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Loss of historical immigration and the unsuccessful rehabilitation of extirpated salmon populations

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Abstract Comprehensive evaluations of multiple genetic factors are rarely undertaken in rehabilitation attempts of extirpated populations, despite a growing need to address why some rehabilitation projects succeed and others fail. Using temporally-spaced samples of microsatellite DNA, we tested several genetic hypotheses that might explain an unsuccessful attempt to re-establish Atlantic salmon populations (*Salmo salar*) in two rivers of the inner Bay of Fundy, Canada. Census sizes (N) in both populations plummeted to near zero from initial increases after reintroduction/human-mediated recolonization occurred. Over the same period (1974–1996), both populations were characterized by low or relatively low effective sizes (N_e) and temporally unstable genetic structuring, whereas neighbouring populations, known historically for their significant salmon production, were not. Despite evidence for genetic bottlenecking and continual linkage disequilibrium over time in both populations, neither exhibited detectable inbreeding or a significant loss of allelic diversity or heterozygosity relative to known donor/source populations. Ratios of N_e to N also increased with decreasing N in both populations, implying a buffering capacity against losses of genetic diversity at depressed abundances. Most significantly, multiple lines of evidence were consistent with the hypothesis that there has been substantial and recurrent asymmetric migration (migration rate, m) from neighbouring areas into both populations even after initial rehabilitation. This included migration from a historically productive population that became extirpated during the course of rehabilitation efforts, indicating that both populations might have naturally depended on immigration from neighbouring areas for persistence. Our results highlight the value of incorporating temporal genetic data beyond commonly used metrics of neutral genetic diversity (F_{ST}, allelic richness, heterozygosity) to evaluate rehabilitation successes or failures. They also illustrate how the joint evaluation of multiple genetic concerns in rehabilitation attempts, at spatial scales beyond donor and rehabilitated populations, is useful for focusing future rehabilitation efforts.

Keywords Rehabilitation · Recolonization · Reintroduction · Atlantic salmon · Metapopulation · Asymmetric gene flow · Effective population size · Temporal stability · Genetic compensation · Effective-census size ratio

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Introduction

From a genetic perspective, different options must be considered when a population (or group of populations) becomes severely depleted or extirpated. If intervention in the form of a reintroduction is deemed necessary to rehabilitate the population, what constitutes an appropriate donor population? Should one evaluate the possibility that natural recolonization might occur from elsewhere? Even after a population is reintroduced or recolonized, other genetic concerns still loom because the new population often will initially have small census (N) and effective population

sizes (N_e) (Franklin 1980; Frankham et al. 2002). For example, the rate of loss of genetic diversity per generation via random genetic drift is greater when N_e is small and, in the absence of migration, this rate is expected to increase as N_e becomes smaller (Frankham et al. 2002). Inbreeding and its potential effects also increase as populations become smaller (Franklin 1980; Frankham et al. 2002; Keller and Waller 2002). Initial reintroductions themselves might not establish enough genetic variation into populations (Frankham et al. 2002). Additionally, the mating system or biology of some species may result in a reduced capacity of populations to 'buffer' against losses of genetic diversity at depressed sizes, a process referred to as genetic compensation (Ardren and Kapuscinski 2003).

Although not mutually exclusive, all of the above factors might augment the susceptibility of populations to environmental change and thus increase the probability of extirpation. Consequently, their joint evaluation over time is crucial to decipher if particular genetic factors are implicated in any reintroduction attempt, whether it is successful or unsuccessful. Such evaluations, however, are rarely undertaken, because they require multiple genetic samples of both donor and reintroduced populations, knowledge of other aspects of population structure and biology (e.g. N_e, abundance trends) over time, and details of the reintroduction history, all of which are frequently unknown (but see Latch and Rhodes 2005).

Here we use a comprehensive series of long-term genetic analyses to jointly address the role of genetic factors in an unsuccessful rehabilitation of two Atlantic salmon (*Salmo salar*) populations in the inner Bay of Fundy (iBoF), Canada. Phylogeographic, life history and demography studies have suggested that salmon spawning in rivers of the iBoF are differentiated from other regional salmon groups in North America (DFO 2002; COSEWIC 2006). As early as the 1800s, iBoF rivers were often used to transport timber, frequently with sawmills and associated dams (Perley 1851; Jones and Clay 1995). These complete barriers prevented seaward migration of juveniles and breeding migrations of returning adults, resulting in the extirpation of salmon populations from several rivers. As with other salmonid fish species, some iBoF populations have been the recipients of significant rehabilitation efforts (COSEWIC 2006). As a consequence, insights gained from a study of these populations may be relevant to many rehabilitation programs.

Two such populations were the geographically proximate Point Wolfe and Upper Salmon Rivers (Fig. 1). A reintroduction program implemented in Point Wolfe followed dam removal in this river in the early 1980s and consisted of introduced juveniles from the nearby Big Salmon River (Fig. 1). Reintroduction was deemed more desirable than natural recolonization because of associated genetic concerns from low numbers of founders, and the lengthy time required for establishing a self-sustaining population (Alexander and Galbraith 1982). It is unclear whether the purported advantages of reintroduction in Point Wolfe were justified. Notably, natural recolonization of salmon occurred in Upper Salmon after removal of dams in this river in the late 1960s (Dadswell 1968). Nevertheless, following adult peak returns in Point Wolfe and Upper Salmon of 200 (1985) and 1200 (1967), respectively, N declined to near zero in each river by the mid 1990s for unknown reasons (Fig. 2). In fact, virtually all other iBoF populations began to decline considerably by the late 1980s. By the late 1990s, these rivers contained very few, if any, Atlantic salmon (COSEWIC 2006).

It is notable from this standpoint that other iBoF rivers, known historically to contribute large components of the Bay's salmon production, were affected by human influences at approximately the same time as the rehabilitation efforts within Point Wolfe/Upper Salmon Rivers, and the general iBoF population decline, commenced. In particular, the construction of a causeway on the Petitcodiac River in 1968 is estimated to have negatively affected 20–28% of the salmon production in the iBoF (70% in Chignecto Basin, where Point Wolfe and Upper Salmon Rivers are located; Fig. 1) (Hutchings 2003, references therein; COSEWIC 2006). Such an impact might have subsequently affected the sustainability of iBoF salmon, if smaller iBoF populations have historically depended on immigrants from larger populations for local persistence (Hutchings 2003). The extent to which this may have contributed historically to iBoF salmon declines has not previously been addressed.

Using microsatellite DNA, we firstly evaluated the degree to which salmon from several iBoF rivers were genetically differentiated and to what extent this population structuring was temporally stable over the long term in the donor population (Big Salmon), the population of reintroduction efforts (Point Wolfe) and the naturally recolonized population (Upper Salmon). We then addressed several questions pertaining to the potential role of genetic factors in the unsuccessful rehabilitation of Point Wolfe and Upper Salmon populations: (1) Did reintroduction or recolonization lead to sufficient genetic variation within populations? (2) Did populations (reintroduced or recolonized) experience genetic bottlenecking or inbreeding? (3) Did populations (reintroduced or recolonized) exhibit a lack of genetic compensation at depressed abundance

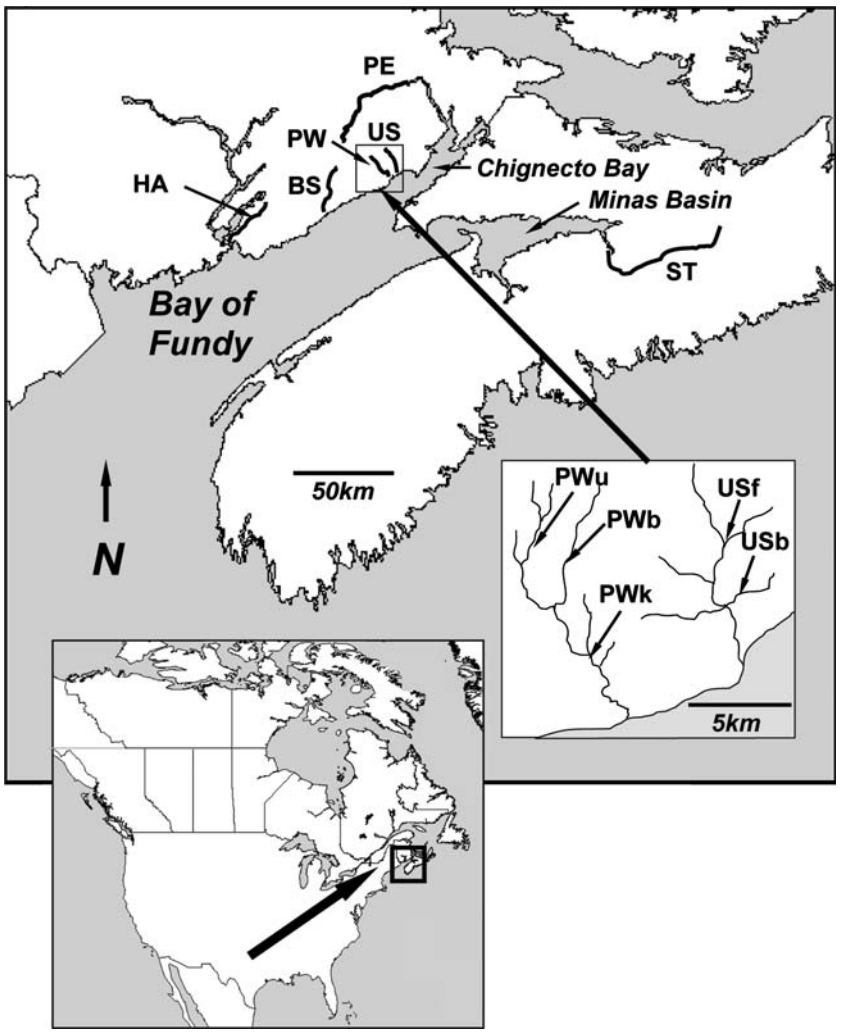

Fig. 1 Map of the location of the six Bay of Fundy Rivers sampled for the study. BS = Big Salmon R.; PW = Point Wolfe R.; US = Upper Salmon R.; HA = Hammond R.; PE = Petitcodiac R.; ST = Stewiacke R. The small inset map shows the locations of samples collected within rehabilitated rivers for particular years (Point Wolfe River: PWb, at Bennett Creek; PWk, at Key Hole; PWu, upper PW above the confluence with the east branch; Upper Salmon River: USb, at Black Hole; USf, at the forks with Haley River)

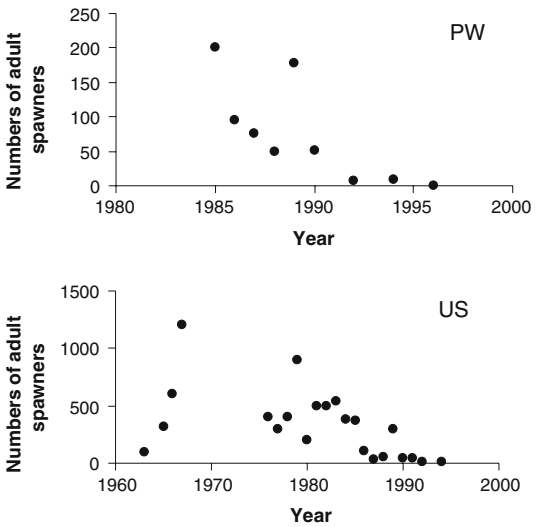

Fig. 2 Estimated number of returning, adult spawning salmon in Point Wolfe (PW) and Upper Salmon (US) Rivers, between 1985–1996 and 1963–1995, respectively

levels? (4) Can processes beyond the spatial scale of the source and reintroduced/recolonized populations explain the persistence of the latter populations?

Methods

Background on sampled rivers

A total of 32 spatial and temporal samples of Atlantic salmon were collected from 5 iBoF rivers and 1 outer BoF River (Hammond R.) from 1970 to 1996 (Fig.1). Four iBoF Rivers flow into Chignecto Bay: Point Wolfe R. (PW), Upper Salmon R. (US), Big Salmon R. (BS) and Petitcodiac R. (PE). A fifth river, Stewiacke (ST), flows into Minas Basin (Fig. 1). Dried scale samples of adults originated from individuals captured during spawning migrations. Adipose fin tissue samples of parr (salmon prior to their migration to sea) were obtained from electrofished individuals captured across multiple locations within each river and stored in 95% ethanol;

in some cases, sample sizes in specific areas were sufficient to compare genetic differentiation between them (PW1992–96; US1996; see Fig. 1 for details; Table 1).

The PW (drainage area: 130 km^2) and US (174 km^2) populations were extirpated by the 1930s (Dadswell 1968). Between 1982 and 1985, 42,000 fingerling progeny of salmon from BS were introduced annually into PW, before dam removal occurred in 1985 (Jones and Clay 1995). Salmon began to naturally recolonize US in the mid 1960s after the removal of remaining dams. No recorded stocking of salmon (reintroductions) in US occurred up to the latest temporal sample in the

Table 1 Summary of genetic diversity at five microsatellite loci among samples from six Bay of Fundy Rivers. Numbers within sample codes represented the year that samples were collected (e.g. BS70 = 1970). Within-river samples from PW and US are as follows: PWu (upper PW, above the confluence with the east branch); PWb (at Bennett Creek); PWk (at Key Hole); USb (at Black Hole); USf (at Forks). Sample size = N; mean sample size genotyped = $N\hat{}$

River	Sample Code	N	$N\hat{}$	HWE	LD	LD$\hat{}$	H_O	H_E	A	A_C	L_{EX}	P_{sign}	P_{Wilk}	F_{IS}
Big Salmon R.	BS70	36	34.0	202(d)*	1		0.665	0.748	10.6	10.2	2	0.67	0.69	**0.127**
	BS74	18	15.8		1		0.756	0.759	8.2	–	2	0.66	0.50	0.004
	BS84	14	12.4				0.814	0.762	7.6	–	3	0.65	0.59	– 0.024
	BS88	31	30.0	85(d)			0.742	0.756	10.2	10.1	1	0.28	0.89	0.036
	BS89	43	41.6		1	1	0.761	0.750	10.8	10.1	2	0.41	0.69	– 0.003
	BS90	15	14.8				0.771	0.782	8.2	–	4	0.33	0.15	0.049
	BS93–95*	90	90.0		2	2	0.760	0.768	12.8	10.2	3	0.66	0.41	0.017
Point Wolfe R.	PW82–84*	26	22.0	85(d)	2		0.770	0.744	9.0	–	2	0.32	0.59	– 0.008
	PW88*	13	11.2		1		0.778	0.723	7.6	–	1	0.09	0.92	– 0.025
	PWu92*	60	60.0	171(e)	4	1	0.721	0.683	7.6	6.7	3	0.68	0.69	– 0.048
	PWk92*	30	30.0	171(e)	1		0.813	0.775	10.2	10.2	1	0.33	0.31	– 0.032
	PWu93*	58	58.0		7	3	0.762	0.731	8.2	7.1	4	0.32	0.08	– 0.011
	PWk93*	33	31.6		2		0.821	0.777	10.4	10.2	3	0.67	0.41	– 0.046
	PWb93*	50	46.0		1		0.760	0.757	10.4	9.6	4	0.33	0.10	0.011
	PWk94*	47	45.6	85(d)	4	2	0.745	0.775	11.0	9.4	4	0.29	0.10	0.005
	PWb94*	46	44.0	12(d)	1		0.769	0.764	10.2	10.1	3	0.66	0.31	0.048
	PWu96*	62	61.8	85(e)*	6	2	0.780	0.683	6.0	5.5	4	0.31	0.03	– 0.133
	PWk96*	27	26.8		4		0.798	0.774	9.8	–	3	0.66	0.10	0.002
	PWb96*	40	39.0	171(e)	3		0.718	0.706	9.8	9.3	4	0.33	0.31	– 0.018
Upper Salmon R.	US74	34	30.0		1		0.836	0.775	9.4	8.4	4	0.33	0.08	– 0.061
	US84	38	36.6		2		0.781	0.769	10.8	9.6	3	0.66	0.41	0.002
	US89	15	14.8				0.812	0.776	10.8	–	4	0.32	0.06	– 0.011
	US93*	30	30				0.792	0.789	9.8	9.8	5	0.07	0.01	0.014
	USb96*	37	37.0	171(e)	9	2	0.746	0.701	9.8	9.6	3	0.65	0.42	– 0.050
	USf96*	62	62.0	171(e)	7	2	0.787	0.766	9.9	9.8	2	0.32	0.40	– 0.019
Petitcodiac R.	PE83	35	31.6	197(d)			0.771	0.783	8.4	8.3	5	0.08	0.02	0.038
	PE84	20	16.8				0.852	0.799	7.8	–	5	0.06	0.02	– 0.021
Stewiacke R.	ST78	42	35.8				0.771	0.802	8.8	8.6	5	0.09	0.06	0.041
	ST84	37	34.8				0.785	0.802	10.2	9.9	3	0.67	0.19	0.021
	ST88	27	27.0	197(e)	3	3	0.815	0.778	8.8	–	4	0.32	0.06	– 0.047
	ST90	15	14.6				0.836	0.787	8.2	–	4	0.32	0.31	– 0.063
Hammond R. (Outer Bay of Fundy)	HA96*	54	51.4	202(d)			0.751	0.795	10.6	10.5	4	0.33	0.18	0.021
Mean		37.0	35.5											
Total		1185	1137	(95.9%)										
Total (archival)		420	400.6	(95.4%)										

Samples with asterisks are parr samples. Loci showing significant deviations from Hardy–Weinberg Equilibrium (HWE) within samples ($P < 0.05$) are indicated by the locus name (i.e. *Ssa202* = 202), followed by the type of deviation in parentheses (d = heterozygote deficit; e = heterozygote excess). HW deviations with asterisks were still statistically significant following Bonferroni corrections. LD indicates the number of loci-pairs out of a total of 10 tests that showed evidence of linkage disequilibrium in each sample (LD$\hat{}$= following Bonferroni correction). Also highlighted are observed (H_O) and expected (H_E) heterozygosities, the mean number of alleles per locus (A), and the corrected mean number of alleles per locus (A_C) within each sample (corrected to $N = 30$). L_{EX} indicates the number of loci with a heterozygote excess out of 5 in each sample, where H_E exceeds H_{Eq} (see Material and Methods on bottleneck tests). Significance (P) of heterozygote excess tests associated with bottlenecking were assessed using sign and Wilcoxon tests implemented in BOTTLENECK (Piry et al. 1999) and are indicated by P_{sign} and P_{Wilk} (one-tail for heterozygosity excess), respectively. F_{IS} represents the inbreeding coefficient over all loci within each sample of Weir and Cockerham (1984), with those samples having F_{IS} values significantly exceeding zero in bold (following 1000 permutations in GENETIX: Belkhir et al. 2000; level of significance, $P = 0.05$)

present study (1996). Although BS (332 km^2) was also dammed intermittently from the 1800s to 1920s, salmon continued to gain access to the spawning grounds via a fish passageway; complete dam removal occurred by 1963 (Jessop 1986). BS was stocked intermittently with non-native salmon from rivers outside the BoF from 1938 to 1969 (Gibson et al. 2003). Declines from several thousand to a few hundred returning adults occurred in BS from the late 1960s to the early 1990s (Jessop 1976; DFO 2002). Temporal samples from PE (3000 km^2) and ST (2700 km^2) were included in our sampling because these rivers have by far the largest habitat areas for salmon of any rivers within the iBoF, and because they were the most productive iBoF salmon populations in Chignecto Bay/Minas Basin in terms of production in the past (Gibson and Amiro 2003; Hutchings 2003). Thus, historically, PE and ST were the most probable candidate sources of immigrants within the iBoF. PE became extirpated by about 1990 following the construction of a causeway (1968) and a poorly designed fish passageway. A remnant population is believed to still exist in ST. Extensive stocking of mixed progeny from PE and other BoF populations (BS and Saint John R., New Brunswick) occurred during rehabilitation attempts in the 1980s; ST has been stocked with native progeny since 1965. The Hammond River (HA), within the Saint John R. basin, was stocked with Saint John R. juveniles until the late 1970s (Gibson et al. 2003).

Molecular genetic analyses

For DNA extractions, archived scales (1–3 per individual) or fin tissue (50 mg) were digested in 200 ul of buffer (10 mM Tris, 50 mN KCl, 0.5% tween20) and proteinase K (0.1–0.4 μg). Samples were incubated at 45–55°C for 2–16 h and vortexed intermittently. Samples were then heated at 94°C (10 min) to eliminate proteinase K, centrifuged (5 min), and diluted to final concentrations of 3–300 ng/μl). DNA from each individual was then genotyped at five microsatellite loci (*Ssa12*, *Ssa85*, *Ssa171*, *Ssa197*, *Ssa202*; O'Reilly et al. 1996; O'Reilly 1997). Polymerase chain reaction (PCR) and electrophoresis conditions followed those of O'Reilly et al. (1996), but with two duplexes (*Ssa85*/*202* and *Ssa171*/*191*) and one uniplex (*Ssa12*). Alleles were scored manually against a standard of combined PCR product from eight individuals of known allele sizes.

Genetic diversity within samples

Both the reintroduction program (PW) or natural recolonization process (US) might have failed to establish sufficient genetic variation into populations. If true, we might expect lower genetic diversity in PW relative to its source (BS) and in US relative to its candidate sources (other iBoF rivers, including BS). We thus firstly quantified allelic richness (A) and heterozygosity (observed: H_O; expected: H_E) in all temporal/spatial samples. Using the rarefaction method of FSTAT 2.9.3 (Goudet 2001), we then standardized A to a sample size of $n = 30$ (PWk92) to increase the power of detecting differences in A (Leberg 2002) and to approximate the mean n of each sample in our study (Table 1). We finally compared A, H_O, and H_E between the 22 of 32 samples with corrected sample sizes ($n = 30$), using two-way analyses of variance (ANOVAs; factors: river, time period: 1970s, 1980s or 1990s).

Tests of Hardy–Weinberg and linkage equilibrium within samples

We verified Hardy–Weinberg equilibrium (HWE) expectations of genotypic frequencies across loci in each sample and at each locus, as well as genotypic linkage equilibrium between all loci pairs, using GENEPOP 3.3 (Raymond and Rousset 1995). We were particularly interested in whether considerable linkage disequilibrium existed among loci within rehabilitated populations (PW, US) relative to other populations, as linkage disequilibrium is expected within populations experiencing recent bottlenecks (McVean 2002) or population turnover (extinction-recolonization dynamics) (Ohta 1982), or where a recent admixture of populations occurs (Ardlie et al. 2002; Tero et al. 2003).

Bottleneck tests within samples

Genetic bottlenecking can be associated with periods of small census population size and can occur in newly founded populations (Cornuet and Luikart 1996; Frankham et al. 2002). These conditions might have typified the early stages of reintroduction in PW or recolonization in US, wherein bottlenecking could have increased the susceptibility of each rehabilitated population to environmental change (and thus extirpation) through a loss of genetic diversity. We thus tested for evidence of recent genetic bottlenecks in all temporal/spatial samples. Although several approaches exist for this task, a recent simulation study concluded that one method was particularly suited to the biological conditions likely found in this study's rehabilitated populations. Namely, Williamson-Natesan (2005) found that the heterozygote excess test of Cornuet and Luikart (1996) had the highest precision for detecting

bottlenecks under a two-phase mutation model of microsatellite evolution (TPM; see below) and when (i) bottlenecks were more recent (last few generations), (ii) mutation rates were low, (iii) pre-bottleneck population sizes were likely low (probable for PW and US at the onset of rehabilitation), and (iv) demographic recovery of populations did not occur. The approach of Cornuet and Luikart (1996) considers that A is lost faster than H_E during a bottleneck due to a loss of rarer alleles that do not contribute significantly to H_E. To test for a heterozygote excess in each temporal/spatial sample, we compared H_E to a simulation-generated distribution of that expected from the observed number of alleles at mutation-drift equilibrium (H_{Eq}), using 5000 iterations in BOTTLENECK 1.2.12 (Piry et al. 1999). The standard deviation (SD) of H_{Eq} was used to compute the standardized difference for each locus (($H_E - H_{Eq}$)/SD) and enabled the computation of a P-value for H_E. Significance of the P-value was assessed using sign and Wilcoxon tests, as suggested for studies with a small number of loci (Cornuet and Luikart 1996). We assumed a two-phased model of mutation (TPM) for our microsatellites, with 70% and 30% of the mutations following the step-wise mutational model (SMM) and the infinite allele model (IAM), respectively. We adopted the TPM with such frequencies for two reasons. First, the TPM is probably closest to the true mode of mutation at microsatellite loci (Di Rienzo et al. 1994; Ellegren 2000), including in other salmonid fishes (Angers and Bernatchez 1998). Second, there is a predominance of SMM mutations in microsatellites (e.g. Shriver et al. 1993). We treat our bottleneck tests with caution, however, since the number of loci used in our study is low (five), which can reduce the statistical power necessary to detect bottlenecks (Cornuet and Luikart 1996; Williamson-Natesan 2005). As a result, we consider a less conservative level of significance of $P = 0.10$ as an indication of genetic bottlenecking, particularly because the heterozygote excess test can be too conservative under certain conditions (Williamson-Natesan 2005). Note that these bottleneck tests also assumed well-defined population structure with no immigration (Cornuet and Luikart 1996), which might be unlikely in iBoF salmon populations (see below).

Potential inbreeding within rehabilitated population samples

If inbreeding has occurred within rehabilitated populations (PW, US), its consequences might have increased their susceptibility to extirpation (e.g. Frankham et al. 2002; Keller and Waller 2002). We therefore estimated inbreeding coefficients (Weir and Cockerham's 1984 F_{IS}) over loci in different temporal samples of PW and US, using GENETIX 4.0 (Belhkir et al. 2000). Several definitions of inbreeding exist in the literature (reviewed in Keller and Waller 2002). Our use of F_{IS} follows the definition of inbreeding wherein inbred individuals have parents that are more closely related than two randomly chosen individuals in a population, relative to a randomly mating population of the same size (Crow and Kimura 1970). Under this nonrandom mating definition of inbreeding, F_{IS} values exceeding zero signify more inbreeding than expected by chance (Crow and Kimura 1970; Keller and Waller 2002).

Genetic differentiation, population structure and temporal stability

As a first step in evaluating population structure among our samples, we tested for genetic differentiation between spatial samples within rivers in the same year in rehabilitated rivers (PW, 1992–96; US, 1996; Fig. 1, Table 1), using Weir and Cockerham's (1984) θ_{ST}. Spatial samples within rivers showing little evidence of genetic differentiation (PWb and PWk for 1993–96) were pooled for simplicity in subsequent assignment tests and phylogenetic analyses (see below). As a second step, we compared genetic differentiation (θ_{ST}) between all temporal samples. We evaluated temporal stability in within- or between-river population structure using θ_{ST} because the differences in sample size in our study (n = 13–90) have no appreciable effect on this estimator (Ruzzante 1998; Kalinowski 2002). Similarly, we estimated distance-based relationships among temporal samples from an unrooted neighbour-joining (NJ) clustering analysis of Nei's (1978) unbiased genetic distance (D), since bias with this distance metric is negligible with varying sample sizes as long as sample sizes are not too small (see Kalinowski 2002). We used POPULATIONS 1.12.14 to estimate distance-based relationships (Langella 2001; 2000 bootstraps over loci).

Effective population sizes

We estimated generational effective population sizes (N_e) within rehabilitated populations (PW, US) to determine whether either population might have been susceptible to losing genetic diversity through genetic drift due to low N_e (Frankham et al. 2002). Where sufficient sample sizes permitted (for any population), we estimated N_e using three contrasting methods that considered temporal changes in allele frequencies

between sampling periods given that no currently available method accommodates all major intricacies of the Atlantic salmon's overlapping generation life history (circa within PW: 1982–1996; US: 1974–1996; BS: 1970–1996; ST: 1978–1988). Each method thus makes different assumptions that might not completely conform to this species' life cycle (in addition to assuming random sampling within populations). Since there was also good evidence for subpopulation structure within PW (PWu versus PWb and PWk) and US (USb versus USf) (see Results) after rehabilitation efforts were initiated, N_e was estimated with each method separately for different subpopulations where permissible.

The first method, described by Waples (1990a), assumed that populations were closed to migration and was the only method to accommodate for overlapping generations and age structure in Atlantic salmon (as most temporal methods of N_e estimation assume discrete generations). That is, assuming discrete generations when generations overlap can lead to bias in the estimation of N_e (Waples 1990a, 2002a). Such bias can be reduced in salmonid fishes with overlapping generations by estimating the effective number of breeders per year N_b, another measurable parameter that has a signal determined by N_e (i.e. N_e per generation = gN_b, where g is the generation length: Waples 1990a). However, the N_b overlapping generation model also assumes semelparity or very low iteroparity (<10%), and iteroparity is known to be greater within iBoF populations than Atlantic salmon populations elsewhere (≈20–30%) (Jessop 1986; Amiro 2003). We thus utilize this approach with caution in the iBoF, but note that iteroparity is particularly a concern when temporal samples are collected less than one generation apart. This can lead to genetic correlations between allele frequencies if the temporal samples involve some of the same adult individuals or progeny of the same adult individuals (Waples 1990a,b, 2002a). Despite a mixture of juvenile and adult samples, this is probably unlikely in our study (Table 1), given that N_e estimates were only based on temporal samples exceeding one generation (Table 2).

We followed the methodology of Waples (1990a) to estimate N_b and subsequent generational N_e within populations, using Microsoft^{TM} Excel spreadsheets. Low-frequency alleles (<0.02 over both sampling years) were pooled into a single allele class to reduce the downward bias that rare alleles can have on N_e estimation with this method (Waples 1990a). Values of b, an analogue to the average number of generations elapsed between temporal samples, followed those outlined for various years between samples for a 4-year generation time (to approximate the 3.7 year generation time of iBoF salmon: COSEWIC 2006), based on similar age-class distributions observed in two iBoF rivers (BS, ST: COSEWIC 2006; see Waples 1990a). The 95% confidence intervals associated with N_e were calculated following Waples (1989).

The second method for estimating N_e assumed discrete populations and similarly, that populations were closed to migration (Wang's 2001 pseudo-likelihood method; implemented in MLNE: Wang and Whitlock 2003). We specifically employed this method to make general comparisons with the first method and the third method, which also assumed discrete generations but had the advantage of jointly estimating both N_e and m, the migration rate per generation, for the population under consideration, within the time interval sampled (also in MLNE). Indeed, the assumption of no migration in most temporal methods is clearly unrealistic in many cases, and Wang and Whitlock (2003) have shown that migration can introduce a significant bias in N_e estimates. The Wang and Whitlock (2003) method also required allele frequency data for a source population thought to be contributing migration (per generation) to the population in question. For this purpose, pooled allele frequency data from all BS, PE, ST and HA samples were used. For comparison, we also estimated N_e in each of the other populations for which there were adequate temporal data (BS, ST), adjusting the source population allele frequencies each time by excluding allele data from the population in consideration. Note that Wang and Whitlock's (2003) model assumes constant migration from a source population of infinite size and fixed allele frequency. Although simplistic, it might be roughly applicable to rehabilitated populations in iBoF for two reasons. First, the likely sources of rehabilitated populations (e.g. BS, PE, ST) were continuously abundant iBoF salmon populations over time (Gibson and Amiro 2003; Hutchings 2003). Second, these same populations show evidence for temporal stability in within-population structure (see Results). This suggests that these populations were likely continual sources of immigrants to other iBoF rivers, and that more gradual changes in allelic frequency have occurred within each. Additionally, there was a danger in only estimating N_e assuming that a population was closed to immigration (as the first two methods do), when *a priori* we had anecdotal evidence that the historical propensity of dispersal in iBoF populations might have been quite high (Dadswell 1968; Jones and Clay 1995; COSEWIC 2006).

When running MLNE, a maximum N_e of 9000 was implemented. Upper confidence intervals reaching 9000 were assumed to be ∞. We also assumed a

Table 2 Summary of genetic differentiation (θ_{ST}) between samples of Atlantic salmon within rivers of the Bay of Fundy. Above the diagonal are mean θ_{ST} values over all pairwise comparisons (range in parentheses) and the number of significant comparisons of the total number of comparisons after the commas

	BS	PW	PWu	US	PE	ST	HA
BS	**0.006 (0–0.022), 3/21**	0.013 (0–0.025), 32/63	0.079 (0.044–0.119), 21/21	0.021 (0–0.062), 25/42	0.027 (0.011–0.045), 12/14	0.025 (0–0.055), 22/28	0.031 (0.007–0.037), 6/7
PW	All BS-PW82/84, 88	**0.006 (0–0.031), 6/36**	0.039 (0.021–0.096), 27/27	0.025 (0–0.069), 49/54	0.025 (0.018–0.044), 16/18	0.026 (0.006–0.047), 33/36	0.033 (0.027–0.056), 9/9
PWu			**0.029 (0.026–0.032), 3/3**	0.082 (0.05–0.144), 18/18	0.064 (0.047–0.083), 6/6	0.079 (0.054–0.102), 12/12	0.083 (0.059–0.098), 3/3
US	All BS-US74/84			**0.032 (0.009–0.061), 10/15**	0.030 (0.008–0.089), 9/12	0.032 (0.006–0.107), 22/24	0.038 (0.020–0.078), 6/6
PE				US84/89 – PE83	**0.001, 0/1**	0.006 (0–0.011), 1/8	0.028 (0.027–0.029), 2/2
ST					All except PE83-ST88	**0.004 (0–0.012), 0/6**	0.022 (0.017–0.030), 4/4
Within River Trends							
BS: BS93/95 versus BS70,88,89			PW: PWk92 versus PWb96/k96; PWb93 versus PWb96/k96			US: All US96 versus before US96 (16 comparisons)	

Bold values are within-river temporal comparisons. Below the diagonal are notable trends of non-significant differentiation *between* certain rivers, as well as significant differentiation between temporal samples *within* certain rivers. PW = PW82–84, PW88, PWb and PWk samples (abbreviations from Table 1 and Fig. 1)

generation time (T) of 3.7 years (see above), though it might be an overestimate because it did not account for the contribution of mature male parr. Given that pseudo-likelihood methods allow only whole (T) and not partial (T') integers for sampling intervals, N_e and m estimates (N_e', m') were converted by $N_e = (T/T')N_e'$ and $m = 1 - e^{(T'/T)log(1-m')}$ following Wang and Whitlock (2003).

Effective-census size ratios, genetic depensation/compensation within rehabilitated populations

We evaluated whether there was evidence for genetic compensation/depensation in either rehabilitated population (PW, US), reflected by an increase/decrease in effective/census size ratios (N_e/N) as N decreased in each population. Under genetic depensation, for instance, genetic variation related to fitness and population viability might have been lost in either rehabilitated population as they declined. Point estimates of N (Fig. 2) originated from Jones and Clay (1995) and were based on swim-through (snorkel) counts conducted annually each autumn during the spawning period in PW and US (details of methodology in Jones and Clay 1995). Subsequently, we followed Waples (2002b, 2005) and used generational estimates of N for the given temporal period within PW and US to obtain corresponding estimates of the ratio between N_e/N. For time periods where it was warranted, an overall population estimate of N_e (N_{eT}) that accounted for subpopulation structure in each rehabilitated population (e.g. PWu versus PWb/PWk; see Results) was calculated from Wright's (1943) finite island model equation: $N_{eT} = N_T/(1 - F_{ST})$, where $N_T = nN$ (number of subpopulations X N_e of each subpopulation, which for simplicity we took as the mean N_e between subpopulations), and F_{ST} is the degree of genetic differentiation between subpopulations (between PWu and PWb/PWk: mean $\theta_{ST} = 0.039$; between USb and USf: mean $\theta_{ST} = 0.059$). While this model makes simplistic assumptions about metapopulation structure (e.g. subpopulations have equal N and receive the same fraction of migrants drawn randomly from the migrant pool), other models of structured population N_e (e.g. Hedrick and Gilpin 1997; Whitlock and Barton 1997; Nunney 1999) require information on subpopulation productivity that is unavailable for rehabilitated rivers.

We note that the swim-through counts of N did not account for the degree of uncertainty around point estimates, and this method for estimating abundance can be prone to bias (Korman et al. 2002; Thurow et al. 2006). Although this makes it difficult to compare

our N_e/N ratios with other Atlantic salmon populations (including other iBoF rivers), we emphasize that we were mainly interested in the *magnitude* of the difference in N_e/N ratios over time in rehabilitated rivers (PW, US). The degree of difference in N_e/N ratios should, thus, be at least comparable within and between PW and US, given that a similar protocol for estimating N was applied over time in each river. The N estimates were also based on the number of adult anadromous spawners only, so they did not include numbers of potential mature male parr within each river (whose abundance can be difficult to estimate in wild Atlantic salmon populations). Our N_e/N ratios, therefore, also incorporated the assumption that the number of male mature parr within each river fluctuated over time in a similar fashion to adult spawner abundance. This assumption seems reasonable given that parr densities have declined in both rivers as adult spawner abundance has declined since the early 1980s (FNP2002), although we acknowledge that the relative success of parr might vary depending on absolute densities (Jones and Hutchings 2001, 2002).

Processes beyond the spatial scale of the donor and rehabilitated populations

Processes beyond the spatial scale of the donor (BS), reintroduced (PW) or recolonized (US) populations might have affected the latter's persistence, in which case evidence must be provided that dispersal/migration from additional populations had an impact on the demographics/genetics within that population (Fraser et al. 2004; Hanski and Gaggiotti 2004). We therefore estimated historical migration rates (m) among temporal samples from PW, US, and BS, as well as PE and ST, the most historically productive iBoF populations and thus the most probable historical sources of immigrants in the iBoF (see above). Estimates of m were obtained using three different historical data sets that corresponded to different time-periods and the various sampling years available for each river: (1) 1974–1984; (2) 1984–1988; (3) 1988–1992 (Table 1). We used a Bayesian method for estimating m (BAYESASS 1.1; Wilson and Rannala 2003) that specifically considered 'short-term' migration in each time-period (the past 1–3 generations from the given historical data set 1, 2 or 3). This method accounted for the fact that immigrants (and individuals of recent immigration ancestry) show temporary disequilibrium in their genotypes relative to the population in consideration, allowing their identification as immigrants or offspring of immigrants. BAYESASS yields unidirectional estimates of m for each population pair. Additionally, it did not assume migration-drift equilibrium, which might not have been reached for recently founded or colonized salmon populations. For each historical data set, we ran the program three times to evaluate the consistency of results, using 4×10^6 iterations (burn-in: 1×10^6 iterations). Estimates of m involving inter-population comparisons for which some samples were juveniles (e.g. mainly within rehabilitated rivers; Table 1) might be biased using BAYESASS, because they can only evaluate the component of m involving immigrant ancestry and not actual immigration itself. For example, immigrants in populations for which juveniles were sampled might have reduced fitness if local individuals are better adapted to the local environment (and have increased reproductive success: Fraser and Bernatchez 2005), in which case m might be underestimated. Conversely, m might be overestimated if immigrants have greater fitness than local individuals, or m might be close to true values if immigrants and local individuals have equal fitness. Unfortunately, as in many other systems, the data necessary to distinguish between these possibilities are currently unavailable for the iBoF populations.

Origin of individuals within rehabilitated populations

To complement the migration analyses above, we also used assignment tests to determine the origin of all individuals within rehabilitated population (PW, US) samples. Here, all other populations (BS, PE, ST, HA) were considered as sources of rehabilitated populations, with temporal samples within these rivers pooled because of strong evidence for their temporal stability (see Results). We were particularly interested in whether some individuals from the reintroduced population (PW) originated from other populations than just its donor (BS), as this would suggest that immigration from elsewhere has occurred since reintroduction. A first assignment test determined the precision of correctly assigning individuals between source populations; each individual was removed from the data set, allele frequencies were recalculated, and the individual was assigned to a population (implemented in GENECLASS; Cornuet et al. 1999). An exclusion threshold of 0.01 was adopted, wherein individuals that did not fit into the 99% highest likelihood tail of the assignment distribution of any population were rejected from all populations. A second assignment test (with a 0.01 exclusion threshold) was then used to assign PW and US individuals to source populations, following the elimination of excluded individuals from candidate source populations (in the first assignment test).

Results

Lack of genetic variation established in reintroduced/recolonized populations?

All five loci were moderately to highly polymorphic, with 7–29 alleles observed per locus (mean: 17.4 alleles/locus): *Ssa12* (7), *Ssa*85 (13), *Ssa197* (18), *Ssa202* (20), *Ssa171* (29). Only six private alleles observed in four rivers were found; only one of these had a frequency of >0.05 (0.15: *Ssa85*139*, USb96). Mean H_E across samples was also high for each locus (0.50, *Ssa12*; 0.75, *Ssa*85; 0.81, *Ssa197*; 0.86, *Ssa171/202*). Of the 22 samples with sample size ≥30, no differences in corrected allelic richness (A_C) were detected between different rivers (ANOVA: $F = 1.83$, $P = 0.10$) or different time periods ($F = 0.04$, $P = 0.95$). The marginal P value (0.10) between rivers was most likely attributable to the lower A_C in all three PWu samples (1992, 1993, 1996) relative to all other samples (range: 5.5–7.1 vs. 8.3–10.5, respectively; Table 1).There were also no differences in H_E or H_O between samples from different rivers ($F = 0.56$, $P = 0.76$; $F = 0.31$, $P = 0.93$) or different time periods ($F = 0.007$, $P = 0.99$; $F = 0.09$, $P = 0.91$). Note that A in PW82–84 and PW88, years during and just following the reintroduction, was no lower than in any BS samples with $N < 30$ (Table 1). Over all PW samples, only 2 of a total of 75 alleles (frequencies < 0.01) were not found in the donor population BS (*Ssa12**99 and *Ssa197**155 in PW82–84, found also in either ST and/or US, data not shown).

Hardy–Weinberg equilibrium but linkage disequilibrium in rehabilitated populations

Exact tests of global HWE (heterozygote deficiency or excess, for all loci and temporal/spatial samples) were not significant ($P > 0.05$). No loci departed from theoretical expectations ($P > 0.05$), and only 1 of 32 samples displayed a HWE departure (heterozygote deficiency in BS70). Only 14 of 320 individual locus tests within samples showed heterozygote deficiencies (7 of 160) or excesses (7 of 160); these were found over multiple samples (14), in all rivers (6) and loci (5), and only 2 tests retained such departures after Bonferroni correction ($\alpha = 0.0003$, $k = 160$, heterozygote deficiencies and excesses treated separately) (Table 1). Thus, HWE departures could not be attributed to scoring errors or locus-specific problems. However, the proportion of exact tests showing evidence of genotypic linkage disequilibrium between loci was greater than expected by chance (63 of 320 tests; Table 1). This same trend was observed following Bonferroni correction (18 of 320 tests; Table 1). Nevertheless, as Bonferroni correction can be too conservative when there are a large number of tests (this study; Rice 1989; Ryman and Jorde 2001), we consider the results uncorrected for multiple testing to be more biologically meaningful. Here, linkage disequilibrium was partitioned among populations as follows: 30.6% of tests (55/180) were significant in rehabilitated populations (PW, US), whereas only 5.7% of tests (8/140) were significant in other populations (BS, PE, ST, HA) (Table 1). These tests thus revealed a signal for considerable linkage disequilibrium between loci in rehabilitated populations but not in other populations, although this was mainly evident in US for only later temporal samples (1996) (see Table 1).

Bottlenecking in rehabilitated populations?

At a less conservative significance level of $P = 0.10$ (see Materials & Methods), evidence for genetic bottlenecking (where $H_E > H_{Eq}$) was detected in a total of 13 of 32 samples (5 of 32 samples and 12 of 32 samples, using sign and Wilcoxon tests, respectively), including 9 of 18 samples from rehabilitated populations (PW or US) (Table 1). We contend that this constitutes evidence for recent genetic bottlenecking within reintroduced (PW) and recolonized (US) populations. Note that at the standard level of significance of 5%, only 2 of 32 samples (PE83, 84) showed evidence of bottlenecking, suggesting that the power to detect bottlenecks with the five loci was low at this level (Table 1).

Inbreeding in rehabilitated populations?

In accordance with the definition of inbreeding as nonrandom mating within populations, we found no detectable indication for inbreeding coefficients (F_{IS}) to exceed zero in either rehabilitated population, despite having multiple spatial and temporal samples from each river (PW, US: Table 1). The values of F_{IS} within samples from rehabilitated populations ranged from – 0.062 to 0.048 (Table 1).

Genetic differentiation, population structure and temporal stability

Significant global tests for genetic differentiation over all loci ($\theta_{ST} = 0.03$: $P < 0.001$) indicated population structure among sampled rivers. Spatial differentiation was greatest between PWu and all other rivers, including other PW samples, as evidenced by mean pairwise θ_{ST} values (mean 0.071, range 0.039–0.083; Table 2). Less pronounced but significant spatial

differentiation was also evident between most other rivers (BS, PE, ST, US, HA; θ_{ST} range: 0.021–0.038). Two notable exceptions of non-significant differentiation were between (1) several samples of PE and ST, and (2) reintroduced (PW) or recolonized (US) populations and BS (the donor of PW) for mainly earlier temporal periods (e.g. PW82–84, PW88, US74, US84) (Table 2). Overall temporal stability in genetic structure across samples within BS, PE and ST was evidenced by low mean pairwise θ_{ST} values (range: 0.001–0.006) (Table 2). Conversely, temporal stability was less evident within reintroduced or recolonized rivers (PWu, PW, US), based on the number of significant pairwise comparisons within these rivers (θ_{ST} range: 0.006–0.032) (Table 2). Despite overall weak clustering support for distance-based relationships among temporal/spatial samples in the *D* topology, similar patterns emerged as with θ_{ST}: (1) PWu samples clustered together strongly; (2) earlier temporal samples of PW and US clustered with BS samples, whereas their later samples generally clustered separately from BS; and (3) temporal samples within PE and ST each clustered together (Fig. 3).

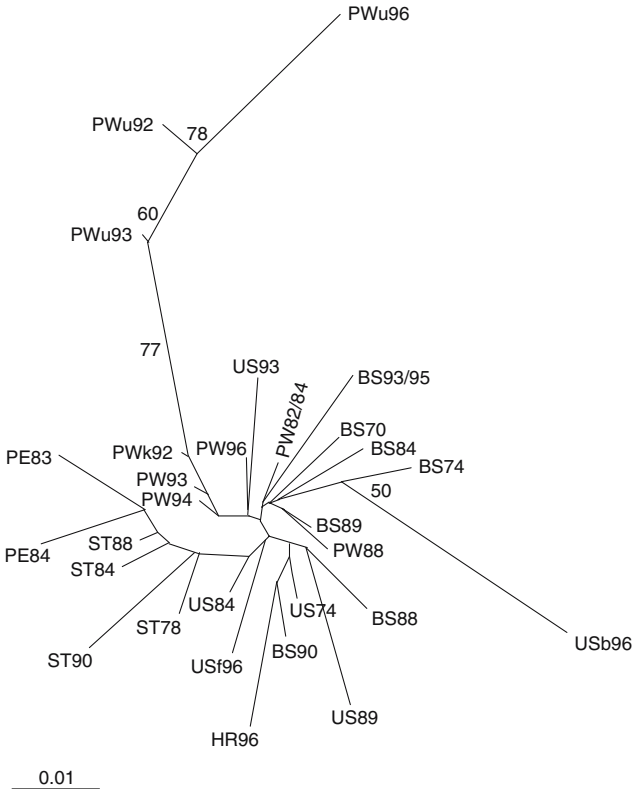

Fig. 3 Unrooted neighbour-joining tree of samples collected from the six Bay of Fundy Rivers (abbreviations from Fig. 1 or Table 1), using five microsatellite loci and based on Nei's (1978) unbiased genetic distance (*D*). The phylogenetic tree was bootstrapped over loci with replacement and 2000 replicates, with numbers indicating branches with at least 50% support. The tree was visualized in TREEVIEW (Page 1996). Certain spatial samples within Point Wolfe River (PW 1993–1996: PWb, at Bennett Creek; PWk, at Key Hole) were pooled together within sampling years because they showed little evidence of genetic differentiation (see Materials & Methods; Table 2)

Estimates of effective population sizes

Estimates of N_e within populations using different temporal methods almost unanimously followed the same order from largest to smallest: overlapping generation, no migration model of Waples (1990a) > discrete generation, no migration model of Wang (2001) > discrete generation, gene flow model of Wang and Whitlock (2003) (Table 3). Estimates of N_e in reintroduced (PW) and recolonized (US) populations ranged from 10 to 111 and from 15 to 233, respectively, depending on the method used and the time period considered (subpopulations treated separately; Table 3). Estimates of N_e within BS and ST were on the order of 2 to 10 times higher than in PW and US for various methods (Table 3). The 95% confidence intervals for N_e estimates in PW and US were generally narrow, except for certain estimates assuming no migration (Table 3).

Genetic depensation/compensation in rehabilitated populations?

Ratios of N_e/N, after accounting for subpopulation structuring within each rehabilitated population, ranged from 0.07–0.39 and 0.01–0.45 in PW and US, respectively, depending on the model of N_e estimation applied (Table 4). Regardless of the N_e method utilized, N_e/N ratios increased as *N* decreased, suggesting genetic compensation in both populations as they declined, particularly within US (Table 4).

Processes beyond the spatial scale of source and rehabilitated populations?

Estimates of migration rates (*m*) between populations showed consistent asymmetries across all three historical data sets. Historically productive iBoF populations, PE and ST, were always net sources of migrants (rather than receivers) to all other populations, with the exception of ST to PE and PWu92 (Table 5). For certain historical data sets, BS was a net source of migrants to PW (1974–1984, 1988–1992) and US (1988–1992), as was US to PW in earlier data sets (1974–1984, 1984–1988). Thus, reintroduced (PW) and recolonized (US) population samples were generally net receivers of migrants from all other populations (10 of 12 and 7

Table 3 Estimates of effective population size (N_e), migration rate (m) and their 95% CI, as described in Waples 1990a (overlapping generations, no migration model), and Wang and Whitlock (2003) (discrete generations, migration or no migration models)

River	Temporal period	Overlapping gen. N_e, no migration (95% CI)	b	Discrete gen. N_e, no migration (95% CI)	Discrete gen. N_e, migration (95% CI)	m (95% CI)	S	T
BS	1970–1993/95	256 (171–758)	2.68	326 (180–856)	100 (65–133)	0.09 (0.07–0.20)	3	6.5
	1970–1989	430 (167–5820)	2.68	205 (63–260)	67 (38–100)	0.10 (0.11–0.21)	2	5.1
	1989–1993/95	251 (138–489)	2.05	81 (53–146)	55 (42–91)	0.24 (0.16–0.32)	2	1.4
PW	1982/84–1996 (PWu)	44 (26–70)	2.68	19 (15–23)	15 (12–18)	0.10 (0.09–1)	4	3.2
	1982/84–1996 (PWb + PWk)	111 (43–317)	2.68	65 (41–120)	21 (17–28)	0.56 (0.47–1)	4	3.2
	1982/84–1988	68 (33–321)	2.05	31 (15–444)	10 (7–15)	0.57 (0.54–1)	2	1.1
	1988–1992 (PWu)	28 (17–49)	1.43	15 (12–20)	11 (8–12)	0.70 (0.46–1)	2	1.1
	1988–1992 (PWk)	83 (32–416)	1.43	45 (20-∞)	15 (10–21)	0.68 (0.37–1)	2	1.1
	1992–1996 (PWu)	43 (22–80)	1.43	17 (13–21)	12 (10–15)	0.03 (0–0.16)	2	1.1
	1992–1996 (PWb + PWk)	58 (32–106)	1.43	26 (21–45)	17 (14–23)	0.66 (0.62–1)	2	1.1
US	1974–1996 (USb96)	76 (43–148)	2.68	55 (39–79)	19 (14–24)	0.70 (0.53–1)	3	5.9
	1974–1996 (USf96)	26 (16–40)	2.68	77 (54–120)	23 (17–28)	0.63 (0.48–1)	3	5.9
	1974–1984	233 (108–692)	2.68	75 (40–253)	20 (14–29)	0.87 (0.28–1)	2	2.7
	1984–1996 (USb96)	71 (42–119)	2.68	64 (41–107)	23 (17–30)	0.32 (0.16–0.88)	2	3.2
	1984–1996 (USf96)	152 (83–291)	2.68	29 (22–40)	15 (11–18)	0.41 (0.22–1)	2	3.2
ST	1978–1988	240 (90–487)	2.68	243 (77–∞)	84 (46–164)	0.18 (0.02–0.48)	3	2.7

b = generation length in the overlapping generation model, according to the number of years between samples (from Waples 1990a). S = the number of temporal samples implicated in the N_e estimate; for instance, 1982/84–1996 (PWu) = 4 (1982/84, PWu92, PWu93, PWu96). T = the estimated number of generations covered between temporal samples

Table 4 Generational effective population sizes (N_e) according to three temporal methods and census size estimates (N) for Point Wolfe (PW) and Upper Salmon (US) Rivers, as well as associated N_e/N ratios. Estimates of N_e account for subpopulation structure in each rehabilitated population, following Wright's (1943) finite island model of metapopulation structure (e.g. PWu and PWb/PWk; USb and USf; see Materials and Methods for details)

River	Temporal Period	N_e, no migration*	N_e, no migration^	N_e, migration	N	N_e/N, no migration*	N_e/N, no migration^	N_e/N, migration
PW	1988–1992	116	62	27	388	0.299	0.160	0.070
	1992–1996	104	45	30	264	0.394	0.170	0.114
US	1974–1984	248	80	21	1850	0.134	0.043	0.011
	1984–1996	248	99	40	545	0.455	0.182	0.073

Methods of N_e estimation assuming no migration: *Waples (1990a), ^Wang (2001)

of 12 pairwise comparisons from all three data sets, respectively; Table 5).

Origins of individuals within rehabilitated population samples

Assignment tests conducted on potential source populations of rehabilitated population (PW, US) individuals led to correct reclassification rates of 81.5% (HA), 75.7% (BS), 58.1% (PE) and 57.0% (ST) (Table 6). Most misclassifications in PE and ST were with one another, and their low correct reclassification rates were expected given their low degree of genetic differentiation at the five loci (Table 2; Cornuet et al. 1999; Fraser and Bernatchez 2005). Nevertheless, we note that at the level of BS vs. PE/ST vs. HA, the mean reclassification rate was 79.8% (381/477 individuals) (Table 6).

Subsequent assignment of PW/US individuals to these potential source populations revealed that individuals from PW samples following reintroduction (1982/84, 1988) were assigned at a rate to the donor population (BS) that was similar to that expected if they all originated from it (all $\chi^2 \leq 0.27$, $P \geq 0.50$), whereas all later PWu samples (1992–1996) and certain other PW samples (PWb94+PWk94) did not (all $\chi^2 \geq 7.2$, $P < 0.01$) (Table 6). Individuals from recolonized US were also assigned to BS at rates similar to that expected (all $\chi^2 \leq 2.62$, $P \geq 0.50$) in most temporal samples, but not for US84 ($\chi^2 = 4.03$, $P < 0.05$). Furthermore, there were more individuals excluded from available source populations in reintroduced (PW and

Table 5 Historical migration rates (*m*) between Atlantic salmon (*Salmo salar*) populations and their 95% confidence intervals according to three different temporal periods, following Wilson and Rannala (2003)

N	Receiver	Source				
1974–1984						
		BS74	PE83/84	PW82/84	ST78	US74
18	BS74	0.828 (0.751–0.861)	**0.038 (0.028–0.051)**	0.030 (0.014–0.036)	**0.027 (0.018–0.032)**	0.097 (0.083–0.165)
55	PE83/84	0.007 (0.005–0.009)	0.974 (0.969–0.977)	0.005 (0.004–0.006)	0.007 (0.005–0.009)	0.007(0.005–0.009)
26	PW82/84	**0.069 (0.055–0.085)**	**0.031 (0.027–0.039)**	0.750 (0.739–0.762)	**0.019 (0.015–0.024)**	**0.131 (0.112–0.152)**
42	ST78	0.011 (0.008–0.013)	**0.233 (0.214–0.248)**	0.008 (0.004–0.011)	0.735 (0.711–0.764)	0.013 (0.011–0.015)
34	US74	0.058 (0.041–0.089)	**0.086 (0.072–0.098)**	0.044 (0.029–0.059)	**0.027 (0.022–0.032)**	0.785 (0.769–0.802)
1984–1988						
		BS84	PE83/84	PW88	ST84	US84
15	BS84	0.693 (0.693–0.701)	**0.164 (0.141–0.188)**	0.023 (0.015–0.031)	**0.075 (0.052–0.101)**	0.045 (0.031–0.055)
55	PE83/84	0.006 (0.005–0.007)	0.932 (0.921–0.944)	0.007 (0.005–0.008)	0.045 (0.029–0.059)	0.01 (0.008–0.011)
13	PW88	0.025 (0.017–0.04)	**0.086 (0.067–0.105)**	0.705 (0.693–0.721)	**0.073 (0.063–0.097)**	**0.109 (0.087–0.134)**
37	ST84	0.013 (0.01–0.016)	**0.266 (0.251–0.276)**	0.008 (0.001–0.012)	0.701 (0.695–0.705)	0.012 (0.009–0.014)
38	US84	0.014 (0.011–0.017)	**0.170 (0.141–0.197)**	0.022 (0.017–0.027)	**0.077 (0.060–0.094)**	0.716 (0.699–0.731)
1988–1992						
		BS88/89/90	PWu92	PWk92	ST88/90	US89
87	BS88/89/90	0.928 (0.918–0.938)	**0.025 (0.023–0.041)**	0.020 (0.014–0.027)	**0.023 (0.018–0.030)**	0.004 (0.005–0.007)
60	PWu92	0.003 (0.002–0.005)	0.989 (0.976–0.994)	0.003 (0.001–0.005)	0.003 (0.002–0.005)	0.002 (0.001–0.003)
30	PWk92	**0.132 (0.085–0.165)**	**0.146 (0.122–0.169)**	0.682 (0.656–0.721)	**0.029 (0.021–0.034)**	0.011 (0.008–0.014)
42	ST88/90	0.012 (0.008–0.016)	0.012 (0.003–0.015)	0.009 (0.007–0.011)	0.961 (0.948–0.972)	0.006 (0.004–0.008)
15	US89	**0.228 (0.211–0.251)**	**0.042 (0.032–0.054)**	0.016 (0.013–0.027)	**0.022 (0.017–0.026)**	0.699 (0.686–0.711)

Bold values indicate asymmetries in *m* (and the direction of the asymmetry) where 95% confidence intervals did not overlap in pair-wise comparisons between rivers
Sample size = *N*

Table 6 Summary of individual assignment tests conducted on potential source populations of Point Wolfe R. (PW) and Upper Salmon R. (US) samples (at top), and subsequently, the proportion of individuals from different PW/US samples assigned to each of these donor populations (below). Sample size = *N*

Potential source populations	Proportion of individuals assigned to (number of individuals in parentheses)					
	N	BS	PE	ST	HA	Unknown
BS (1970–95)	247	**0.757 (187)**	0.081 (20)	0.089 (22)	0.057 (14)	0.016 (4)
PE (1983–84)	55	0.110 (6)	**0.581 (32)**	0.273 (15)	0.036 (3)	
ST (1978–90)	121	0.099 (12)	0.281 (34)	**0.570 (69)**	0.050 (6)	
HA (1996)	54	0.073 (4)	0.056 (3)	0.056 (3)	**0.815 (44)**	
Receiving samples						
PW82–84	26	0.846 (22)	0.077 (2)	0.077 (2)		
PW88	13	0.692 (9)	0.231 (3)	0.077 (1)		
PWu92	60	0.350 (21)	0.316 (19)	0.300 (18)	0.017 (1)	0.017 (1)
PWu93	58	0.155 (9)	0.310 (18)	0.328 (19)	0.052 (3)	0.155 (9)
PWu96	62	0.097 (6)	0.435 (27)	0.355 (22)	0.048 (3)	0.065 (4)
PW92 (PWk)	30	0.567 (17)	0.133 (4)	0.200 (6)	0.033 (1)	0.067 (2)
PW93 (PWb + PWk)	83	0.590 (49)	0.205 (17)	0.120 (10)	0.060 (5)	0.025 (2)
PW94 (PWb + PWk)	93	0.485 (45)	0.269 (25)	0.171 (16)	0.065 (6)	0.010 (1)
PW96 (PWb + PWk)	67	0.642 (43)	0.164 (11)	0.134 (9)	0.045 (3)	0.015 (1)
US74	34	0.647 (22)	0.176 (6)	0.147 (5)	0.03 (1)	
US84	38	0.474 (18)	0.185 (7)	0.289 (11)	0.026 (1)	0.026 (1)
US89	15	0.600 (9)	0.333 (5)		0.067 (1)	
US93	30	0.500 (15)	0.233 (7)	0.100 (3)	0.100 (3)	0.067 (2)
USb96	37	0.541 (20)	0.135 (5)	0.054 (2)	0.135 (5)	0.135 (5)
USf96	62	0.614 (38)	0.065 (4)	0.161 (10)	0.112 (7)	0.048 (3)

Proportions of individuals correctly assigned among source populations are highlighted in bold. Proportions of individuals that were excluded at a threshold of 0.01 from source populations and PW/US samples are found in the last column (see Materials and Methods for details on methodology)

PWu: 20/492) or recolonized (US: 11/216) populations than expected based on the proportion of excluded individuals in the sources (4/477) or from BS alone (4/247) (all $\chi^2 \geq 7.1$, $P < 0.01$) (Table 6).

Discussion

Immigration, genetic compensation and the maintenance of genetic diversity

Reduced genetic diversity through genetic drift, bottlenecking and/or inbreeding are common and theoretically predicted attributes of small populations (Frankham et al. 2002). Here, over the long-term, we have monitored these and other genetic characteristics (e.g. linkage disequilibrium, temporal stability in population structure, N_e) in unsuccessful rehabilitation attempts of two extirpated Atlantic salmon populations. Both populations have had low to relatively low N_e since reintroduction/human-mediated recolonization events occurred. Both populations also had unstable genetic structuring over time and continuously showed signs of genetic bottlenecking, suggesting that conditions within each population were conducive to strong genetic drift. Yet, we found little evidence over a 14–22 year period (3–6 generations) that either population experienced detectable inbreeding or a significant loss of allelic diversity or heterozygosity relative to probable source populations. Although such common genetic attributes of small populations could become more of a concern in subsequent generations, they do not appear to have been major factors in the decline of either population. Shortly after the reintroduction in Point Wolfe R., there was also little difference in genetic diversity relative to that of its donor (Big Salmon R.). Thus, the unsuccessful reintroduction could not be attributed to a reduced level of initial genetic diversity.

We did, however, find evidence that N_e/N ratios increased as N steadily declined in Point Wolfe and Upper Salmon Rivers. Such genetic compensation might have helped to buffer populations from reductions in N and thus slowed the rate of loss of genetic diversity in either river. Nevertheless, the exact causes of such compensation remain uncertain. Some authors have argued that because migratory salmonid females are highly territorial, fewer females can spawn successfully at higher female spawner densities and thus contribute less progeny to the next generation (Chebanov 1991; Ardren and Kapuscinski 2003). For Atlantic salmon, we speculate that reduced variance in reproductive success among mature male parr might also occur at low census sizes because of relaxed competition from lower numbers of anadromous

males, as has been observed experimentally (Jones and Hutchings 2002). Alternatively, the increase to N_e provided by male parr could be larger in smaller populations since their mean reproductive success (relative to anadromous males) would likely be higher than when more anadromous males were around.

The most salient feature of our data was the evidence from multiple historical periods for substantial and recurrent asymmetric migration (migration rate, m) into both reintroduced and recolonized populations from other populations in the iBoF. Historical immigration rates into Point Wolfe R., following the initial reintroduction (1982–1985), and Upper Salmon R., following recolonization (early-mid 1960s), might have been quite high ($m \approx 0.1$–0.3, from Wilson and Rannala 2003; $m \approx 0.03$–0.87, from Wang and Whitlock 2003). In Point Wolfe R., immigration has included populations additional to its source population (Big Salmon R.). In both Point Wolfe and Upper Salmon R., a small percentage of the parr sampled probably were also descendants of immigrants from unknown populations. Such high levels of migration would counter strong genetic drift in both rivers. In addition, we found considerable evidence for recurrent linkage disequilibrium within temporal samples from both rehabilitated populations. Given the evidence for immigration from neighbouring areas and temporal changes in rehabilitated population structuring, a recent admixture of individuals from different populations and/or some degree of population turnover are probable reasons for the observed linkage disequilibrium in both populations (Ohta 1982; Ardlie et al. 2002; Tero et al. 2003). Collectively, it thus appears that genetic compensation, coupled with immigration from beyond both the spatial scale of individual rivers (Point Wolfe or Upper Salmon Rivers) and that of the reintroduced population's source (Big Salmon R.), can account for the maintenance of genetic diversity within Point Wolfe and Upper Salmon Rivers.

A potential exception to the lack of reduced allelic diversity was found in temporal samples of the Upper Point Wolfe River. Despite temporal variability in their allelic frequency composition, Upper Point Wolfe samples clustered together and distantly relative to spatial/temporal samples of all other rivers in the tree topology (Tables 1–3, Fig. 3). Though preliminary, these results suggest that additional factors related to founder effects and the initial reintroduction might have been stronger in this section of river, or that the local environment in this section could differ or be partially isolated from other river sections.

Potential caveats relating to migration rate (m) and effective population size (N_e) estimation

If reintroduced and recolonized populations had not yet reached migration-drift equilibrium, migration rates would have been overestimated. We cannot entirely dismiss this possibility, although our migration estimates avoided the assumption of migration-drift equilibrium (see Wang and Whitlock 2003; Wilson and Rannala 2003). Comparable studies have also obtained high, and probably unrealistic m estimates with Wang and Whitlock's (2003) method (e.g. $\theta_{ST} \approx 0.01$–0.08; 5–7 microsatellite loci: $m = 0.23$–0.99, Ostergaard et al. 2003; $m = 0.05$–1, Ford et al. 2004; $m = 0.51$–1, Hoffman et al. 2004; $m = 0.58$–0.72, Consuegra et al. 2005). However, our Wang and Whitlock m estimates were considerably higher in populations where appreciable allele frequency change over time was observed (e.g. rehabilitated populations). Such patterns are consistent with other situations in which small population sizes, strong drift and migration characterized population structuring (Ostergaard et al. 2003). Additionally, immigration rates in these other studies were substantial where known, though lower than the Wang & Whitlock m estimates (up to 27.4% vs. up to 72%, respectively; Consuegra et al. 2005). The observation of 100 to 300 adult salmon in Upper Salmon River in 1963 and 1965, the years that salmon first arrived in the river following removal of dams (Jones & Clay 1995), suggests a historically high propensity for dispersal in iBoF salmon. We conclude that m was likely overestimated, at least with Wang and Whitlock's (2003) method, but migration has still likely had an important effect on genetic diversity within and among iBoF populations.

Despite relatively narrow confidence intervals in many cases, estimates of N_e typically varied two to fourfold between methods assuming no migration (either overlapping or discrete generation models) and the Wang and Whitlock (2003) method assuming migration. Similar discrepancies have been noted in other studies (Ostergaard et al. 2003; Hoffman et al. 2004; Consuegra et al. 2005). At least for N_e estimates assuming no migration, we might expect that those based on discrete generations in our study are biased downwards because the samples have been taken from only part of a generation (see Waples 2002a). It is also possible that the particularly low N_e values assuming migration might have arisen from deviations of Wang and Whitlock's (2003) migration model, namely, that constant immigration occurs from an infinite-sized source population of fixed allele frequency. This model might be roughly applicable to rehabilitated populations in iBoF because the likely sources of rehabilitated

populations (e.g. Big Salmon, Petitcodiac, Stewiacke) were (1) continuously abundant iBoF salmon populations over time and (2) temporally stable in their population genetic structure. Nevertheless, a small percentage of individuals in rehabilitated populations were descendants of immigrants from unknown populations in later temporal samples, implying slight changes in the source populations of immigrants over time.

Unfortunately, incomplete theoretical predictions of the influence of m on N_e estimation make it difficult to clearly assess which method might have yielded N_e values closer to 'true' N_e values in our study. For example, when migration is present but is ignored, it results in greater short-term changes in allele frequencies and, in turn, an underestimation of N_e (Wang and Whitlock 2003). However, if migration is continuously from the same source population (s), which probably more typifies the situation of our rehabilitated populations, allele frequencies of the population in consideration become more and more similar to those of the source population (s) of immigrants (Wang and Whitlock 2003). If such migration is ignored, N_e will be overestimated, because the little apparent allele frequency change detected is wrongfully assumed to reflect little genetic drift with models of N_e estimation that assume no migration (Waples 1990a). If in such a case m is sufficiently high, N_e estimates for the population in consideration actually then reflect the larger, metapopulational N_e (Waples 2002a). The fact that N_e values are lower when m is high with the Wang and Whitlock (2003) method in our study suggests that the low N_e values might reflect low self-recruitment of individuals in these vicinities under this model. Given that iBoF populations only have moderate genetic differentiation ($\theta \approx 0.01$–0.08; Table 2), an additional consideration is that migration might have less of an effect on population allele frequencies if the allele frequencies are already similar to source populations (see Wang and Whitlock 2003). Despite these ambiguities, we note that both rehabilitated populations showed continual signs of bottlenecking and linkage disequilibrium. Indeed, such phenomena are typically expected within populations that have smaller N_e (Cornuet and Luikart 1996; Tero et al. 2003). This suggests that N_e estimates that assume no migration were overestimated for certain time periods in this study.

The extent to which historical introductions of hatchery-reared fish into our study rivers (see Materials and Methods) might also have affected our migration estimates is unknown. Nevertheless, the fact that genetic diversity was temporally stable within and between rivers known historically for their salmon production suggests that principle iBoF populations have largely maintained their genetic integrity despite past introductions. This is also consistent with results from other genetic studies on the species which have found that populations have retained their genetic uniqueness and natural patterns of gene flow under extensive introductions of hatchery fish (King et al. 2001; Consuegra et al. 2005).

Effective-census population size ratios

Based on the above discussion, it is difficult to compare actual N_e/N ratios estimated for Point Wolfe and Upper Salmon Rivers with other Atlantic salmon populations. Nevertheless, the values we obtained (0.01–0.45) are consistent with N_e/N ratios reported in other Atlantic salmon populations and salmonids with similar life histories (Heath et al. 2002; Shrimpton and Heath 2003; Consuegra et al. 2005). The observation that none of the N_e/N ratios in our study approaches even 0.5 is of note because they all likely reflect overestimates, given that the reproductive contribution of mature male parr in these populations is unknown. At a minimum, the degree of change in N_e/N ratios should be comparable between Point Wolfe and Upper Salmon Rivers if parr abundance has fluctuated proportionally with spawner abundance. This assumption is consistent with available data in both rivers since the early 1980s (FNP 2002), although again it could be that the relative success of parr might vary depending on absolute densities of parr (see Jones and Hutchings 2001, 2002). More generally, the changes in N_e/N ratios over only a couple of generations, as recently observed elsewhere (Ardren and Kapuscinski 2003; Shrimpton and Heath 2003), illustrate the difficulty in characterizing 'standard' N_e/N ratios within natural populations at even contemporary time scales (see Waples 2002a).

Relevance of the scale of local adaptation to future rehabilitation

Continuing rehabilitation efforts for reintroduced (Point Wolfe R.) and recolonized (Upper Salmon R.) populations are hampered by low numbers of local individuals that can be used to regenerate each of them (FNP 2002). If remaining individuals in each river are locally adapted, interbreeding with salmon from other iBoF populations to regenerate each population might impede population recovery rather than help it. There is also ambiguity in setting minimum viable population sizes in both rivers because historical population sizes are unknown (Hutchings 2003).

We wish to emphasize two points here based on our study's results. First, it seems highly probable that

effective population sizes (N_e) of between 50 and 100, and migration rates (m) of 0.05 to 0.10, have been historically characteristic of the Point Wolfe and Upper Salmon River populations of Atlantic salmon. The potential for local adaptation under such conditions is more likely at geographic scales of several rivers (e.g. larger areas of the iBoF) than individual rivers, unless selection within rivers is very strong (Adkison 1995; Hansen et al. 2002; Fraser et al. 2004). Local adaptation was thus unlikely in Point Wolfe and Upper Salmon Rivers, even if it has been shown to manifest itself in other fish populations over periods similar to the histories of each population (4–9 generations; Reznick et al. 1990; Koskinen et al. 2002). Second, it is debatable whether salmon in either river ever sustained or could sustain populations of $N_e = 500$ (considered sufficient to maintain additive genetic variation in quantitative traits and thus evolutionary potential: Franklin 1980; Waples 1990b), given that other iBoF populations inhabiting rivers with considerably more salmon habitat might not even harbour populations of this size (Table 4). Populations of Atlantic salmon might persist at lower N_e if they have done so for long periods of time (e.g. see O'Connell et al. 2002) because deleterious alleles related to inbreeding would probably have been purged in such populations (Waples 1990b). Although we do not discount that this possibility might explain historical populations within Point Wolfe and Upper Salmon Rivers, their present genetic makeup consists of individuals that are recent descendants from populations with likely larger historical N_e. Consequently, we caution that either rehabilitated population today might only persist at lower N_e in the short term if gene flow from elsewhere occurs.

Relevance of historical immigration to future rehabilitation

Perhaps one of the biggest challenges to interpreting population declines in Point Wolfe and Upper Salmon Rivers, as well as the iBoF as a whole, has been in determining the degree to which historical impacts were relevant to population collapses (Hutchings 2003; COSEWIC 2006). Current rehabilitation efforts focus on threats that correspond to recent declines (late 1980s to late 1990s), as well as the maintenance of genetic diversity upon which the future evolutionary potential of iBoF salmon is dictated (COSEWIC 2006). Clearly, all iBoF populations, including rehabilitated populations in this study, declined almost simultaneously, suggesting a common cause for iBoF population declines. Nevertheless, if recurrent immigration from other iBoF populations found in this study was historically vital to the persistence of Point Wolfe and Upper Salmon River populations (Hutchings 2003), it also has important consequences for future rehabilitation efforts here, and for the rest of the iBoF regional group. Specifically, future rehabilitation would also require the re-establishment of important historical patterns of immigration among populations. This would only be achieved by re-allowing accessibility to habitats found in presently-impacted rivers which historically harboured larger salmon populations (e.g. Petitcodiac River). The possibility that iBoF salmon form one or more metapopulations, where population persistence depends in part on recurrent immigration (and resulting gene flow), is not improbable given increasing evidence from empirical studies on salmonids that many regional groups, including in Atlantic salmon, exhibit such characteristics (Heath et al. 2002; Ostergaard et al. 2003; Fraser et al. 2004; Consuegra et al. 2005). The risks of not considering potential metapopulation dynamics in contemporary salmon conservation and management have also been raised (Policansky and Magnuson 1998; Cooper and Mangel 1999; Rieman and Dunham 2000; Fraser et al. 2004). The results of the present study therefore suggest that further investigations into historical levels of immigration between iBoF populations, using earlier samples (if they exist) and incorporating additional populations, are merited.

General rehabilitation and conservation genetic implications

Our joint evaluation of the potential roles of multiple genetic factors in a failed rehabilitation attempt raises some concerns for similar rehabilitations elsewhere. It may seem encouraging that despite evident declines in census size over time, no reduction of genetic diversity occurred within rehabilitated populations relative to donor or neighbouring, historically-productive populations. On the other hand, we observed a lack of congruence between neutral genetic diversity and population size among populations from the same regional group. We also detected temporal instability in genetic structure within rehabilitated populations. Consequently, the maintenance of genetic diversity in rehabilitated populations must be considered judiciously because, in the absence of temporal data, it reiterates that commonly used metrics of genetic diversity (allelic richness, heterozygosity) may paint misleading pictures about the true status of rehabilitated populations (sensu Lande 1988). Such patterns implicate aspects of species' biology that delay the loss of genetic diversity in declining, isolated populations (e.g. genetic compensation) or more likely in the

present case, persistent interpopulation gene flow, as key mechanisms that maintain genetic diversity. Distinguishing between these mechanisms in particular systems is thus crucial for focusing rehabilitation efforts at appropriate spatial scales.

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