ATLANTIC SALMON FEDERATION

FINAL REPORT

The genetic identity of the Clearwater Brook population of Atlantic salmon (*Salmo salar*) ; a temporal and spatial study of Atlantic salmon population genetic structure in the Miramichi, St. John and Margaree Rivers

Julian Dodson and Françoise Colombani,

Centre Interuniversitaire de Recherche sur le Saumon Atlantique,

Université Laval,

Ste-Foy, Québec

Presented to Dr. F. Whoriskey,

Atlantic Salmon Federation,

St. Andrews, New Brunswick

November, 1997

Table of contents

Appendices

Introduction

The idea that species should be managed at subspecific levels dates back to the beginning of the century with the establishment by Heincke and Hjort of the local, self-sustaining population as the unit for fisheries management (Sinclair 1988). The resultant stock concept has given rise to many definitions of what these subspecific management units should be. At one extreme, 'harvest stocks' (Gauldie 1988) are locally accessible fish which may have no genetic or phenotypic differences with adjacent stocks, but whose abundance is independent of adjacent stocks. At the other extreme, 'genetic stocks' are reproductively isolated units, genetically different from other stocks (Carvalho and Hauser 1994). Although fishery management aims to maintain the benefits from a local fish resource and thus targets the 'harvest' stock, the demands of intraspecific biodiversity conservation also requires identifying the genetic stock (Carvalho and Hauser 1994).

For anadromous fishes, the genetic stock is considered to be defined by the time and place of spawning. Thus, fish like Atlantic salmon are considered to be population rich, with as many genetic stocks as spawning tributaries and spawning periods within tributaries. However, this concept is so ingrained in the mythology of fish biology that we often forget that little evidence exists to support it. There are numerous studies documenting morphological and meristic differences among salmon stocks, but such characters are notoriously plastic under the influence of environmental variation (Claytor et al 1991). The use of electrophoretic allele frequency data from allozyme studies has revealed that heterozygosity is low in salmonids and that approximately 60 to 80% of the total electrophoretically detectable genetic diversity

in Atlantic salmon as a species occurs within populations (Davidson et al 1989). Protein electrophoresis often lacks the resolution to yield differences between salmon stocks (Davidson et al. 1989). The use of polymorphisms in mitochondrial DNA (mtDNA) has not solved the problem as low levels of variation in mtDNA of Atlantic salmon has also been observed (Wright and Bentzen 1994).

By far the most promising DNA marker now available to solve this problem is the group of 'variable numbers of tandem repeats (VNTR)' loci known as microsatellites. These are short stretches of DNA composed of di-, tri- or tetranucleotide repeats arrayed in tandem (reviewed in Wright and Bentzen 1994). In species such as Atlantic salmon showing little genetic differentiation with allozymes or mtDNA, the high level of allelic variation revealed by microsatellites makes them potentially more useful markers to study population genetic structure (McConnell et al. 1995a; b; Angers et al. 1995; Tessier et al 1995, Fontaine et al. in press).

Genetic differentiation among stocks of salmon may be based on either spatial and/or temporal segregation. A recent analysis of 7 anadromous populations of Atlantic salmon in 7 Quebec rivers clearly demonstrated that each run involved a genetically distinct population (Fontaine et al. in press). All of these rivers are characterized by a single anadromous run occurring mainly in July and August. However, in many rivers in the Gulf of St. Lawrence, early and late-run groups of Atlantic salmon are common (Chadwick and Claytor 1989). If early and late-run groups of Atlantic salmon segregate on the spawning grounds in different sections of a river system resulting in genetic isolation, then we may expect early and late runs to evolve into genetically distinct populations. Two alternative hypotheses are possible ; (1) genetic segregation may occur within a drainage system independent of differences in the timing of

upstream migration, and (2) no genetic segregation occurs within the river regardless of differences in run timing or location of spawning.

The objective of this study was to test the hypothesis that the early and late runs of Atlantic salmon previously documented in the Miramichi R. represent 2 genetically discrete populations. Secondly, we tested the hypothesis that salmon migrating into a tributary of the southwest branch of the Miramichi, Clearwater Brook, located 145 km upstream from the head of tide, are genetically closer to the early-run fish who have the greatest opportunity to migrate furthest upstream in time for spawning in October. We also tested the genetic distinction of early and late-run salmon in two additional east coast rivers, the St. John and the Margaree. This provided an assessment of the ability of microsatellites to discriminate salmon populations at a larger spatial scale (between rivers) and provided a test of the generality of classifying early and late-run salmon as distinct genetic populations.

Methods and Materials

The populations and sampling schedule : In 1996, approximately 25, 000 grilse and 23, 500 M.S.W. (Multi-Sea-Winter) salmon spawned in the Miramichi River. In the St. John River, the annual average number of spawning salmon based on 1991-1995 data is approximately 4, 300 grilse and 2, 500 MSW fish. In the Margaree R., the annual average number of spawning salmon based on 1991-1995 data is approximately 1, 000 grilse and 3, 500 MSW fish. Thus, the approximate relative abundance of salmon is 10 :2 :1 for the Miramichi, St. John and Margaree Rivers, respectively. In order to characterize the

genetic diversity of these salmon, 50 anadromous fish were sampled at the mouth of each river between July 20 and August 24, 1995 (early run salmon) and between September 19 and October 12, 1995 (late run salmon) for DNA analysis. An additional 126 fish were sampled between September 13 and October 11, 1996 at Avenor Bridge, Clearwater Brook, and at a counting fence located upstream from Avenor Bridge. Between 200 and 400 salmon spawn annually in Clearwater Brook (F. Whoriskey, personal communication). Mature adults of different age classes were sampled to reduce the potential effect of genetic differences among cohorts.

Laboratory analyses : Tissue sampling for DNA analysis involved cutting a piece of the adipose fin and storing it in 95% ethanol. Genomic DNA was extracted using a standard phenol-chloroform protocol (Sambrook et al. 1989). Population genetic diversity was analyzed on the basis of allelic frequencies at four microsatellite loci. Four pairs of primers Ssa 202, 197, 171 (O'Reilly et al. 1996) and SSOSL85 (Slettan et al. 1995) were used to amplify the microsatellite loci using PCR (Polymerase Chain Reaction).

The PCR reactions were performed in 10-µL volumes: 1µL of buffer (Boerhinger, 10 x with 15mM $MgCl_2$), 200 µM for each dNTP, 0.5 Taq unit, 4.5 pmol and 0.5 pmol end-labelled (γ-^{32}P) of one primer, 5 pmol of the other primer, ~60 ng of DNA and water to 10 µL for the Ssa microsatellites. All PCR amplification cycles were preceded by five minutes of denaturation (94°C) and ended with an extension (72°C) of 5 minutes. The denaturing/annealing(x°-temperature)/extension cycle times for the SSA microsatellites were 30/30(58°C)/30 seconds. The SSOSL85 PCR conditions are available in Slettan et al. (1995). The reaction products were resolved by electrophoresis on a 6% denaturing

polyacrylamide sequencing gel and visualized by autoradiography. These microsatellite loci were selected based on the quality of the amplifications and the ease by which they were scored. The Ssa loci were mutltiplexed in the same PCR reaction and on the same gel as described by O'Reilly and Wright (1995). However, we ran the locus Ssa171 separately in certain cases to avoid overlapping with the locus Ssa202. The sizes of alleles of PCR products were determined with a standard M13 sequence (USB, Sequenase Version 2.0 DNA Sequencing Kit). However, our scale differs by +20 base pairs from that used by O'Reilly et al. (1996).

Statistical analyses: Standard measures of genetic variation within samples, including allelic frequency, observed heterozygosity (Ho), expected heterozygosity (He) and assessment of Hardy-Weinberg equilibria were calculated using the GENEPOP program version 1.2 (Raymond and Rousset 1995). The homogeneity of allelic frequencies among samples was evaluated using X^2 randomization tests (Roff and Bentzen 1989) with 1000 permutations performed by the Monte program of the REAP software package (McElroy et al. 1992).

Gene diversity among temporal and spatial samples were evaluated by computing Phi statistics (ϕ_{st} or Phi_{st}) with a test of their significant differences. Phi_{st} is a measure of the proportion of the total genetic variation imputable to the inter-populational component. Significant differences were detected using a random allelic permutation procedure (AMOVA (Analysis of Molecular Variance) computer package, Excoffier et al. 1992; Michalakis and Excoffier 1996). The Phi statistic differs from Wright's F statistic (Wright 1951) by the incorporation of molecular information based on the differences in the number of repeats among microsatellites estimated by squared Euclidean distances between pairwise alleles. It

therefore incorporates the nature of the principal mutational mode of microsatellites, best described by a stepwise mutation model (Kimura and Otha 1978; Shriver et al. 1993).

The effective number of migrants per generation (N_em) among sites was evaluated by replacing F_{st} by Phi_{st} in the following formula $F_{st}=1/(4N_em+1)$ (Michalakis and Excoffier 1992). The effective number of migrants per generation provides an estimate of the relative importance of gene flow among the putative populations.

Results

All loci were highly variable as revealed by levels of heterozygosity which ranged from 0.82 to 1. Twenty, 18, 35 and 13 alleles were detected at the locus Ssa 202, 197, 171 and SSOSL85, respectively. None of the 40 tests (locus x sample) showed significant departures from Hardy-Weinberg equilibria at the p = 0.05 level (appendix 1, 2 and 3). The allelic frequencies of loci are presented in appendix 4, 5 and 6.

The genetic comparison of early and late run Atlantic salmon groups within rivers

The chi-square analysis of the frequency distribution of alleles of each locus revealed no significant differences between runs (Table 1). Allelic frequencies were homogenous for each locus tested between each early- and late-run group of salmon.

	Ssa171	Ssa197	Ssa202	SSOSL85	
	X^2 (P)	X^2 (P)	X^2 (P)	X^2 (P)	nb of X^2 P<0,05
St. John early vs late	30,86 (0,070)	15,17 (0,280)	9,39 (0,970)	15,94 (0,142)	0
Miramichi early vs late	37,65 (0,120)	18,45 (0,223)	14,69 (0,640)	10,79 (0,569)	0
Margaree early vs late	25,93 (0,730)	14,92 (0,490)	15,17 (0,410)	6,48 (0,850)	0

Table 1. Test of genetic homogeneity (X^2) between the early- and late-run salmon sampled in each of the three east-coast rivers for each of the 4 microsatellite loci tested. No significant differences in allelic frequencies were observed between runs in each river

Based on these results, the early- and late-run salmon sampled within each river cannot be considered as separate genetic populations. This conclusion is reinforced by the results of the analysis of molecular variance (AMOVA). The proportion of total genetic variance imputed to differences between early and late-run salmon is not significant for any locus within any river (Table 2).

	Ssa171	Ssa197	Ssa202	SSOSL85			
	Phi_{st} (P)	Phi_{st} (P)	Phi_{st} (P)	Phi_{st} (P)	nb of Phi_{st} P<0,05	Mean of Phi_{st}	N_em
St. John early vs late	0,000 (0,730)	0,000 (0,400)	0,000 (0,360)	0,016 (0,117)	0	0,0040	62,25
Miramichi early vs late	0,000 (0,900)	0,000 (0,840)	0,010 (0,159)	0,000 (0,889)	0	0,0025	99,75
Margaree early vs late	0,000 (0,880)	0,000 (0,810)	0,002 (0,270)	0,000 (0,950)	0	0,0005	499,75

Table 2. Genetic variance (Phi_{st}) and numbers of effective migrants per generation (N_em) between early- and late-run salmon in 3 east-coast rivers for the 4 microsatellite loci. In all cases, genetic divergence between the early- and late-run salmon were not significant.

Divergence between early and late-run salmon accounted for between 0 and 1.6% of total genetic variation, depending on the locus and river (Table 2). In the Miramichi, the mean inter-group variance estimate was 0.25% which was not statistically different from zero. Inter-group variances in the St. John and Margaree Rivers were also not significantly different from zero. The large numbers of effective migrants per generation between runs in each river thus represent sufficient gene flow to prevent genetic segregation. The effective number of migrants rapidly approaches infinity as Phi_{st} approaches values of 0.

The genetic comparison of Atlantic salmon among rivers

In order to compare rivers, allelic frequency information was calculated for the 100 fish sampled at the mouth of each river. The chi square analysis of the frequency distribution of these alleles at each locus revealed significant differences among rivers (Table3).

	Ssa171	**Ssa197**	**Ssa202**	**SSOSL85**	
	X^2 (P)	**X^2 (P)**	**X^2 (P)**	**X^2 (P)**	**nb of X^2 P<0,05**
St. John vs Miramichi	47,61 **(0,014)**	41,42 **(0,000)**	26,68 (0.090)	89,99 **(0,000)**	3
St. John vs Margaree	71.09 **(0,000)**	55,29 **(0,000)**	35,97 **(0,002)**	80,77 **(0,000)**	4
Miramichi vs Margaree	38,99 (0,194)	20,74 (0,183)	29,11 **(0,040)**	40,75 **(0,000)**	2

Table 3. Test of genetic homogeneity (X^2) between the salmon populations sampled in three east-coast rivers for each of the 4 microsatellite loci tested. Bold lettering indicates significant differences in frequency distributions in pairwise comparisons.

The frequency distributions of alleles at 3 loci were significantly different between the Miramichi and the St. John R. whereas the distributions of alleles at 2 loci were significantly different between the Miramichi

and the Margaree R. The St. John and Margaree Rivers differed significantly at all 4 loci. Based on these results, the three rivers can be considered to house three genetically distinct populations of salmon, although the Margaree is surprisingly similar to the Miramichi despite their geographic distance. This conclusion is reinforced by the results of the analysis of molecular variance (AMOVA). The proportion of genetic variance imputed to differences between rivers is significant for a variable number of loci. Divergence between the St. John and Miramichi R. fish accounted for between 0.5% ($p = 0.112$) and 13.6% ($p < 0.001$) of total genetic variation, depending on the locus (Table 4).

	Ssa171	**Ssa197**	**Ssa202**	**SSOSL85**			
	Phi_{st} (P)	**Phi_{st} (P)**	**Phi_{st} (P)**	**Phi_{st} (P)**	**nb of Phi_{st} P<0,05**	**Mean of Phist**	**N_em**
St. John vs Miramichi	0,005 (0,112)	**0,136 (<0,001)**	0,000 (0,360)	**0,061 (<0,001)**	2	0,050	4,75
St. John vs Margaree	**0,035 (0,004)**	**0,144 (<0,001)**	0,002 (0,250)	0,000 (0,450)	2	0,045	5,30
Miramichi vs Margaree	0,003 (0,190)	0,000 (0,730)	**0,017 (0,045)**	**0,031 (0,002)**	2	0,012	20,58

Table 4. Genetic variance (Phi_{st}) and numbers of effective migrants per generation (N_em) between the three east-coast rivers for the 4 microsatellite loci. Bold lettering indicates significant genetic divergence in pairwise comparisons.

This translated into an overall inter-group variance estimate of 5.0%, with 2 of 4 loci exhibiting significant genetic variation between rivers. The relatively small number of effective migrants per generation thus represents a degree of gene flow insufficient to cause genetic homogenization of the two populations. Divergence between the St. John and Margaree R. fish accounted for between 0 ($p = 0.450$) and 14.4% ($p < 0,001$) of total genetic variation, depending on the locus. This translated into an overall inter-group variance estimate of 4.5 %, with 2 of 4 loci exhibiting significant genetic variation between rivers.

Again, the relatively small number of effective migrants per generation reflects a minor degree of gene flow insufficient to cause genetic homogenization of the two populations. Divergence between the Miramichi and the Margaree R. fish accounted for between 0 ($p = 0.73$) and 3.1% ($p < 0.002$) of total genetic variation, depending on the locus (Table 3). This translated into an overall inter-group variance estimate of 1.2 %, with 2 of 4 loci exhibiting significant genetic variation between rivers. Thus, gene flow between the rivers is not sufficient to overcome genetic differentiation at 2 loci, although the greater number of effective migrants per generation suggests a greater degree of connectivity between the Miramichi and the Margaree than between the Miramichi and the St. John. Nevertheless, the three rivers may be considered as three genetically distinct populations with a limited and variable amount of gene flow among them.

The genetic identity of Clearwater Brook salmon

The frequency distributions of alleles at each locus were first compared between Clearwater Brook salmon and the early and late-run salmon sampled at the mouth of the Miramichi River (Table 5). Allelic frequencies at locus Ssa171 differed significantly between Clearwater Brook salmon and early- and late-run Miramichi salmon (Table 5). Allelic frequencies at locus SSOSL85 differed at the limit of significance between Clearwater and Miramichi late run salmon. The comparison between pooled Miramichi fish and Clearwater Brook fish revealed significantly different allelic frequencies at 2 of the 4 loci (Table 5).

	Ssa171	**Ssa197**	**Ssa202**	**SSOSL85**	
	X^2 (P)	**X^2 (P)**	**X^2 (P)**	**X^2 (P)**	**nb of X^2 P<0,05**

Miramichi vs Clearwater	59,88 **(0,000)**	18,85 (0,347)	29,90 (0,237)	22,50 **(0,032)**	2
Clearwater vs Miramichi early	52,46 **(0,005)**	22,64 (0,182	22,94 (0,230)	18,13 (0,076)	1
Clearwater vs Miramichi late	60,46 **(0,000)**	11,91 (0,788)	18,61 (0,357)	20,46 **(0,05)**	2

Table 5. Test of genetic homogeneity (X^2) between Clearwater Brook salmon and the early run, late run and pooled Miramichi samples. Bold lettering indicates significant differences ($P < 0.05$) in allelic frequencies between groups.

Based on these results, Clearwater Brook salmon are genetically distinct from Miramichi salmon sampled both early and late in the season. This conclusion, however, is not supported by the results of the analysis of molecular variance (AMOVA). The proportion of total genetic variance imputed to differences between Clearwater Brook salmon and early and late-run salmon (Phi_{st}) is not significant for any locus (Table 6). Divergence between Clearwater Brook salmon and early-run Miramichi salmon accounted for between 0 and 1.2% of total genetic variation, depending on the locus (Table 6). This translated into a mean inter-group variance estimate of 0.3% which was not statistically different from zero. Similarly, divergence between Clearwater Brook salmon and late run Miramichi salmon was not significant for any locus within any river (Table 6). Divergence between Clearwater Brook salmon and late-run Miramichi salmon accounted for between 0 and 0.1% of total genetic variation, depending on the locus. This translated into a mean inter-group variance estimate of 0.025% which is not statistically different from zero.

	Ssa171	**Ssa197**	**Ssa202**	**SSOSL85**			
	Phi_{st} (P)	**Phi_{st} (P)**	**Phi_{st} (P)**	**Phi_{st} (P)**	**nb of Phi_{st} P<0,05**	**Mean of Phi_{st}**	**N_em**

Miramichi vs Clearwater	0,000 (0,512)	0,000 (0,646)	0,000 (0,299)	0,003 (0,202)	0	0,00075	333,00
Clearwater vs Miramichi early	0,000 (0,634)	0,000 (0,513)	0,012 (0,092)	0,000 (0,356)	0	0,0030	83,08
Clearwater vs Miramichi late	0,000 (0,556)	0,000 (0,913)	0,000 (0,895)	0,001 (0,259)	0	0,00025	999,75

Table 6. Genetic variance (Phi_{st}) and numbers of effective migrants per generation (N_em) between Clearwater Brook salmon and the early run, late run and pooled Miramichi samples. In all cases, genetic divergence between the Clearwater Brook salmon and the Miramichi samples were not significant.

Divergence between Clearwater Brook salmon and the pooled Miramichi sample accounted for between 0 and 0.3% of total genetic variation, depending on the locus. This translated into a mean inter-group variance estimate of 0.075% which is not statistically different from zero. The large numbers of effective migrants per generation between Clearwater salmon and the Miramichi groups indicate sufficient gene flow to prevent genetic divergence. As mentioned before, the effective number of migrants rapidly approaches infinity as Phi_{st} approaches values of 0 such that no particular significance can be attached to the difference between 83 and 999 effective migrants. In all cases, the analysis of molecular variance indicates that gene flow is sufficient to genetically homogenize the two putative populations despite significantly different allelic frequencies at 2 of the 4 microsatellite loci.

Discussion

The genetic differentiation observed among Atlantic salmon occurring in the Miramichi, St. John and Margaree Rivers is consistent with the results obtained in other studies that have used microsatellites to discriminate Atlantic salmon populations. As with protein and mitochondrial DNA, clear evidence of discrimination was demonstrated between Canadian and European fish populations (McConnell et al. 1995a, b). These authors also found significant differences in allele frequencies and/or genetic variances (X^2 and G_{st} analysis respectively) for five populations of salmon from Nova Scotia (Gold, Salmon, Stewiacke, LeHave, Isaac's Harbour). G_{st} estimates of inter-population genetic variance were 0.089 for the Ssa 4 locus and 0.058 for the Ssa 14 locus (McConnell et al. 1995a). The mean inter-population variance estimate (Phi_{st}) for 7 Quebec rivers was 0.075 with the number of effective migrants per generation varying from a low of 1.3 between the Koksoak and Natashquan Rivers to 16.4 between the Cascapedia and Trinité Rivers (Fontaine et al. in press). Two rivers, the Bonaventure and the Cascapedia were genetically distinct based on significant differences in allelic frequencies at 4 of 5 microsatellite loci but shared 59.3 effective migrants per generation. Thus, the estimates of inter-population genetic variance between the Miramichi, St. John and Margaree Rivers are consistent with similar estimates among rivers of Quebec and among rivers of Nova Scotia.

The genetic homogeneity of the early and late-run fish seen in the three east-coast rivers clearly indicates that they cannot be considered as separate genetic populations. This suggests that despite differences in the timing of runs, early and late-run fish mingle on the spawning grounds. Although it is believed that early-run fish penetrate furthest into the river systems, late-run fish in the Miramichi have been observed to run far upstream before spawning (R. Cunjak, personal communication). Even if early and late-run fish were to segregate spatially in some years, just one or two mixing events during a generation would

effectively prevent any genetic divergence between the two groups. The results of this study also suggest that the timing of the spawning run is not genetically determined. Rather, variance in growth and development at sea and/or differences in marine migratory routes (Chadwick and Claytor 1989) may determine run timing in these stocks of Atlantic salmon.

Although significant differences in allelic frequencies at 2 of 4 loci were observed between Clearwater Brook fish and the pooled sample of salmon caught at the mouth of the Miramichi, the genetic divergence imputed to differences between Clearwater and Miramichi salmon was not significant. Such contradictions between comparisons of allelic frequencies (X^2 analyses) and analyses of genetic divergence (Phi_{st}) are not uncommon and may be due to the sensitivity of the X^2 analysis. Nevertheless, the failure to detect significant genetic divergence between salmon spawning in Clearwater Brook and those captured at the mouth of the Miramichi is surprising. Given the size of the Miramichi river and the number of tributaries, we presumed homing to natal tributaries and thus expected significant divergence through genetic drift. For example, in the Ste-Marguerite R., Saguenay, Québec, the proportion of genetic variance imputed to differences between a tributary and 3 sites located within the main branch of the river was significant. In addition, the proportion of genetic divergence imputed to differences between a tributary and two sites within the northeast branch of the river were also significant (D. Garant, J. Dodson, L. Bernatchez 1997. First CIRSA symposium, November, 1997), although these observations require replication. It seems likely that some degree of genetic divergence would have developed within a system as complex and large as the Miramichi R.

One possible explanation for the contradictory results concerning genetic variance between Clearwater and Miramichi salmon concerns the scale of the sampling program. Each group of fish analyzed in this study was composed of adult salmon intercepted either at the mouth of the sampled rivers or in Clearwater Brook during their upstream migration over a period of approximately 30 days in each case. It seems likely that this sampling scheme may have captured fish bound for different spawning grounds located throughout the system. The possibility thus exists that different genetic populations defined by spatial segregation rather than by temporal segregation were artificially lumped together in one sample. As such, the total genetic variation within Miramichi fish may have been so amplified by pooling several genetic groups that the genetic variance imputed to a difference between Clearwater Brook and the so-called early- or late- run fish was diluted to insignificance in spite of significant differences in allelic frequencies at some loci. It is important to note however that if such were the case the tests of Hardy-Weinberg equilibria should have revealed a deficiency in heterozygotes, indicating sub-structuring in the samples. This was not the case.

A second possible explanation of these results concerns the stocking history of Clearwater Brook and other areas of the Miramichi River. Between 1952 and 1970, tens of thousands of juvenile salmon (fry and parr) were released in Clearwater Brook from the Department of Fisheries and Oceans Southesk hatchery. The origin of the brood stock used to generate these fish could not be identified at the time of writing this report. Since 1985, the stocking of juveniles in Clearwater has been derived from Clearwater brood stock. Thus, the possibility exists that if Clearwater Brook was stocked in the past with juveniles derived from brood stock obtained from outside Clearwater Brook, then gene flow has been artificially increased between the Clearwater and other Miramichi populations.

Two recommendations are suggested to resolve this apparent contradiction and test the possibility that several genetic groups occupy the Miramichi and that Clearwater Brook may indeed house a genetically divergent group of fish. First, we recommend that the complete stocking history of New Brunswick salmon rivers be reconstructed from historical records to assess the magnitude and direction of human-induced gene flow among populations in general, and within the Miramichi in particular (eg. Fontaine et al. in press). Secondly, any future sampling for genetic studies must reduce the spatial scale of sampling. Rather than sampling adults intercepted during their migration, emergent fry should be captured on known spawning grounds. The analysis of genetic variance would thus be focused on the most likely ecological basis of genetic divergence; the spatial segregation of reproductive activities. The number of known spawning grounds to be sampled and assayed would no doubt be limited by budget, but the effort required to carry out such a project would necessitate the creation of a molecular ecology laboratory dedicated to elucidating the genetic structure of the Miramichi and other east coast salmon stocks. A New Brunswick university would be the most logical choice for the establishment of such a laboratory.

Conclusions and Recommendations

(1) All loci were highly variable as revealed by levels of heterozygosity which ranged from 0.82 to 1. No departures from Hardy-Weinberg equilibria were documented. The allelic frequencies of the 4 loci presented here represent the basis of any future assessments of the genetic diversity of Atlantic salmon in New Brunswick using microsatellite loci.

(2) The early- and late-run salmon sampled within the Miramichi, Margaree and St. John rivers cannot be considered as separate genetic populations. The proportion of total genetic variance imputed to differences between the two runs is not significant for any locus within any river.

(3) The three rivers can be considered to house three genetically distinct populations of salmon. The proportion of genetic variance imputed to differences between rivers is significant for a variable number of microsatellite loci. The three rivers represent three genetically distinct populations with a limited and variable amount of gene flow among them.

(4) Clearwater Brook salmon cannot be considered as genetically divergent from Miramichi salmon sampled both early and late in the season based on the present data set. The proportion of total genetic variance imputed to differences between Clearwater salmon and early and late-run salmon is not significant for any locus, although significantly different allelic frequencies were observed at 2 loci. Such contradictory results require further analyses.

(5) We suggest that the apparent absence of genetic structuring within the Miramichi R. may be due to two confounding factors ;

(a) past stocking practices ; it is recommended that the stocking history of Clearwater Brook and the Miramichi R. as well as that of other east-coast Rivers be reconstructed to assess the possible role of man-induced gene flow among putative populations.

(b) an inappropriate scale of sampling ; the spatial scale of sampling should be reduced to reduce the possibility of pooling genetically distinct groups. Emergent alevins should be sampled on known spawning grounds, thus focusing on the most likely ecological basis of genetic divergence; the spatial segregation of reproductive activities.

(6) We recommend the creation of a molecular biology laboratory at a New Brunswick university dedicated to carry out extensive studies on the genetic structure of Miramichi and other east coast salmon populations.

Acknowledgments

This research was supported by grants from the Atlantic Salmon Federation, the Natural Sciences and Engineering Research Council (NSERC)/ Department of Fisheries and Oceans (DFO) subvention program and the NSERC individual research grant program. We thank Gerald Chaput, DFO and Fred Whoriskey, ASF for providing samples, and Pierre-Michel Fontaine for reviewing this report. This is a contribution to the program of CIRSA (Centre Interuniversitaire de Recherche sur le Saumon Atlantique).

References

Angers, B., Bernatchez, L., Angers, A. and DesGroseillers, L. 1995. Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographical scale. J. Fish Biol. 47 (Suppl. A): 177-185.

Carvalho, G.R. and L. Hauser 1994. Molecular genetics and the stock concept in fisheries. Rev. in Fish Biol. and Fisheries 4 : 326-350.

Claytor, R., H. MacCrimmon and B. Gots 1991. Continental and ecological variance components of European and North American Atlantic salmon (Salmo salar) phenotypes. Biol. J. Lin. Soc. 44 : 203-229.

Davidson, W.S., Birt, T.P. and Green, J.M. 1989. A review of genetic variation in Atlantic salmon *Salmo salar* L. and its importance for stock identification, enhancement programs and aquaculture. J. Fish Biol. 34: 547-560.

Excoffier, L., Smouse, P.E. and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics. 13: 479-491.

Fontaine, P-M., Dodson, J.J., Bernatchez, L., Sletan, A. in press. A genetic test of metapopulation structure in Atlantic salmon (*Salmo salar*) using microsatellites. Canadian Journal of Fisheries and Aquatic Sciences.

Gauldie, R.W. 1988. Taking stock of genetic concepts in fisheries management. Canadian Journal of Fisheries and Aquatic Sciences 48 : 722-731.

Kimura, M. and. Ohta, T. 1978. Stepwise mutation model and distribution of allelic frequencies in a finite population. Proc. Natl. Acad. Sci. USA 75: 2868-2872.

McConnell, S.K., Hamilton, L., Morris, D., Cook, D., Paquet, D., Bentzen, P. and Wright, J. 1995a. Isolation of salmonid microsatellite loci and their application to the genetics of Canadian east coast stocks of Atlantic salmon. Aquaculture. 137: 19-30.

McConnell, S.K., O'Reilly, P., Hamilton, L., Wright, J.M. and Bentzen, P. 1995b. Polymorphic microsatellite loci from Atlantic salmon (*Salmo salar*) : genetic differentiation of the North American and European population. Can. J. Fish. Aquat. Sci. 52: 1863-1872.

McElroy, D,. Moran, P., Bermingham, E. and Kornfield, I. 1992. REAP. An integrated environment for the manipulation and phylogenetic analysis of restriction data. J. Heredity. 83: 157-158.

Michalakis, Y. and Excoffier, L 1996. A generic estimation of population subdivision using distances between alleles with special reference to microsatellite loci. Genetics. 142: 1061-1064.

O'Reilly, P.T. and Wright, J.M. (1995). The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. J. Fish Biol (Suppl. A) : 29-55.

O'Reilly, P.T., Hamilton, S.K., McConnell, S.K., Wright, J.M. 1996. Rapid analysis of genetic variation in Atlantic salmon (Salmo salar) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Can. J. Fish. Aquat. Sci. 53:2292-2298.

Raymond, M. and Rousset, F. 1995. GENEPOP (v. 1.2) A population genetics software for exact tests and ecumenicism. J. Heredity. 89: 248-249.

Roff, D.A. and Bentzen, P. 1989. The statistical analysis of mitochondrial DNA polymorphisms : X^2 and the problem of small samples. Mol. Biol. Evol. 6: 539-545.

Sambrook. J., Fritsch, E.F. and Maniatis, T. eds. 1989. Molecular cloning : A laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Shriver, M.D., Jin, L., Chakraborty, R. and Boerwinkle, E. 1993. VNTR allele frequency distribution under the Step-Wise mutation model: Genetics, 134: 983-993.

Shriver, M.D., Jin, L., Boerwinkle, E. Deka, R., Ferrell, R.E. and Chakraborty, R. 1995. A novel measure of genetic distance for highly polymorphic tandem repeat loci. Mol. Bio. Evol. 12(5): 914-920.

Sinclair, M. 1988. Marine populations. University of Washington Press

Slettan, A., Olsakek, I. and Lie, O. 1995. Atlantic salmon, *Salmo salar*, microsatellites at the SSOSL25, SSOSL85, SSOSL311, SSOSL417 loci. Anim. Genet.. 26: 281-282.

Tessier, N., Bernatchez, L. Presa, P. and Angers, B. 1995. Gene diversity analysis of mitochondrial DNA, microsatellites and allozymes in landlocked Atlantic salmon. J. Fish Biol. 47 (Suppl. A): 156-163.

Wright, S. 1951. The genetical structure of populations. Ann. Eugen. 15: 323-354.

Wright, J.M. and Bentzen, P. 1994. Microsatellites : genetic markers for the future. Rev. Fish Biol. Fisheries. 4: 384-388.

Appendix 1. Observed (Ho) and expected (He) heterozygosity for each locus and group of fish sampled in the Miramichi River. Ho and He for 'Miramichi total' was based on the pooled early- and late-run salmon captured at the river's mouth. Observed and expected heterozygosities were not significantly different ($P > 0.05$) for any of the comparisons. a= number of alleles detected in each group, n= number of fish sampled in each group. Unequal sample sizes (n) indicates that certain loci in some fish could not be successfully amplified.

Loci	**Miramichi early**	**Miramichi late**	**Miramichi total**	**Clearwater**
	(a=23 n=50)	(a=26 n=50)	(a=32 n=100)	(a=25 n=124)
Ho	0,920	0,940	0,930	0,935
Ssa171 (a=35)				
He	0,912	0,928	0,920	0,930
	(a=14 n=50)	(a=14 n=50)	(a=16 n=100)	(a=17 n=125)
Ho	0,860	0,880	0,870	0,880
Ssa197 (a=18)				
He	0,880	0,900	0,897	0,910
	(a=18 n=50)	(a=14 n=45)	(a=18 n=95)	(a=18 n=122)
Ho	1,000	0,911	0,957	0,893
Ssa202 (a=20)				
He	0,923	0,905	0,914	0,903
	(a=11 n=50)	(a=13 n=50)	(a=13 n=100)	(a=12 n=124)
Ho	0,900	0,860	0,880	0,887
SSOSL85 (a=13)				
He	0,869	0,890	0,879	0,868

Appendix 2. Observed (Ho) and expected (He) heterozygosity for each locus and group of fish sampled in the St. John River. Ho and He for 'St. John total' was based on the pooled early- and late-run salmon captured at the river's mouth. Observed and expected heterozygosities were not significantly different ($P > 0.05$) for any of the comparisons. a= number of alleles detected in each group, n= number of fish sampled in each group. Unequal sample sizes (n) indicates that certain loci in some fish could not be successfully amplified.

Loci		St John early	St John late	St John total
		(a=21 n=48)	(a=18 n=48)	(a=23 n=96)
Ssa171 (a=35)	Ho	0,833	0,916	0,875
	He	0,891	0,846	0,872
		(a=12 n=48)	(a=11 n=48)	(a=14 n=96)
Ssa197 (a=18)	Ho	0,833	0,810	0,823
	He	0,825	0,836	0,833
		(a=15 n=48)	(a=17 n=48)	(a=18 n=96)
Ssa202 (a=20)	Ho	0,790	0,958	0,875
	He	0,885	0,893	0,889
		(a=11 n=48)	(a=12 n=48)	(a=12 n=96)
SSOSL85 (a=13)	Ho	0,875	0,875	0,875
	He	0,865	0,873	0,873

Appendix 3. Observed (Ho) and expected (He) heterozygosity for each locus and group of fish sampled in the Margaree River. Ho and He for 'Margaree total' was based on the pooled early- and late-run salmon captured at the river's mouth. Observed and expected heterozygosities were not significantly different ($P > 0.05$) for any of the comparisons. a= number of alleles detected in each group, n= number of fish sampled in each group. Unequal sample sizes (n) indicates that certain loci in some fish could not be successfully amplified.

Loci	**Margaree early**	**Margaree late**	**Margaree total**
	(a=27 n=46)	(a=23 n=50)	(a=31 n=96)
Ho	0,944	0,980	0,947
Ssa171 (a=35)			
He	0,947	0,946	0,943
	(a=16 n=47)	(a=15 n=49)	(a=16 n=96)
Ho	0,936	0,897	0,9166
Ssa197 (a=18)			
He	0,900	0,900	0,905
	(a=14 n=40)	(a=15 n=43)	(a=16 n=83)
Ho	0,950	0,860	0,903
Ssa202 (a=20)			
He	0,897	0,898	0,896
	(a=12 n=44)	(a=12 n=48)	(a=12 n=92)
Ho	0,930	0,833	0,880
SSOSL85 (a=13)			
He	0,860	0,863	0,859