portneuf Reserve were higher than in the Mastigouche Reserve. These observations are potentially due to the fact that fishes used for stocking in the Portneuf Reserve are pure domestic, which are not completely homogenized with their wild counterparts, thus generating a deficit in heterozygotes. For the Mastigouche Reserve, hybrids (crosses between domestic females and wild-origin males) from three hatcheries are used for stocking, which is likely to generate an excess of heterozygotes. Admixture results also suggest that at high stocking intensity, domestic individuals substantially contribute to the local genetic pool (e.g. HS lakes in Portneuf). Moreover, our observation of a more pronounced level of admixture in MS lakes from Mastigouche than MS lakes from Portneuf raises the possibility that stocking hybrids may have accelerated introgression rate relative to domestic stocking at moderate stocking intensity (but see also Griffiths *et al.*

2009). On the other hand, we should emphasize that, MS lakes of the Portneuf reserve have not been stocked on average since 18.1 (±14.0) years, whereas lakes in Mastigouche reserve are still stocked on average every 3.7 (±3.3) years. Taylor *et al.* (2007) suggested that one potential factor explaining low level of admixture they observed in rainbow trout was the long lapse of time since the most recent introduction of hatchery fish in their system (30–80 years). They also argued that in most stocked localities natural selection could have potentially eliminates hatchery genotypes (Taylor *et al.* 2007). This further suggests that when stocking stops, the genetic integrity of native populations may be at least partly restored (see also Ruzzante *et al.* 2004; Eldridge & Naish 2007). Indeed, in our study system the extent of genetic differentiation of MS lakes of the Portneuf Reserve is closer to that of NS lakes, whereas it is closer to that of HS lakes in the Mastigouche Reserve (Fig. 2).

Another possibility for the low rate of introgression being detected in MS lakes of Portneuf is that the hatchery samples we analysed are not representative, genetically, of the hatchery fish that were stocked in Portneuf lakes more than 30 years ago. Such difference would have impeded our current discrimination of hatchery and indigenous fish. However, even though we have limited information on the actual strain of fish being stocked over the period preceding the current study, this possibility seems unlikely since the most common domestic strains used for stocking in Quebec are very similar genetically to each other relative to their wild counterparts (Martin *et al.* 1997; see also Taylor *et al.* 2007).

To our knowledge, this study represents the first large-scale analysis of the consequences of stocking on patterns of genetic diversity and genetic structure of populations of brook charr. Brook charr is the most important species used to support recreational fishing in Quebec and is widespread throughout Eastern Canada. Thus, this study has several implications for the conservation of exploited fish populations and the management of stocking practices. Currently, regulations concerning stocking and exploitation of brook charr in lakes exist, but implementations of the importance of maintaining the genetic diversity of populations of this species are limited. Our results suggest that stocking may lead to a loss of local adaptation and eventually a net loss in biodiversity, although this remains to be corroborated by the analysis of variation at potential 'adaptive genes'. Our results thus imply that the management of all non stocked lakes and their fish populations should be treated as independent units, to maintain the maximum of genetic diversity, and thus the local adaptations. However, lakes with hydrographical connections and naturally migrating fish could be considered as a same unit given the importance of this factor in shaping the observed level of genetic differentiation. Stocking activities could arguably be maintained in the heavily stocked lakes since these lakes are already severely impacted by such activities. For the moderately stocked lakes, we suggest to avoid stocking in lakes which have not been stocked for a long time as the level of genetic structuring in such lakes approaches that of the un-stocked lakes. Quite clearly, future management plans must take into account the preservation of the genetic diversity and structure of populations of wild brook charr.

In summary, this study clearly showed that the loss of genetic integrity of wild brook charr populations, both in terms of genetic diversity and population structure, is strongly associated with the extent of stocking intensity. Admittedly however, these results do not allow to decipher the dynamics of introgression which will require a more detailed study of patterns of introgressive hybridization observed in each population and a statistical assessment of the effects of variation in abiotic and biotic factors that characterized each lake. We are currently conducting such investigation.

Acknowledgements

We are grateful to Fabien Lamaze, Bruno Mayot, Marc-André Poulin, Andréanne Lessard and Mélissa Lieutenant-Gosselin for their help with field sampling and laboratory analyses. Thanks also to Eric Taylor and two anonymous reviewers for their constructive comments on a previous version of this paper. We acknowledge the important contributions of the Ministère des Ressources Naturelles et de la Faune du Québec (MRNF), the Société des Établissement de Plein-Air du Québec (SÉPAQ) and the Réseau Aquaculture Québec (RAQ) to the project. This research was financially supported by a strategic project grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada to LB and DG.

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This study is part of A.D.M.'s doctoral research in D.G.'s laboratory, which aims to document the genetic consequences of stocking in Brook Charr. The research interests of both L.B. and D.G. lie in the understanding of the patterns and processes of molecular and organismal evolution, as well as their significance to adaptation and conservation.

Supporting Information

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

Table S1 Microsatellites loci details including the size range in base pairs (bp), the annealing temperature in degrees Celsius (°C), the number of cycles used in the polymerase chain reaction (PCR), the GenBank accession number and references

Table S2 Pairwise F_{ST} (above diagonal) and associated *P*-value (below diagonal) for (a) the Portneuf reserve and (b) the Mastigouche reserve

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