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APPLICATION OF GENETIC MARKERS TO CONSERVATION BIOLOGY: SALMONID FISH IN EASTERN CANADA

by

Matthew William Jones

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

at

Dalhousie University
Halifax, Nova Scotia
April 2001

DALHOUSIE UNIVERSITY

DATE: April 10, 2001

Author: Matthew William Jones

TITLE: Application of genetic markers to conservation biology: Salmonid fish in eastern Canada

DEGREE: Ph. D. Convocation: May Year: 2001

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For my mother,
for instilling confidence,
and the memory of my father,
whose ghost I'll never catch.

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ABSTRACT

I used genetic markers to address questions fundamental to the conservation of two salmonids native to Eastern Canada. These questions centre on effective population size, some factors that influence it, and the effect of low population size on genetic diversity. Genetic variability is believed to be an important determinant of a population's long-term persistence in the face of changing environments. To test the generality of previously observed low levels of heterozygosity in lake populations of brook trout (*Salvelinus fontinalis*), I examined genetic variation within and among nine pairs of adjacent lake and stream populations. Lake populations generally had lower heterozygosity than their adjacent stream populations. There were negative associations between metrics of fishing mortality and the difference in heterozygosity between lake and adjacent stream populations.

Mating systems can strongly influence effective population size. Examining factors that affect fertilization success of mature male Atlantic salmon (*Salmo salar*) parr when competing with a single anadromous male for the opportunity to fertilize eggs of a single anadromous female, I found parr body size was an important predictor of the probability of an individual being involved in spawning. I found a negative relationship between total parr fertilization success and intensity of anadromous male competition. I also established two experimental replicates each involving multiple anadromous males and females and mature male parr in a semi-natural spawning environment. There was some evidence of size being an important determinant of both the frequency of spawning and the overall individual parr fertilization success among those parr identified as having spawned. Fertilization of eggs by parr can significantly increase the effective number of males on a nest-by-nest basis, however, the variance in individual anadromous male fertilization success appears to have the greatest overall influence on effective population size.

Using temporal and spatial microsatellite variation of threatened Atlantic salmon in the Inner Bay of Fundy, I found that while a previous reintroduction programme achieved its goals of introducing genetic variation similar to that of the source population to the new population, similar results were found for a population that had been recolonized naturally. Heterogeneity among year groups within some Inner Bay rivers was found, as was a decline in genetic variation in some populations. Finally, while there is some degree of reproductive isolation between Inner Bay salmon and other salmon populations, they do not appear to compose a unique phylogenetic lineage.

ACKNOWLEDGEMENTS

The years of my PhD were a time of much personal and, I would like to think, academic change and growth. I owe many for making this the experience it was and I will always have fond memories of my time in both Halifax and Fundy National Park.

I would first like to thank my supervisor, Jeff Hutchings, who gave me encouragement and support throughout and from whose office I always left with renewed enthusiasm. Jeff also played a major role in the establishment of the salmon reproductive success experiments and provided excellent comments and insights on all of the versions of each of these chapters.

I thank the members of my advisory committee, Rick Cunjak, Christophe Herbinger and Mark Johnston for the time and effort they put into reading proposals, essays and various versions of this thesis; their critical comments invariably led to marked improvements and their encouragement helped me at various times along the way. I thank Julian Dodson for his insights on various aspects throughout the entire thesis. I would also like to thank Roger Doyle, Chris Taggart and Mike Hart for their input at earlier stages of this work during different examinations. Comments from many people on different chapters of this thesis are also much appreciated; I thank Mick O'Connell and Steve Mockford (Chapter 1), Roy Danzmann, Pat O'Reilly and 2 anonymous reviewers (Chapter 2), Anders Klemetsen, Bror Jonsson and 2 anonymous reviewers (Chapter 3), Andrew Hendry and an anonymous reviewer (Chapter 4) and Bob Latta (Chapter 5). I am also most grateful to both Ellen Kenchington and Chris Taggart for their patience with me while the completion of this thesis dragged on.

I am deeply indebted to the many people who have helped me collect the data that was used in my work; I am especially thankful to those who put up with working in the field when conditions were less than ideal. Doug Clay convinced me to return to Fundy National Park after my MSc, provided the initial impetus for the salmon population genetics work, helped obtain funding for the trout and salmon population work and provided much support during the fieldwork phase of this research. The brook trout fieldwork, sample and data collection was possible only with the help of many people. Tara McParland ran the majority of the allozyme samples and got to share the joys of

sampling fish in sub-zero weather. The assistance of Reagan Sutherland in the summer of 1996 is gratefully acknowledged. Additional help by Shirley Humphries, Alain Caissie, Yves Lanteigne, George Sinclair, Sedgewick Sinclair, and Nancy Mason is much appreciated. Tom Pettigrew (New Brunswick Department of Natural Resources and Energy) provided information regarding lake size, depth and angling pressure and without whose advice and gill nets, collection of brook trout outside of Fundy National Park would not have been possible. These collections also required the consent and assistance of Lawrence McFarlane and Alison Forsythe (Pleasant Lake Fishing Club), Reginal McKenzie (Chisholm Fishing Club - Dick's Lake), and Burt Miller and Edwin Patterson (Hampton Fishing Club - Wood Lake). Comments by Mark Johnston at the planning stages of this project led to the paired sampling scheme employed. I thank Roy Danzmann for letting me back into his lab year after year. This work was funded by Parks Canada through Science Advisory Board research grants to Fundy National Park and by NSERC Research Grants to Jeff Hutchings and Roy Danzmann.

The work on reproductive success would not have been possible without the help of many people. Leonard Forsythe provided advice on various aspects of the raceway set up and monitored the raceways during the experiment. Leonard Forsyth, Larry Forsyth, Jeff Hutchings, Larry Marshall, Paul LeBlanc, Mike Mason, and Kevin Davidson participated in the collection of the anadromous fish. Patrick O'Reilly provided primer sequences prior to their publication and offered lab advice. Doug Cook was a source of insight in the lab. Tillmann Benfey provided possible explanations for the triploids. Tara McParland helped sample the post-spawned fish, offered valuable assistance in the lab and with scoring, and read various versions of these chapters. Financial support for this study came from an NSERC Research Grant to Jeff Hutchings

I cannot possibly thank all the people who have been responsible for the collection of samples used in the study of temporal and spatial genetic variation in Atlantic salmon. Mick O'Connell was instrumental in formulating the ideas behind this work and I would not have attempted it without the groundwork laid by Pat O'Reilly. Tara McParland was responsible for the nearly impossible job of obtaining microsatellite data from scale samples. Recent sample collections would not have been possible

without George Sinclair, Reagan Sutherland and Tara McParland; additional help from Betty Betts, Alain Caissie, Shirley Humphries, Yves Lanteigne, Sedgewick Sinclair, John Underwood, Jane Watts and Thane Watts is much appreciated. Peter Amiro (DFO, Halifax) provided most of the historical Inner Bay of Fundy samples, was always a source of novel insight, and kindly gave me an electronic copy of the map of the Bay of Fundy. The historical samples from the Point Wolfe and Upper Salmon rivers were collected and preserved by the Fundy National Park wardens. Tom Pettigrew provided the 1974 Big Salmon River scales. Additional samples were collected during assessment surveys. John Mallory (DFO Fredericton) and Ross Jones (DFO Moncton) collected the 1994-1996 Hammond and Big Salmon river samples. Shane O'Neil (DFO, Halifax) provided me with samples that, through not fault of his, never made it into the dataset. Arran McPherson was the first to point out to me that the high heterozygosity generally found with microsatellite loci results in maximum F_{ST} values of much less than 1. This work was funded by Parks Canada through Science Advisory Board research grants to Fundy National Park and an NSERC research grant to Jeff Hutchings.

I would also like to thank those who have enriched my life outside the decaying yet unfinished "Life" Sciences complex. The support of my mum, my sibs and my in-laws was always important and appreciated. Mary Dillon, Lou Robitaille, Marcel and Madeleine are an inspiration; Kerri Oseen and Rob MacKinnon try to keep me young; the return of Tim Jackson, Alison Luke and Poppy was most welcome. Members of the extended Hutchings lab, especially Janice MacDonald, Jim Eddington, Blair Adams and Dee Galway and MGPLers past and present, in particular Mick O'Connell, Pat O'Reilly, Arran McPherson, Shelley Lang, Lorraine Hamilton and Doug Cook have all contributed to my mental health, such as it is. I cannot possibly express my good fortune for and gratitude to Tara McParland. Her contributions and support during these years, especially for reminding me to enjoy today while encouraging me to pursue my dreams, as off the wall as they often become, mean the world to me. Finally, for her ability to lift my spirits from the lows of these past months, I thank our sleep deprivation experiment, Hannah the Haligonian.

SCIENTIFIC CONTRIBUTIONS

Much of the following work has been presented or published in some form during my PhD. As of April 10th, 2001:

Presentations

Jones, M.W., T.L. McParland, J.A. Hutchings, R.G. Danzmann & D. Clay. 2000. Low genetic variability in brook trout lake populations. Canadian Conference For Fisheries Research. Fredericton, NB. January, 2000. (Chapter 2)

Jones, M.W., J.A. Hutchings, R.G. Danzmann, & D. Clay. 1996. Implications of low genetic variability in brook trout populations in Fundy National Park, New Brunswick. Canadian Society of Zoologists; Measuring fitness in the wild and in the laboratory symposium. St. John's, NF. May, 1996. (Chapter 2)

Jones, M.W., & J.A. Hutchings. 1999. Reproductive success in Atlantic salmon: effects of parr size and intensity of mate competition. *Invited.* American Society of Ichthyologists and Herpetologists; Reproductive success in salmonids symposium. State College, PA. June 1999. (Chapters 3 and 4)

Jones, M.W., & J.A. Hutchings. 1999. Reproductive success in Atlantic salmon: effects of parr size and intensity of mate competition. Canadian Society of Zoologist. Ottawa, ON. May 1999. (Chapters 3 and 4)

Jones, M.W. Reproductive success in Atlantic salmon: effects of parr size and intensity of mate competition. Department of Biology Friday Seminar Series. Dalhousie University. April 1999. (Chapters 3 and 4)

Jones, M.W., T.L. McParland, J.A. Hutchings, & D. Clay. 1998. Temporal changes in genetic variation in Atlantic salmon populations in the Inner Bay of Fundy. Canadian Conference For Fisheries Research. Kingston, ON. January, 1998. (Chapter 5)

Publications

Jones, M.W., T.L. McParland, J.A. Hutchings, R.G. Danzmann, & D. Clay. 1998. Low levels of genetic variation in brook trout lake populations in Fundy National Park: preliminary results. In: *State of the Greater Fundy Ecosystem.* Eds. Woodley, S., G. Forbes, & A. Skibicki: pp. 163-165. (Chapter 2)

Jones, M.W., T.L. McParland, J.A. Hutchings & R.G. Danzmann. In revision. Low genetic variability in lake populations of brook trout (*Salvelinus fontinalis*): a consequence of exploitation? Submitted to *Conservation Genetics*. (Chapter 2)

Jones, M.W., & J.A. Hutchings. 2001. The influence of male parr body size and mate competition on fertilization success and effective population size in Atlantic salmon. *Heredity*. In Press. (Chapter 3)

Jones, M.W., & J.A. Hutchings. In revision. Individual variation in Atlantic salmon fertilization success: implications for effective population size. *Ecological Applications*. (Chapter 4)

Blanchfield, P., & M.W. Jones. 2000. Reproductive success in salmonids. Conference report. *Reviews in Fish Biology and Fisheries* **10:** 119-121. (Tangentially, Chapters 1, 3 and 4)

CHAPTER 1.

General Introduction

CONSERVATION BIOLOGY OF SALMONID FISH: IMPLICATIONS OF EFFECTIVE POPULATION SIZE AND GENETIC VARIATION

The primary goal of conservation biology is to maintain biological diversity in response to human activities. Conservation of exploited species requires the integration of many elements including the amount and distribution of genetic variation, gene flow, life history variation, and responses to population manipulations. Fundamental to conservation biology and these specific issues is a comprehensive understanding of factors influencing effective population size (N_e). N_e is of particular interest and importance because the rate of loss of heterozygosity, a primary metric of genetic variation, is inversely proportional to the effective size. Therefore, an understanding of N_e is fundamental for conservation-oriented management. I am interested in salmonid conservation genetics. In this thesis, I address conservation-related questions centred on achieving a better understanding of the factors that influence effective population size in salmonid populations.

When modelling genetic drift, one assumes the existence of an "ideal" population. This "ideal" population consists of sexually reproducing diploid organisms with an even sex ratio in which each individual has an equal chance of contributing gametes to the next generation, generations are non-overlapping, individuals are divided into many independent subpopulations each with constant size, and there is neither mutation, selection, nor migration among subpopulations (Hartl and Clark 1989). N_e is an abstract parameter that Wright (1931) introduced to evaluate the effects of some differences real populations display relative to "ideal" populations.

The life histories of many fish species violate most assumptions of the "ideal" population. For example, many have long-distance migrations and subsequent homing to natal sites, albeit with some straying, and large variations in subpopulation sizes. In practical terms, N_e is affected by fluctuations in population size, uneven sex ratio, large

variance in reproductive success, overlapping generations, and geographic dispersal of populations (Table 1-1). In most real populations, violation of the assumptions of the "ideal" population lowers N_e.

N_e has traditionally been important to both short- and long-term conservation planning. The immediate concern is often related to decreased fitness associated with inbreeding among small numbers of breeders (e.g., Soulé 1980). The long-term objective of conservation is to maintain sufficient genetic variation to allow for adaptive response to environmental change (e.g., Frankel and Soulé 1981). Rates of inbreeding increase and heterozygosity loss have both been estimated to be $1/(2N_e)$ per generation (Wright 1931, Falconer 1981).

There is empirical evidence that reductions in genetic variation can negatively influence fitness. One of the most familiar examples cited is that of the cheetah (*Acinonyx jubatus*). Extremely low levels of genetic variation in the cheetah, when compared to other felines (O'Brien et al. 1983, 1985, 1987), are associated with low sperm count and high frequency of morphologically aberrant sperm (O'Brien et al. 1983), increased juvenile mortality, and vulnerability to disease outbreaks (O'Brien et al. 1985; but see also Caughley (1994), Caro and Laurenson (1994) for potential weaknesses in this evidence, and O'Brien's (1998) review and rebuttal against most of the raised concerns). There is similar evidence for a wide variety of genera including plants, invertebrates, herpetiles, birds and mammals (see reviews in Soulé 1980, Allendorf and Leary 1986, Mitton 1993). Similarly, some salmonid studies (but not all; Hutchings and Ferguson 1992) have suggested a positive association between heterozygosity and components of fitness (ability to survive and reproduce) (e.g. Leary et al. 1983, Ferguson et al. 1985, Danzmann et al. 1986, 1987, 1988). Furthermore, decreases in heterozygosity in salmonid hatchery strains are thought to have a negative influence on such factors as survival, growth, and development stability (see reviews in Leary et al. 1985, Allendorf and Ryman 1987, Hedrick and Miller 1992).

Much of the debate on the importance of genetic variation is centred upon the purported positive correlation between fitness and heterozygosity. Indeed, it may be

premature, with the low number of markers tested to date, to draw any firm conclusions on this topic (Mitton 1993). Of broader interest may be population heterozygosity values given that genotypic variance is lost at the same rate as heterozygosity, i.e., $1/2N_e$ per generation (Franklin 1980). If there is little or no dominance or epistatic variance, additive variance will be lost at the same, or approximately the same, rate. Therefore, populations with low heterozygosity may have less additive genetic variation, and individuals in such populations may have lower fitness. Quattro and Vrijenhoek (1989) provided evidence of such an association between mean survival, growth, early fecundity, and development stability (used as measures of fitness) and mean heterozygosity in three populations of Sonoran topminnow (*Poeciliopsis occidentalis*).

The implications of low N_e are not limited to potential fitness consequences. N_e is also a key parameter affecting the rate at which populations diverge. For example, small populations will rapidly accumulate allele frequency differences at neutral loci but these differences may reflect no biologically important differences. It is, therefore, of considerable importance, when assessing the potential viability of populations and interpreting population genetic data, to have a clear understanding of N_e and the factors that influence it.

Population genetics is a study of the various forces of evolution, namely mutation, selection, migration, and genetic drift. For the purpose of conservation and short-term management, we can, at least initially, ignore mutation and perhaps selection as forces in the modelling and maintenance of genetic variation. While it is the accumulation of differences due to these forces that we are attempting to conserve, as some of these differences can be adaptive (Taylor 1991), quantification of population differences is generally based on neutral or nearly neutral genetic markers. Many questions of concern in the conservation and management of fish relate to population differentiation from genetic drift and the extent of genetic homogenization as a result of gene flow among populations.

Genetic differentiation among populations has varying degrees of importance to management and conservation efforts. Populations that have had no gene flow for

extended periods of time will accumulate large genetic differences while those that have only recently become separated and/or have maintained some level of gene flow will be much less differentiated (deep versus shallow separations (Avise 1994)). Population differentiation accumulates over time but is, of course, closely related to population size; excluding selection and the small impact of novel mutations, infinitely large subpopulations would not diverge. For example, to create phylogeographic structuring with mtDNA, gene flow would need to be restricted for approximately 2-4 N_e generations (Avise et al. 1984, Neigel and Avise 1986). In the management of salmonids, which have a tendency for natal homing (reviewed in Stabell 1984), the degree of genetic differentiation among populations is less of a concern than the actual presence of identifiable differentiation. However, genetic divergence among populations is of great interest from a conservation perspective.

Temporal stability has implications for evaluating the biological significance of population differentiation. Given that many salmonid effective population sizes, especially within sections of rivers, are small, the expectation for variation in allele frequencies is high. Thus, it is difficult to assess the biological relevance of significant statistical differences in allele frequencies among collections of fish. For example, the relationship between genetic and geographic distances found in Atlantic salmon (*Salmo salar*) (Elo 1993) provides strong evidence for isolation by distance in this anadromous species. Such a general trend would probably be stable over time. However, finding genetic differences among locations, especially when addressing questions on a small geographical scale, does not necessarily have any biological significance. Such studies must be accompanied by tests of the temporal stability of any such differences. Ideally, one would examine the genetic variation of spawning adults at a given location in the same river over time to quantify temporal stability of genetic variation. Few studies to date have examined temporal stability of genetic variation. If temporal heterogeneity within populations was found, previous observations of spatial heterogeneity would need to be reevaluated as they may in fact only be a reflection of temporal fluctuations around a common mean of allele frequencies (Jordan et al. 1992).

Genetic markers have proven very informative in identifying and quantifying the occurrence of natural and anthropogenically-induced interspecific introgression. For example, use of allozyme and mtDNA variation allowed Leary et al. (1993) to identify hybrids and the direction of hybridization between the endangered and native bull trout (*Salvelinus confluentus*) and the introduced brook trout (*Salvelinus fontinalis*) in Columbia and Klamath rivers. By examining one location over eight years, they were able to document an increase in frequency of brook trout alleles over time (Leary et al. 1993). Similar methods have been used to examine introgression between Atlantic salmon and brown trout (*Salmo trutta*); it tends to be lower in European waters where they naturally co-occur than in North America where brown trout have been introduced (e.g., reviewed by Davidson et al. 1989).

Genetic markers are also useful for detecting intraspecific introgression, or lack thereof, between wild and introduced fish. For example, Garcia de Leaniz et al. (1989) used one allozyme locus to reveal low survival of stocked Atlantic salmon ova in two Spanish rivers. Similarly, using both allozyme and mtDNA variation, Jones et al. (1996; see also references therein) found no evidence for introgression of a previously stocked brook trout strain into wild populations. Simulation studies have suggested that under large-scale introductions from a common source, e.g., aquaculture escapees, historical population differentiation will be lost in very few generations (Mork 1994). Thus, such introgression may be inferable by examining temporal genetic variation.

Fish display great variation in reproductive strategies. Such variation within a species or a population can have many important implications. Atlantic salmon exemplify variation in reproductive strategies. Typically, a salmon's life cycle consists of a freshwater component and a period at sea followed by a return to its natal river to spawn. Age at smoltification (physiological changes occurring prior to seaward migration) varies from one to seven years (Chadwick et al. 1987). Time at sea varies from one to five years (Thorpe 1986). Alternatively, some male parr forego or delay migration to sea and mature in freshwater. Parr maturation is believed to be maintained by negative-frequency dependent selection (Gross 1985). Smolt, and subsequently, adult

sex ratios are biased towards females, presumably as a result of increased mortality of post-reproductive mature male parr (Myers 1984). The large number of mature male parr present could effectively even or reverse the operational sex ratio bias towards the males. The level of reproductive success of these parr will therefore influence N_e. It is thus important to quantify individual variance in male reproductive success to obtain more reliable estimates of effective number of males. Although it is unclear whether resident males are involved to the same extent in reproductive competition in brook trout, circumstantial evidence suggests they do. Males compete during spawning (Power 1980) and sex ratios of anadromous brook trout can be highly skewed towards females (e.g., White 1940). Recent field observations of lake resident brook trout support the possibility of greater variance in male reproductive success. Blanchfield and Ridgeway (1997) found males arrived earlier and remained at the spawning area longer, thus in higher numbers, than females.

Genetic markers can clearly be of use in the estimation of individual reproductive success (Blanchfield and Jones 2000). Previous studies on the reproductive success of mature male parr have indicated that parr as a group are successful in fertilizing some eggs (Hutchings and Myers 1988, Jordan and Youngson 1992, Morán et al. 1996). Hypervariable genetic markers are beginning to allow the assessment of individual reproductive success and hence provide better variance estimates and understanding of the factors associated with alternate reproductive strategies (e.g., Thomaz et al. 1997).

The "decision" to mature as parr in the fall is made by the individual several months previously and is largely influenced by lipid reserves at that time (Rowe et al. 1991). The incidence of male parr maturation is also largely dependent on growth rate (Thorpe 1986, Rowe and Thorpe 1990) which can be dependant on food availability. Thus, at low densities when there is little competition for food, the incidence of male parr maturation may be high. This may have an interesting consequence for N_e of salmon populations. At high parr densities, presumably when adult returns are relatively high, the incidence of male parr maturation may be low. Similarly, the incidence of parr maturation may increase as adult returns decrease (especially male returns); such

increases could partially offset the low adult returns. Successful reproduction of mature male parr also has implications for the interpretation of population genetic data. Significant reproductive success of the non-migratory parr would increase the level of among-population genetic differentiation and within-population temporal stability. Thus, a clearer understanding of the reproductive success of mature male parr is important in improving our understanding of N_e as well as better interpreting population genetic data.

SUMMARY OF THESIS CHAPTERS

In each of the following chapters, I address questions fundamental to the conservation of two salmonids native to Eastern Canada (Atlantic salmon and brook trout). These questions centre on effective population size, some factors that influence it, and the effect of low population size on genetic diversity. Specifically, I (1) provide one explanation for previously observed low levels of heterozygosity in lake populations of brook trout, (2) quantify individual reproductive success in Atlantic salmon, (3) consider how mating structure and variance in individual reproductive success affects Atlantic salmon effective population size, (4) evaluate a previous reintroduction effort of Atlantic salmon, and (5) assess the effects of low population size on genetic variation and genetic divergence of a putatively ecologically unique group of Atlantic salmon.

Understanding how recent anthropogenic activities affect a population's persistence in light of genetic, demographic, and environmental stochasticity is of increasing importance to the conservation of populations (Lande 1993). Of these, genetic variability is believed to be an important determinant of a population's long-term persistence in the face of changing environments (Frankel and Soulé 1981, Lande and Shannon 1996). I previously found lower levels of genetic variation in lake than stream populations of brook trout (Jones et al. 1996). In the second chapter, I test the generality of this observation by examining whether brook trout genetic variation differed within and among nine pairs of adjacent lake and stream populations. With one exception, I found that lake populations did have lower heterozygosity than their adjacent stream populations. While no association was found between lake size characteristics and the degree of difference in heterozygosity between lake and their adjacent stream

populations, there were negative associations between metrics of fishing mortality and the difference in heterozygosity between lake and their adjacent stream populations. The greater the estimated fishing pressure on lake-dwelling trout, the greater the reduction in heterozygosity in those populations relative to their adjacent stream populations. These findings indicate that intensive fishing pressure can significantly reduce genetic variation, suggesting that populations be allowed to increase in size as rapidly as possible following a decline.

Mating systems can strongly influence effective population size. Alternative mating strategies in male Atlantic salmon are characterized by variability in body size and mate competition. In the third chapter, I examined whether body size of mature male parr influenced fertilization success and whether such an association was affected by mate competition among parr when competing with a single anadromous male for the opportunity to fertilize the eggs of a single anadromous female. Variation at 3 to 4 hyper-variable microsatellite loci was used to determine individual paternity of 53-60 offspring from 2 or 3 nests from each experimental treatment. Although individual and total parr reproductive success differed significantly among nests within treatments, there was no relationship between parr size and individual reproductive success at any level of competition when anadromous males were involved. However, in a single treatment having no anadromous male, the influence of body size on parr fertilization success was highly significant. Combining data from all treatments, parr body size was an important predictor of the probability of an individual being involved in spawning. I found a negative relationship between total parr reproductive success and intensity of anadromous male competition. By estimating N_e, I illustrate how mature male parr can greatly increase the effective number of males when the latter is estimated from anadromous individuals alone.

By influencing the variance in individual reproductive success, the effect of mating structure on effective population size may be greater than expected from single anadromous pair experiments. To test this, I established two experimental crosses each involving 4 anadromous females, 4 anadromous males, and 20 mature male parr in a

semi-natural spawning environment. I again used hyper-variable microsatellite loci to identify the parents of 755 embryos and to quantify the variance in individual fertilization success. Anadromous males generally dominated fertilization success. There was no relationship between individual mature male parr size and individual fertilization success in any individual nest, nor was there any relationship between anadromous male size and individual fertilization success. There was some evidence of size being an important determinant of both the frequency of spawning and the overall individual parr fertilization success among those parr identified as having spawned, although these relationships were not always significant. Both anadromous males and females were identified as having spawned with multiple partners, although the frequency of multiple anadromous males spawning simultaneously with a female was low. Fertilization of eggs by parr can significantly increase the effective number of males on a nest-by-nest basis, however, the variance in individual anadromous male fertilization success appears to have the greatest overall influence on effective population size.

As salmonid population sizes decline, it is increasingly likely that intervention activities will be implemented by management authorities. In Canada, declines of Atlantic salmon in the Inner Bay of Fundy are so great that reintroduction programmes are currently underway. In the fifth chapter, I tested the utility of a previous reintroduction effort by comparing the genetic variation at five microsatellite loci of Atlantic salmon reintroduced to one river to the genetic variation of salmon in its source population as well as that of salmon in a naturally recolonized river. I also quantified the effects of declining numbers of returning anadromous salmon on genetic diversity. Finally, I quantified the genetic diversity among Atlantic salmon populations in the Inner Bay and the degree of differentiation between Inner Bay salmon populations and populations in other Maritime rivers. I found that, while the reintroduction programme achieved its goals of introducing genetic variation similar to that of the source population to the new population, similar results were found for a previously recolonized population for which no reintroduction had occurred. The use of temporal as well as spatial examination of Atlantic salmon genetic variation revealed heterogeneity among year

groups within some Inner Bay rivers, most likely as a result of population bottlenecks. Atlantic salmon populations appear to maintain high levels of genetic diversity for longer than may be expected based on the number of returning adults, presumably the result of increased reproductive success of large numbers of mature male parr. Despite this ability to maintain higher levels of genetic variation than expected, based only on the number of returning anadromous salmon, genetic diversity did decline over time as the number of returning salmon declined. Finally, while Inner Bay of Fundy Atlantic salmon appear to be reproductively isolated from other salmon populations, I found no evidence to support the hypothesis that they comprise a unique phylogenetic lineage.

Conservation genetics is a broad field that requires the integration of many elements. A central theme common to all elements is related to population size, specifically N_e. Genetic markers can clearly contribute to the understanding of elements of concern to the conservation and management of fish species. The increasing use of genetics markers to address questions of central importance in conservation biology will aid greatly to our understanding of the factors influencing effective population size.

Table 1-1. Factors that influence N_e.

Factor	Effect	Variables	Comments
Fluctuations in population size[1]	$1/N_e = (1/t)\,(1/N_1 + 1/N_2 + \ldots + 1/N_t)$	t is time in generations; N_1 is the population size in generation 1, etc.	Population sizes do vary over generations and N_e is most influenced by the generation which has the lowest number of breed ers. In the absence of immigrants or new mutations, one or few bottlenecks can have long lasting effects on the N_e of a population.
Sex ratio[1]	$N_e=4\,N_m\,N_f/(N_m + N_f)$	N_m is the number of males; N_f the number of females	N_e is most affected by the sex contributing to the next generation in the lowest frequency.
Non-Random Family Size[2]	$N_e=(4N-4)/(V_k+2)$	N is the actual number; V_k is the variance in the number of gametes contributed	The "ideal" population assumes there to be variation in offspring survival to reproductive age. When population size is constant, the mean family size will, on average, be two offspring surviving to reproductive age for each pair of parents. The distribution in family size is approximated by the Poisson distribution (mean and variance are equal, in this case, two). Clearly, as the variance increases, N_e decreases. Assumes sexes separate but have the same variance in recruitment; necessary to also consider when sexes do not have same variance and when individuals contribute to more than on generation - see additional references.

Dispersion of populations[3]	$N_e=4\pi\delta\sigma^2$	δ is the number of breeding individuals per unit area; σ^2 is the one-way variance of distance between birth and breeding sites	Neighbourhood size estimate may be appropriate for resident or territorial fish for which distribution may be uniform but would be inappropriate if spawning locations were isolated or for anadromous fish where dispersion would not follow a normal distribution. An alternate and perhaps more realistic view for salmonids would be to consider the dynamics of metapopulations - see additional references.
Overlapping Generations[4]	$N_e = N_a/G$	N_a is the annual effective size; G is the mean generation time in years	As most species have some component of overlapping generations (i.e., few species have discrete and uniform generation times), this is an important consideration in estimating effective population size. The N_e of a population with overlapping generations is the same as one with non-overlapping generations that has the same number of individuals entering the population each generation and the same variance in reproductive success.

[1]Wright 1938.

[2]Crow and Kimura 1970; see also: Crow and Denniston 1988, Hedrick et al. 1995, Lande and Barrowclough 1987, Rockwell and Barrowclough 1995.

[3]Hartl and Clark 1989; see also: Gilpin 1991, Caballero 1994.

[4]Hill 1972, 1979; see also: Lande and Barrowclough 1987, Jorde and Ryman 1995, Rockwell and Barrowclough 1995, Caballero 1994.

CHAPTER 2.

Low genetic variability in lake populations of brook trout: A consequence of exploitation?

INTRODUCTION

In addressing issues of importance to the conservation of populations, we are frequently most interested in understanding how recent anthropological activities affect a population's persistence in light of genetic, demographic, and environmental stochasticity (Lande 1993). Regarding the first of these, genetic variability is believed to be an important determinant of a population's long-term persistence in the face of changing environments (Frankel and Soulé 1981, Lande and Shannon 1996). The amount of genetic variation present in a population is influenced by mutation, migration, selection and genetic drift (Wright 1931). Of these, the relative influence of genetic drift is likely to dominate, although the role of selection to changed conditions and gene flow among populations could also be important. Understanding the factors that influence genetic diversity in natural populations is thus of fundamental interest to both conservation and evolutionary biologists.

In a previous study of genetic diversity among brook trout (*Salvelinus fontinalis*) populations in the Canadian Maritimes, I found heterozygosity (a metric of genetic variation) within 3 lake populations to be significantly lower than that within 30 stream populations (Jones et al. 1996). Included in this study were 3 streams from the same drainage basin as the lakes. In each of these stream populations, heterozygosity was approximately twice that of the lake populations. I had no reason a priori to expect such differences. More recently, a study consisting primarily of stream populations (Hébert et al. 2000) documented much higher population genetic diversity, based on microsatellites, than a study based on the same microsatellite loci but examining lake populations (Angers and Bernatchez 1998). If the difference in heterozygosity between lake and stream populations is general, it could reflect different demographic or mating structures affected by the two habitat types, or alternatively a fitness advantage of heterozygotes in

stream environments.

Although previous work has suggested that habitat may influence genetic variation in a population, these findings were tangential to the original objectives of those studies and the sampling protocols were not designed to specifically address the question of habitat-specific differences in heterozygosity. For example, Jones et al. (1996) sampled a disproportionate number of streams relative to the number of lakes and, for the lake populations examined, the adjacent stream was not always sampled. In the present study, I examine whether brook trout genetic variation differed within and among nine pairs of adjacent lake and stream populations. I then determine whether the habitat-based differences in heterozygosity are associated with specific physical or anthropological attributes of the lake and stream environments.

MATERIALS AND METHODS

Study Sites

Brook trout were sampled from Bennett Lake, Fundy National Park, its 3 inlet tributaries, and its outlet brook in 1996 (Figure 2-1; Table 2-1). The outlet brook, Bennett Brook, was sampled immediately below the Bennett Lake dam. Kelly Brook was sampled above a small, impassable waterfall (approximately 300 m upstream from the lake). Tracey Lake Brook was sampled between Bennett and Tracey lakes (approximately 700 m upstream from Bennett Lake). Unnamed Brook was sampled in two locations, 150-250 m and 600-700 m upstream from Bennett Lake. A 10 m long, 3/4" mesh gill net (set 2 consecutive nights) was used to sample the lake; the brooks were sampled by electrofishing.

For the present study, sampling was then conducted on additional lakes inside and outside Fundy National Park and, for each lake, an adjacent inlet or outlet stream was sampled within 0.5-1.0 km of the lake (Table 2-1). All lakes were sampled with a combination of fyke and gill nets, except Dick's Lake which was angled (previously studied Tracey and Wolfe lakes (Jones et al. 1996) were also sampled by angling). Nets in Laverty Lake were set overnight, while those in all lakes outside Fundy National Park were set during daylight hours and were monitored continuously. All streams, including previously sampled Laverty Brook and East Branch (Jones et al. 1996), were sampled by electrofishing. For all locations, I attempted to obtain fish from the same year class, specifically the 1+ age class, that is, fish in their second year of life. Sampling occurred during the summer and fall of 1996 in all systems except Henry, Pleasant and Wood lakes, which were sampled in the summer and fall of 1997. The lakes were small (2.5 to 61.9 ha) and shallow (1.5 to 15.2 m maximum depth; Table 2-1). Maximum lake depth and lake surface area data were obtained from Fundy National Park and from the New Brunswick Department of Natural Resources and Energy.

While some information on angling intensity, in the form of creel censuses, exists for some lakes, it does not exist for many. Furthermore, existing information was often not collected in a uniform manner nor was it always collected at the same time of year. I

used instead two indirect, objective metrics associated with angling intensity: lake access (public versus private) and minimum trail distance to each lake from the nearest all-season road. Three lakes (Dick's, Pleasant and Wood) are private with access limited to 10-20 fishing club members per lake (plus guests of members). The remaining lakes are open to the public (lakes in Fundy National Park require a National Park fishing license while all others, including private lakes, require a provincial fishing license; Wolfe and Tracey Lakes were closed to angling in 1995 and 1998, respectively). Shortest trail distance from each lake to an all-season road was estimated from topographical maps (series A 791; maps 21 H/5, /10, and /11; edition 3).

Genetic Analysis

My electrophoretic analyses of allozyme variability followed those described by Jones (1995) and Jones et al. (1996). Specifically, tissue samples from the eye, liver, and white muscle were collected from freshly killed fish and stored in liquid nitrogen. Upon arrival at the University of Guelph, tissues were transferred to an ultracold freezer (-80°C) until analysis. Ten of eleven loci previously found to be variable (Jones et al. 1996) were used in this study (*sAAT-4** (liver), *ADH** (liver), *sIDHP-1** (liver), *LDH-A2** (muscle), *LDH-B1** (eye), *LDH-B2** (eye), *sMDH-B1,2** (muscle), *mMEP-1** (muscle), *mMEP-2** (muscle), and *PGM-1** (muscle)); difficulties visualizing the relatively invariant *G3PDH-1** resulted in its exclusion. The protein products were resolved following the procedures described by Utter et al. (1974), Allendorf et al. (1977), and May et al. (1979) for 12% starch gels (*sIDHP-1**, *sMDH-B1,2**, *mMEP-1**, and *mMEP-2**) and by Hebert and Beaton (1989) for cellulose acetate gels (*sAAT-4**, *ADH**, *LDH-A2**, *LDH-B1**, *LDH-B2**, and *PGM-1**). The genetic nomenclature for the protein-coding loci followed that recommended by Shaklee et al. (1990). Allele designations followed those previously used which were, when possible, standardized to those published (see Jones et al. 1996). Genetic interpretation of observed gel band phenotypes are described elsewhere (Jones 1995).

Population Estimates

Mark-recapture experiments were conducted in Bennett, Wolfe, and Tracey lakes in this and previous work (see Table 2-2 for sample sizes and years). During these experiments, several box nets were moved to randomly assigned locations within each lake. Population sizes were estimated using the Schnabel method (Ricker 1975).

Statistical Analysis

Intrapopulation Analyses

Genotypic proportions were tested for agreement with Hardy-Weinberg expectations by chi-square goodness-of-fit, with Levene's (1949) correction for small sample size, using BIOSYS-1 (Swofford and Selander 1981). *PGM-1** was excluded from the analysis due to the presence of a null (φ) allele; it was not possible to distinguish between a non-φ allele homozygote (e.g., *100/100*) and a φ/non-φ allele heterozygote (e.g., φ*/100*). The *PGM-1** allele frequencies were determined assuming Hardy-Weinberg proportions (i.e., the frequency of the φ allele was calculated as the $\sqrt{}$(# φ/φ individuals in the population); the frequency of the non-φ allele as 1 - the above value).

Interpopulation Analyses

Mean observed heterozygosities were calculated for each population. Differences among population heterozygosity values were tested with the Kruskal-Wallis and Mann-Whitney tests, using MINITAB 12.2. *PGM-1** was again excluded from the analysis due to the null allele. Three individual fish were AABB at *sMDH-B1,2**. I conducted the analyses treating these individuals both as double heterozygotes and as homozygotes at two separate loci.

Associations between heterozygosity differences in adjacent lake and stream populations and various physical and anthropogenic factors (see Table 2-1) were examined. Fish sampled immediately adjacent to each lake (i.e., within 250 m of the lake) were excluded from this analysis. Thus, the stream heterozygosity value used for Bennett Lake was the mean of the Kelly Brook and the upper sample of Unnamed Brook observed heterozygosity values. Tracey Lake and Bennett Lake were treated as independent systems even though they are part of the same tributary; there is little

evidence of movement of Bennett Lake fish beyond 200 m upstream in any tributary and there are small barriers to movement such as beaver dams throughout Tracey Lake Brook (M.W. Jones, unpublished data). Furthermore, the genetic data indicate both lake populations differ significantly from that in Tracey Lake Brook.

To partition genetic variation among sampling sites, I constructed a dendrogram, using the neighbour-joining method (Saitou and Nei 1987) with Cavalli-Sforza and Edwards' (1967) chord distance. The genetic distances were calculated with BIOSYS-1 and were used by MEGA (Kumar et al. 1993) to produce the neighbour-joining dendrogram. To determine if significant heterogeneity across loci existed between adjacent populations or groups of populations in the dendrogram, hierarchical G-tests were performed using G-Stat (Siegismund 1993).

RESULTS

Two of the ten loci previously found to be variable throughout Maritime populations of brook trout (Jones et al. 1996) were invariant in this study (*LDH-A2** and *mMEP-2**). The remaining eight were variable (common allele <95%) in at least 2 populations (Table 2-3).

Intra-population Genetic Variation

There were significant deviations from expected Hardy-Weinberg genotypic proportions in 8 of the 116 tests conducted for the 21 sites in this study. This was slightly greater than the 5.8 significant deviations that would be expected by chance at the 0.05 significance level. When significance levels were adjusted to account for multiple testing (Bonferroni procedure (Rice 1989)), 3 remained significant. One instance involved a single individual heterozygotic for two rare alleles at *sIDHP-1** (*100/120*) in Dick's Lake and was thus likely a result of chance sampling. The other two deviations both occurred in Camp Brook, and both were heterozygosity deficiencies, one at *LDH-B1** and the other at *mMEP-1** . Mis-genotyping could account for this observation, although these banding patterns have been previously interpreted in this manner without such deviations and these deviations were observed at two independent loci. Alternatively, the fish sampled over a 100-200 m length of stream may have originated from two separate breeding groups (Wahlund effect) or were the offspring of few spawning pairs; either possibility could bias genetic relationships among populations but would have little consequence on subsequent heterozygosity analyses.

Inter-population Genetic Variation

Heterozygosity Differences Between Lakes and Adjacent Streams

The heterozygosity of trout sampled from streams adjacent to Bennett Lake (including Tracey Lake Brook) was higher than that of trout in Bennett Lake, although there were differences in the extent of the variation; the values for Kelly Brook and Tracey Lake Brook differed significantly from that of Bennett Lake (Table 2-1; Figure 2-2). The outlet (Bennett Brook) was sampled immediately downstream from Bennett Lake and the lower sample from Unnamed Brook was collected where spawning lake fish were later

observed. The heterozygosity of trout in both locations differed very little and not significantly from that of Bennett Lake. The heterozygosity of upper Unnamed Brook trout was higher than that of the lower sample but it too did not differ significantly from that of Bennett Lake trout.

Overall, heterozygosity within lake populations was lower than that of their adjacent stream population in 8 of 9 cases ($p = 0.017$, paired t-test using observed population heterozygosities); only the population in Laverty Lake had higher heterozygosity than that of the population in its adjacent stream. Not all differences in heterozygosity between paired lake and stream populations were significant (Mann-Whitney test, using individual heterozygosity values; see Figure 2-2). The results were not affected by the designation of AABB individuals at *sMDH-B1,2** as either double heterozygotes or double homozygotes; only 3 such individuals existed (2 in Laverty Lake and 1 in Livingstone Lake). Among all Maritime populations currently and previously examined (i.e., including data from Jones et al. 1996 but excluding populations for which $n < 20$), lake population heterozygosity levels were lower ($p = 0.037$, t-test using observed population heterozygosity values) although they did not always have the lowest observed heterozygosities (Figure 2-3). There was significant heterogeneity in population heterozygosity levels, both among all populations as well as among all lake and among all stream populations (Kruskal-Wallis test, $p < 0.001$ in all cases).

Population Heterozygosity

There was no relationship between heterozygosity differences between adjacent lake and stream populations and either lake surface area (analyses were conducted both including and excluding Laverty Lake because the heterozygosity for this population may be an artifact of other factors (see Discussion): including Laverty Lake and Brook pair, $r^2 = 0.00$ and $p = 0.956$; excluding Laverty $r^2 = 0.11$ and $p = 0.430$) or maximum lake depth (including Laverty Lake and Brook pair, $r^2 = 0.06$ and $p = 0.511$; excluding Laverty $r^2 = 0.00$ and $p = 0.983$). Private lakes had smaller differences in observed heterozygosity with their adjacent streams than public lakes. This association was significant when Laverty Lake was excluded from the analysis (including Laverty Lake and Brook pair $p =$

0.140, excluding Laverty $p = 0.029$; Figure 2-4a).

Trout inhabiting lakes closer to all-season roads tended to have greater differences in observed heterozygosity with their adjacent streams than those in lakes further away; this relationship was significant when Laverty Lake was included ($r^2 = 0.52$ and $p = 0.029$) but not when it was excluded ($r^2 = 0.48$ and $p = 0.058$; Figure 2-4b). When the public and private lakes were analysed separately, this relationship appeared stronger within the public lakes (including Laverty Lake and Brook pair, $r^2 = 0.90$ and $p = 0.004$; excluding Laverty, $r^2 = 0.77$ and $p = 0.052$). The effect of distance was diminished and there was little power in the private lakes analysis ($r^2 = 0.95$ and $p = 0.138$; Figure 2-4b).

Gene Flow Between Lake and Stream Populations

Adjacent lake and stream populations tended to either cluster together or to be associated within the same major grouping (Figure 2-5), suggesting some degree of gene flow between adjacent lake and stream sites. However, all lake and adjacent stream populations differed significantly in allele counts (hierarchical G-tests), suggesting that any contemporary gene flow is limited. During the 1996 spawning season when over 25% of the fish of spawning size in Bennett Lake were marked, none were found further than 200 metres upstream in either Unnamed Brook or Tracey Lake Brook during electrofishing surveys.

Lake Population Sizes

Mark-recapture studies have been conducted for 3 of the lakes in Fundy National Park (Table 2-2). All studies used similar methodology. At both times of year, young-of-the-year were excluded from the population size estimates. However, fish that would be excluded in an autumn estimate would be included in the spring. Thus, the spring 1981 population size estimate for both Bennett and Wolfe Lakes being approximately 3 times larger than subsequent autumn estimates likely only reflects a seasonal shift in year class abundances. The spring 1981 population size estimate for Tracey Lake is likely similarly inflated. However, even accounting for a threefold over-estimate, the Tracey Lake brook trout population was clearly much lower in the autumn of 1997 than is was in 1981.

DISCUSSION

The present study suggests that heterozygosity within lake populations of brook trout may often be less than that of adjacent stream populations. These results suggest that the factors influencing trout population heterozygosity differ between lake and stream habitats. The study populations probably originated from the same glacial refugium less than 10,000 years ago (Danzmann et al. 1998), suggesting that the factors influencing genetic variation in these populations are limited to selection, migration and random genetic drift; the absence of any alleles in the brook trout in this study not seen elsewhere in their range supports the contention that mutation at these loci is not a factor influencing genetic variation in these populations. My work also suggests that anthropological activity, specifically exploitation, may be the most parsimonious explanation for the observed difference in heterozygosities between lakes and adjacent streams.

Population Fluctuations and Genetic Variation

While lake populations tended to have lower heterozygosity than their adjacent stream populations, there was still significant heterogeneity among population heterozygosity levels within habitat types. Such differences are not unexpected because historical factors such as recolonization events and drainage size will differ among populations, underscoring the importance of the use of a paired lake/stream sampling design. It is thus the degree of difference in observed heterozygosity levels between lake and adjacent stream pairs that is important to understand.

Previous population sizes for trout inhabiting the lakes in Fundy National Park have been large, i.e., several thousand for Bennett, Wolfe and Tracey Lakes. However, despite these high population sizes, these lake populations have experienced dramatic short-term declines. In 1974, over 5000 brook trout, close to the 1981 spring population size estimate, were found dead in Wolfe Lake after ice break-up (Hoar 1981). Similarly, dead and dying fish observed in Tracey Lake in the summer of 1997 were associated with a population estimate of less than 400 trout that same autumn. Although I do not know whether such dramatic population declines are common to all lakes, such declines alone will not necessarily result in great losses of genetic variation in a population.

Nei et al. (1975) examined the consequences of severe population bottlenecks (effective population sizes, N_e, declines from 4 X 10^6 to 10 and 2 individuals) followed by different rates of population increase r = 1.0 and 0.1). They found that while rare alleles will be lost, the decline in heterozygosity would be slight if the population size increases rapidly, e.g., a bottleneck of N_e = 10 with r = 1 resulted in a decline in population heterozygosity of only 8%. However, at a slower rate of population increase r = 0.1), 41% of the population heterozygosity was lost. Thus, in the event of a single population bottleneck, the rate at which population size increases is critical in determining the loss heterozygosity in a population.

Bottlenecks in my study lakes may be of a similar order of magnitude as those modelled by Nei et al. (1975). For example, the actual number of fish remaining in Tracey Lake following the summer of 1997 was 400. Frankham (1995) estimated the ratio of effective to actual population size in wildlife populations to be approximately 0.10. Thus, an approximate bottleneck N_e for Tracey Lake, based on the 1997 data, would be 40. The extent to which heterozygosity would be lost from Tracey Lake would depend on the maximum rate of increase for the population (r). Although r is unknown for these lake populations, it is likely to be closer to 0.1 than 1.0. Exploitation would have the additional effect of suppressing any natural population increase or even further reducing the population. This would result in increased number of generations at low population size which would cause a greater loss of heterozygosity than Nei at al.'s (1975) single bottleneck event model would predict. Thus, natural population bottlenecks, compounded by exploitation may be responsible for the low heterozygosity detected in some of my lake populations.

Population Structure, Gene Flow and Genetic Variation

It has been hypothesised that stream populations may be comprised of many sub-populations, interconnected by some level of gene flow, potentially maintaining greater genetic variation than that in a single panmictic population (Hébert et al. 2000). In general, spawning locations for brook trout do appear to be more limited in lakes than in streams (e.g., Ridgway and Blanchfield 1998), potentially decreasing the possibility of

such a population structure in lakes. Hébert et al. (2000) also hypothesised that stream environments may be more stable than that of lakes. However, while my results further support the observations that lake populations of brook trout have lower genetic variation than stream population, they also suggests that an additional factor, anthropological exploitation, may have the greatest influence on the degree to which lake populations loose genetic variation.

Selection and Genetic Variation in Lake and Stream Populations

Several studies have examined associations between individual heterozygosity and components of fitness in salmonids. While some have documented positive associations, many have not. Interestingly, many of the former studies were reported for non-natural populations. Thus, positive associations between fitness traits and heterozygosity could have reflected higher fitness among those more outbred individuals (i.e., hybrid vigour) versus those crossed within inbred lines. By contrast, within five unexploited brook trout populations in the wild, Hutchings and Ferguson (1992) could find no associations between individual heterozygosity and several life history traits. Similar independence between heterozygosity and fitness-related characters has been documented for wild populations of wood frog (*Rana sylvatica*; Wright and Guttman 1995) and Scots pine (*Pinus sylvestris*; Savolainen and Hedrick 1995). Even if selection should favour individual heterozygosity in natural populations, it is unclear why this would be more prevalent in stream than lake populations, or why such selection should be stronger in some lakes than in others.

Laverty Lake Exception

While most lakes had slightly or greatly lower heterozygosities relative to their adjacent streams, one, Laverty Lake, did not. Given this lake's small size and shallow depth, it likely has the smallest population size of all the lakes studied, making this result surprising. Notably, Laverty Lake trout possess alleles that occur in half or less of the populations (i.e., the *100* allele at *sIDHP-1**, the *72* allele at *LDH-B1**, the *275* allele at *LDH-B2**, both the *80* and *120* alleles at *sMDH-B1,2**, and the *140* allele at *PGM-1**; Table 2-3). Laverty Lake trout, with those in Dick's Lake and Lake Brook, have the

greatest number of alleles across all loci. Many of these less common alleles also occur downstream in Laverty Brook, but at lower frequencies. Such findings suggest the possibility of introgression of external alleles.

Laverty Lake, like many of the other lakes in this study, has previously been stocked (18,700 brook trout between 1962 and 1977 (Jones 1995)). Stocked brook trout generally have much poorer survival relative to their wild counterparts (e.g., Fraser 1989 and references therein). Similarly, genetic studies have found little or no evidence of reproductive success of hatchery brook trout in the wild (Danzmann and Ihssen 1995, Jones et al. 1996). However, when hatchery brook trout are stocked in the absence of competitors (inter- or intra-specific), they can experience higher growth and survival (Lachance and Magnan 1990), which may lead to increased reproductive success. The lakes in Fundy National Park can experience extreme population declines. If such declines occurred in the smaller and likely more unstable Laverty Lake between 1962 and 1977 while it was being stocked, hatchery fish may have experienced higher than typical levels of reproductive success. This would result in the introgression of hatchery alleles and would artificially increase the genetic variation in the population. The prevalence of the less common alleles, and their more frequent occurrence in Laverty Lake than downstream in Laverty Brook, provides support for this hypothesis. However, the general observations and conclusions drawn in this study do not depend on acceptance of this hypothesis.

Implications

The enzyme polymorphisms detected at the loci used in this study are not likely to be of great importance to fitness by themselves. Of import is the high probability that the observed variation reflects the level of variation found within a population. Despite brook trout lake population sizes typically being high, lake populations with easy public access have lower than expected heterozygosity (i.e., lower than their adjacent stream population). A plausible explanation is, following a natural mortality event, angling pressure further decreases a lake's population size and causes it to increase very slowly. Prolonged low effective population sizes, which would occur after a natural mortality

event followed by continued angling pressure, could result in the lower than expected levels of genetic variation observed in these populations. Because prolonged low effective population sizes can have implications to a population's long-term sustainability, managers of small lakes should be cautious in allowing any exploitation and, most importantly, should eliminate all anthropological sources of mortality at the first indication of higher than normal natural mortality. Furthermore, my findings underscore the importance of allowing populations which have recently experienced declines to increase in numbers as rapidly as possible.

Table 2-1. Brook trout lake-stream population pairs, sample sizes (*n*), lake latitudes, longitudes, surface areas, maximum depths, type of access and shortest trail distance from lakes to an all-season road.

					Lake Characteristics						
Population pair	**Lake**	***n***	**Stream**	***n***	**Latitude**	**Longitude**	**Drainage**	**Area (ha)**	**Maximum depth (m)**	**Access**	**Nearest all-season road**
A	Bennett	75	i) Unnamed Bk-U	40	45:38:00	65:04:40	Pt. Wolfe R	24.4	9.7	public	10
			ii) Kelly Bk	44							
			iii) Unnamed Bk-L	46							
			iv) Bennett Bk	43							
B	Dick's	41	Felix Bk	44	45:33:00	65:26:00	Big Salmon R	28.7	9.8	private	5200
C	Henry	32	Porter Bk	44	45:24:30	65:36:50	Hammond R	23.9	14.6	public	2200
D	Laverty	44	Laverty Bk[1]	62	45:39:05	65:02:15	Upper Salmon R	2.5	1.5	public	4300
E	Livingstone	41	Lake Bk	45	45:39:20	64:55:40	Upper Salmon R	9.7	3	public	600
F	Pleasant	28	Camp Bk	44	45:39:20	65:15:30	Kennebecasis R	27.1	4.1	private	5600
G	Tracey[1]	22	Tracey Lake Bk	45	45:39:05	65:04:20	Pt. Wolfe R	6.6	2.1	public[2]	3000
H	Wolfe[1]	44	East Branch[1]	38	45:39:00	65:08:20	Pt. Wolfe R	21	8.5	public[3]	10
I	Wood	41	Mosher River	45	45:24:45	65:35:00	Moser R	61.9	15.2	private	10

[1] genetic data from Jones et al. (1996); [2] closed to angling from 1998 to present; [3] closed to angling from 1995 to present.

Table 2-2. Brook trout population sizes estimates (*n*, 1+ and older) based on mark-recapture studies for 3 lakes in Fundy National Park.

Lake	Year	Season	*n*	95% C.I.	Marked	Recaptured	Study
Bennett	1981	spring	21545	19312-24196	3201	302	Hoar 1981
	1985	autumn	10203	6093-20709	403	10	Anon. 1985
	1996	autumn	7111	6324-8056	1852	262	present
Wolfe	1981	spring	6700	5767-7883	1362	157	Hoar 1981
	1985	autumn	2131	1408-3616	266	17	Anon. 1985
Tracey	1981	spring	6154	5672-6701	1888	539	Hoar 1981
	1997	autumn	383	306-493	179	26	present

Table 2-3. Allelic frequencies, excluding the *100* (*-100* for *ADH**) allele, of the eight variable loci in brook trout populations in this study.

	*sAAT-4**		*ADH**		*sIDHP-1**			*LDH-B1**	*LDH-B2**	*sMDH-B1,2**		*mMEP-1**	*PGM-1**	
Site	**133*	**170*	**0*	*-*205*	**120*	**140*	**152*	**72*	**275*	**120*	**80*	**0*	**φ*	**140*
Bennett L	0.060	0.827	0.873	0.000	0.000	1.000	0.000	0.000	0.047	0.000	0.000	0.993	0.611	0.021
Unnamed Bk Up	0.025	0.837	0.863	0.000	0.038	0.962	0.000	0.025	0.000	0.013	0.000	0.913	0.500	0.021
Kelly Bk	0.125	0.784	0.591	0.000	0.000	1.000	0.000	0.000	0.011	0.000	0.000	0.977	0.693	0.000
Unnamed Bk Low	0.022	0.837	0.848	0.000	0.011	0.989	0.000	0.033	0.011	0.011	0.000	0.989	0.587	0.043
Bennett Bk	0.023	0.814	0.802	0.000	0.000	1.000	0.000	0.000	0.012	0.006	0.000	1.000	0.663	0.047
Dick's L	0.366	0.220	0.805	0.000	0.024	0.951	0.000	0.061	0.012	0.000	0.055	0.756	0.390	0.073
Felix Bk	0.352	0.636	0.830	0.000	0.000	1.000	0.000	0.091	0.068	0.000	0.011	1.000	0.341	0.000
Henry L	0.703	0.250	0.375	0.000	0.016	0.969	0.016	0.016	0.000	0.016	0.000	0.750	0.469	0.000
Porter Bk	0.591	0.261	0.534	0.000	0.000	0.966	0.034	0.091	0.011	0.011	0.000	0.557	0.545	0.045
Laverty L	0.114	0.739	0.693	0.000	0.080	0.920	0.000	0.011	0.023	0.051	0.017	0.943	0.534	0.068
Livingstone L	0.244	0.707	0.561	0.012	0.000	0.988	0.012	0.012	0.024	0.016	0.000	0.750	0.469	0.000
Lake Bk	0.233	0.567	0.389	0.000	0.089	0.900	0.011	0.000	0.011	0.006	0.011	0.744	0.696	0.011
Pleasant L	0.714	0.214	0.768	0.000	0.018	0.929	0.000	0.089	0.000	0.000	0.000	0.321	0.625	0.000
Camp Bk	0.455	0.443	0.625	0.000	0.000	0.989	0.000	0.091	0.080	0.000	0.000	0.739	0.648	0.011
Tracey Lake Bk	0.167	0.600	0.811	0.000	0.033	0.967	0.000	0.000	0.000	0.006	0.000	1.000	0.600	0.022
Wood L	0.402	0.427	0.963	0.000	0.000	1.000	0.000	0.220	0.000	0.000	0.006	0.756	0.159	0.000
Mosher River	0.453	0.442	0.953	0.000	0.012	0.988	0.000	0.174	0.105	0.000	0.000	0.640	0.191	0.000

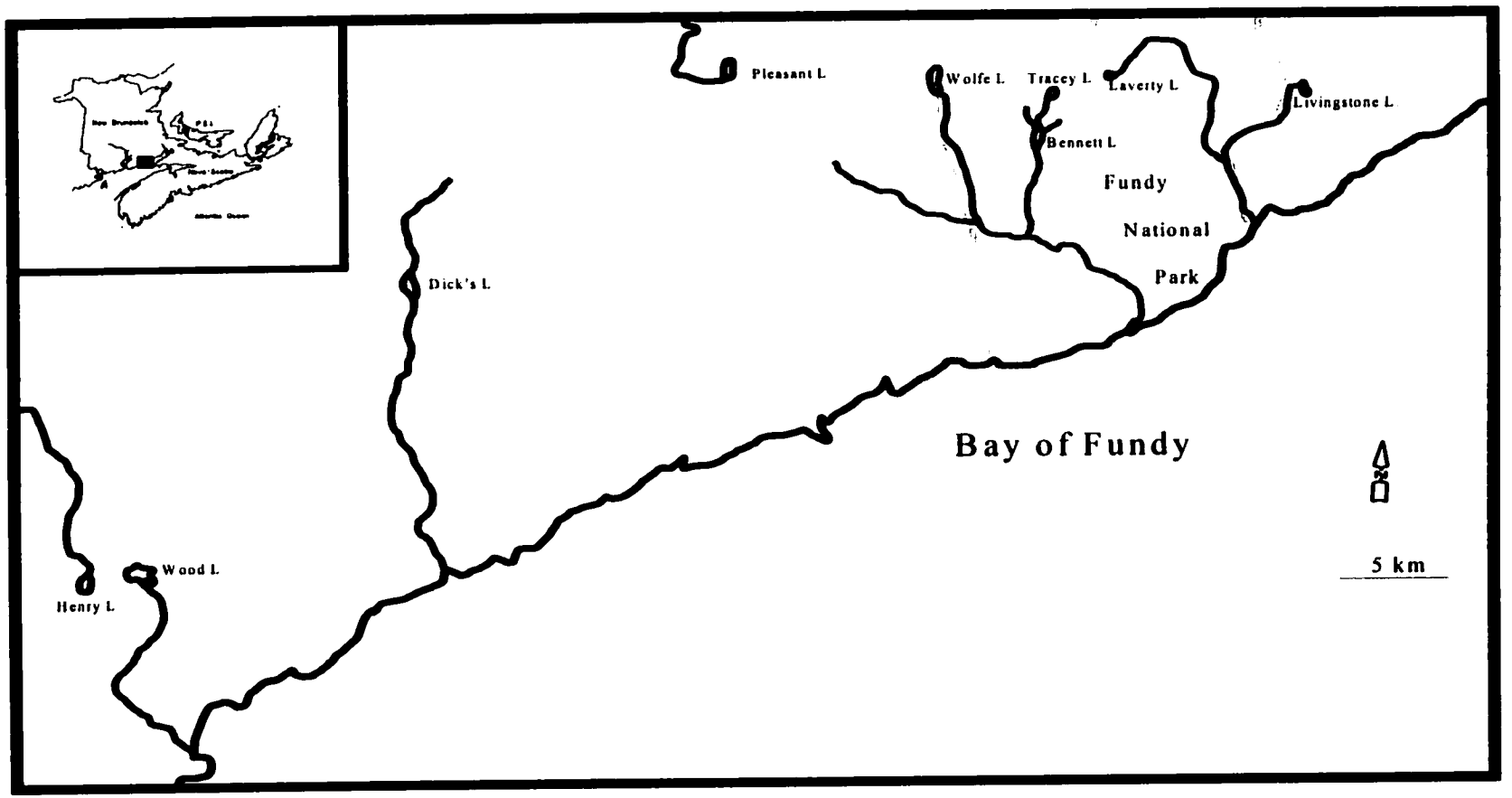

Figure 2-1. Locations of lakes sampled for brook trout; adjacent streams were sampled for each lake. Darkened area of inset indicates enlarged area.

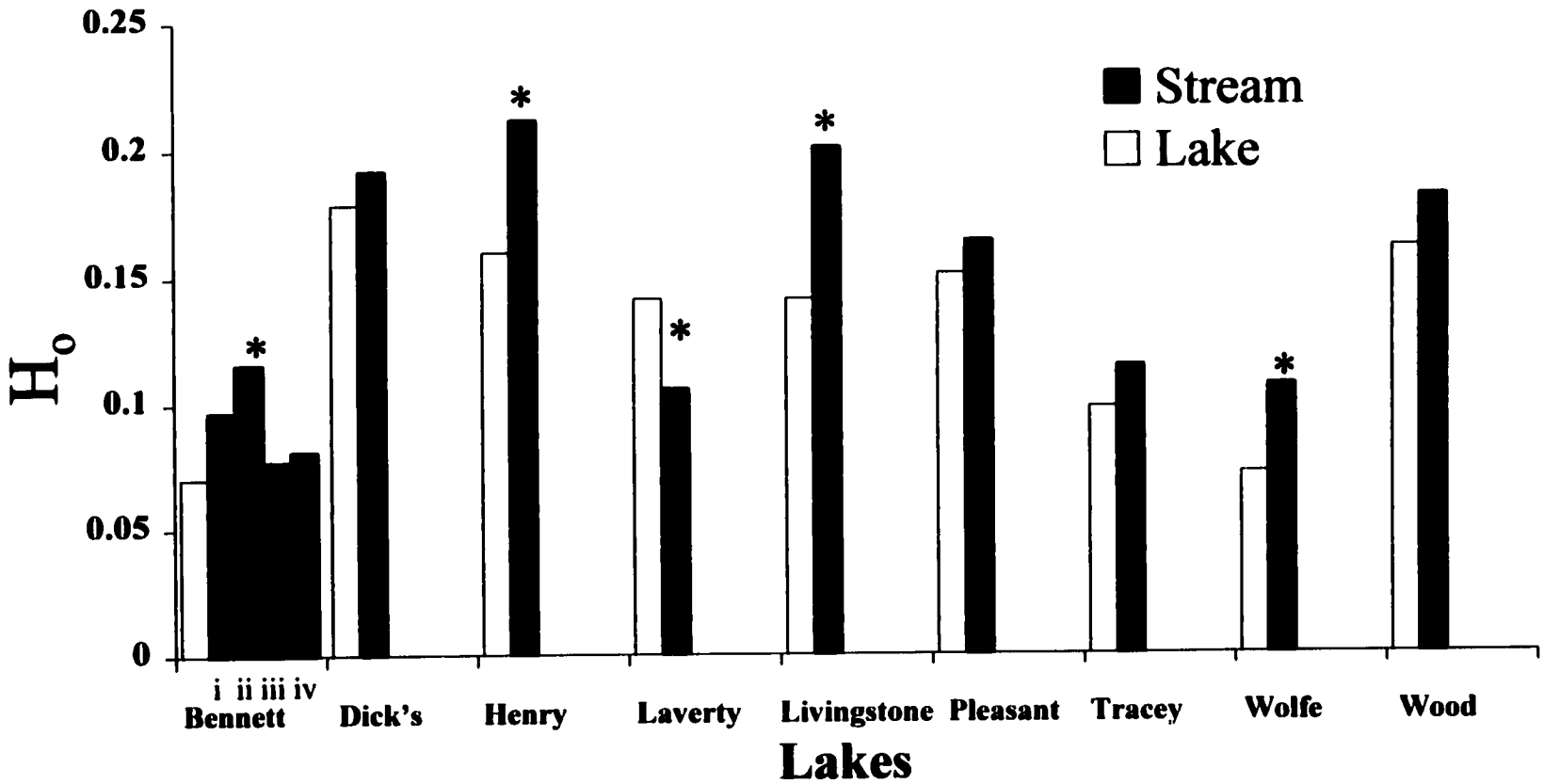

Figure 2-2. Observed heterozygosity (H_o based on 9 loci) for paired lake and stream brook trout populations (see Table 2-1 for stream names). Streams with an asterisk (*) differed significantly, based on the Mann-Whitney test, in heterozygosity levels with their associated lake.

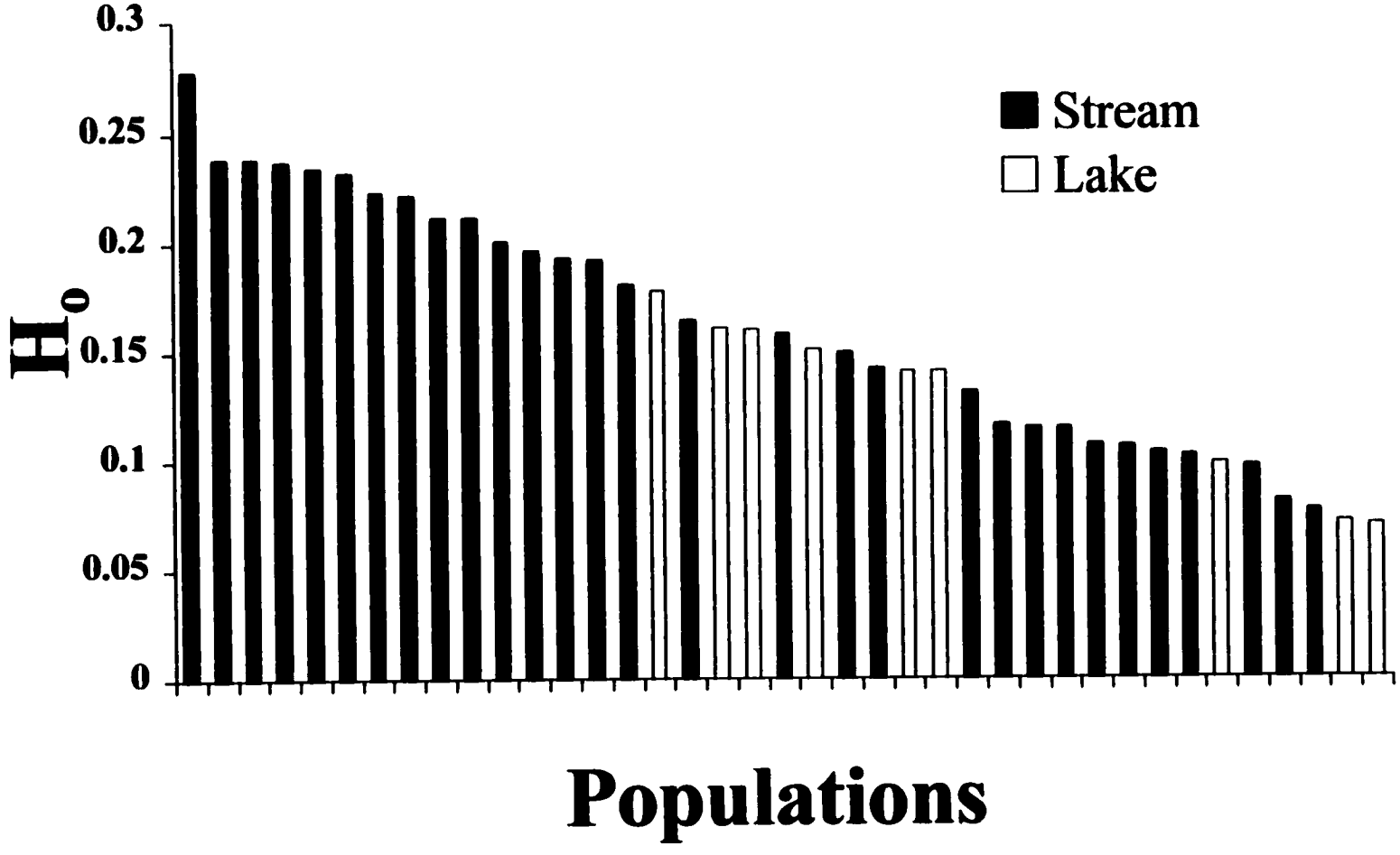

Figure 2-3. Observed heterozygosity (H_o based on 9 loci) for Canadian Maritime brook trout populations (all populations from this study and those from Jones et al. (1996) with $n \geq 20$).

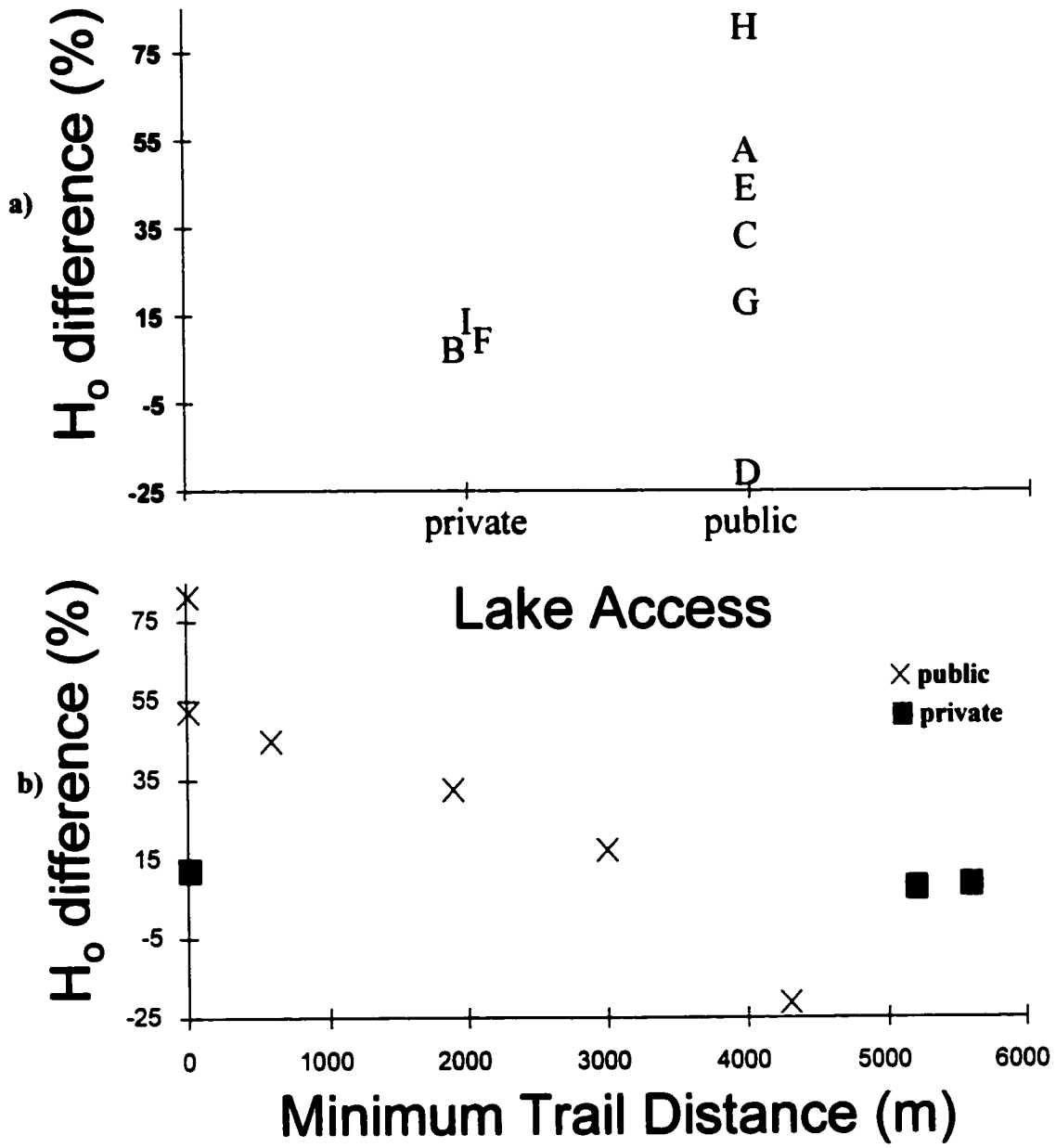

Figure 2-4. Percent observed heterozygosity difference between lakes and their adjacent streams ((stream H_o - lake H_o)/(lake H_o)*100) versus a) lake access (public or private), and b) minimum trail distance to an all-season road. Letters in a) refer to population pairings defined in Table 2-1.

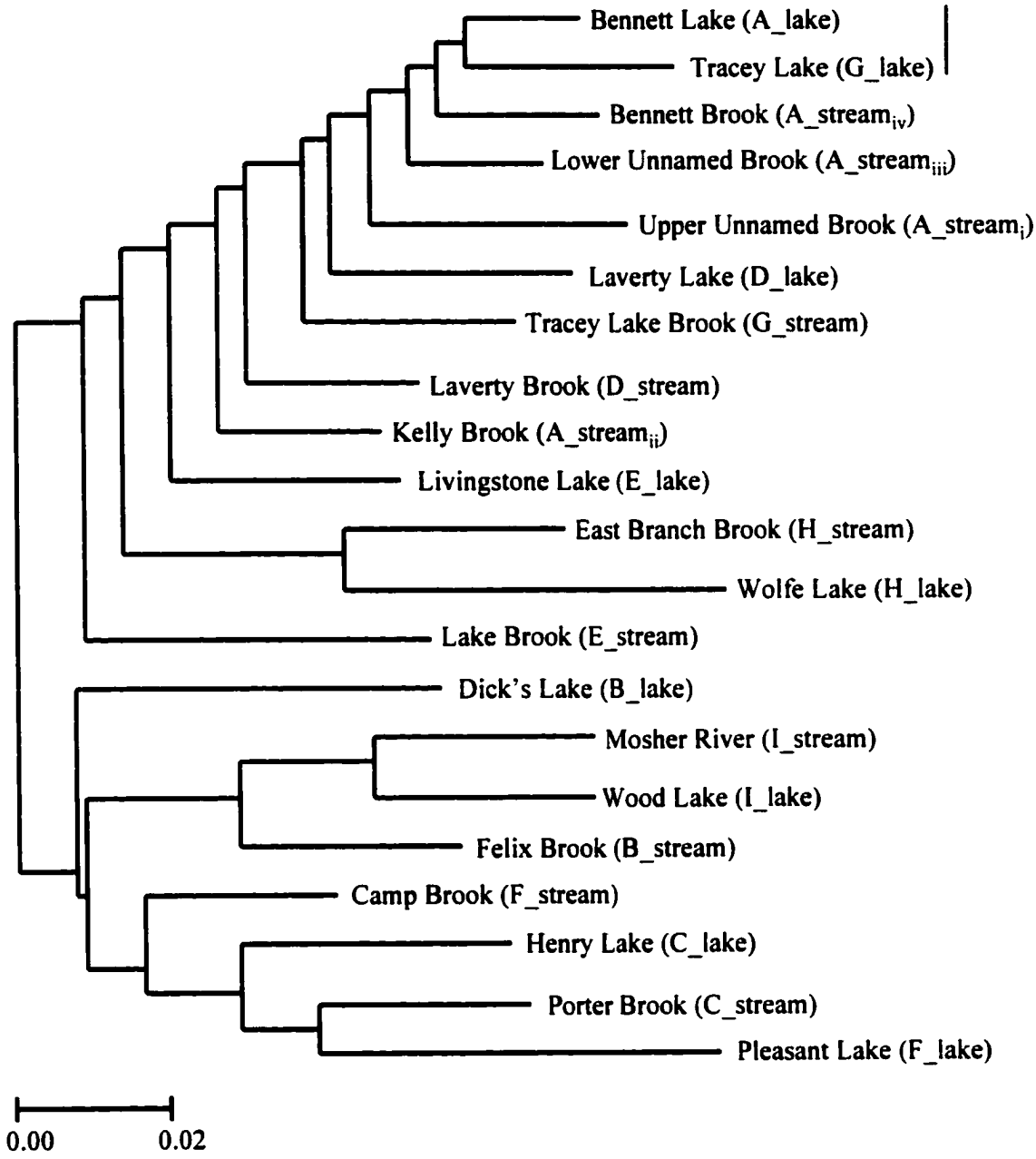

Figure 2-5. Neighbour-Joining dendrogram of the allozyme genetic distance (Cavalli-Sforza and Edwards 1967) calculated among brook trout lake and stream populations. Genetic distances are additive according to the scale shown and are based on 10 loci. Letters in parentheses refer to population pairings defined in Table 2-1. Populations joined by vertical lines do not differ significantly ($\alpha = 0.05$) in allele counts (hierarchical G-tests).

CHAPTER 3

The influence of male parr body size and mate competition on fertilization success and effective population size in Atlantic salmon

INTRODUCTION

Atlantic salmon, *Salmo salar*, have variable life histories. A salmon's life cycle can consist of a freshwater, parr component and a period at sea followed by a return to that salmon's natal river to spawn. Alternatively, some males forego the marine component prior to maturation. Those males that mature in fresh water later can smoltify and become anadromous while others remain in fresh water and later re-mature (Hansen et al. 1989). Parr that previously matured are believed to experience higher mortality than non-maturing parr in fresh water (Hutchings and Myers 1994) and later at sea (Berglund et al. 1992). Notwithstanding a strong environmental influence (e.g., Prévost et al. 1993), there is evidence of a heritable component to male parr maturation (e.g., Herbinger and Newkirk 1990 and references therein). This variation in salmonid male maturation may have evolved as a mixed evolutionarily stable strategy maintained by negative frequency-dependent selection (Gross 1985, Hutchings and Myers 1994).

Mature male parr must compete with larger anadromous males for access to anadromous females for fertilization opportunities. Studies examining the reproductive success of mature male parr have demonstrated that mature male parr, as a group, are successful in fertilizing some eggs (Hutchings and Myers 1988, Jordan and Youngson 1992, Morán et al. 1996, Thomaz et al. 1997). But, given that selection acts at the level of the individual, factors associated with individual reproductive success are also of interest. During spawning, mature male parr are arranged in a hierarchical manner with the largest male nearest the female (Myers and Hutchings 1987). A recent study on mature male parr reproductive success documented a positive relationship between individual parr size and reproductive success when six parr were competing with one anadromous male for fertilization opportunities with one anadromous female (Thomaz et al. 1997). The cost of maintaining this improved access to the female may increase with

larger numbers of competitors to a point at which body size has no influence on fertilization success (Hutchings and Myers 1994).

Reproductive success by mature male parr can have implications for a population's effective size (L'Abée-Lund 1989, Morán and García-Vázquez 1998), subsequently altering the effective rate of gene flow among populations (Morán et al. 1996). The effective number of males in a population can be estimated if individual male reproductive success is known (e.g., Lande and Barrowclough 1987). Previous studies on Atlantic salmon reproductive success were unable to quantify individual reproductive success either due to the limitation of the genetic markers at the time (e.g., Hutchings and Myers 1988), or, due to unsuitable sample sizes, were unable to attempt to quantify the influence parr success has on the effective population size (e.g., Thomaz et al. 1997, Martinez et al. 2000). In contrast, the extensive variation found within and among loci, the minimal tissue requirements, and the relative ease of the analysis render microsatellite loci highly amenable to parentage experiments (O'Reilly and Wright 1995, O'Connell and Wright 1997). This technique permits larger numbers of individuals to be used in experiments, increases the scope of replication, and enables one to quantify individual reproductive success at sample sizes suitable for determining the influence of mature parr reproductive success on the effective number of males.

The objectives of this study were thus two-fold. Firstly, I tested the effects of parr body size and intensity of mate competition on individual reproductive success, and secondly, I quantified the effect mature male parr can have on the effective number of males. Both of these objectives were addressed by using highly variable microsatellite loci to assign paternity.

MATERIALS AND METHODS

Field experiment

Experiments were undertaken in outdoor flow-through raceways at the Margaree Fish Hatchery, Nova Scotia, Canada (46°21'N, 60°58'W), from October 31 to December 9, 1996. The raceways (14.6 m X 1.2 m) were divided equally into four sections using a barrier of 0.9 mm square vexar mesh in a wooden frame. Substrate suitable for spawning was added to a depth of 30 cm. Running ripe mature male parr were electrofished from nearby tributaries of the Margaree River and held in the hatchery facility for one week prior to the experiments. Anadromous males were obtained from the fish trap on Lake O'Law River, the largest of Margaree River's tributaries. Females were obtained by seining the Margaree River.

We established thirteen single anadromous pair experimental treatments with varying numbers of parr (3 replicates of 3 parr and two replicates of 5, 7, 10, and 20 parr) of different sizes (range 81-123 mm). I created this number of treatments to have replication over the wide range of number of parr that typically attend spawning events in the wild as well as in anticipation of not having all treatments being successful. The parr were divided into 3 size classes: 81-90 mm (84.9 ± 2.7 mm), 91-110 mm (97.9 ± 5.7 mm), and 111-123 mm (116.9 ± 3.7 mm). Parr were placed in each section prior to the addition of the anadromous salmon. Each treatment was randomly assigned to a section in the raceways (see Figure 3-1 for treatment placement, details on anadromous salmon lengths, and number of parr from each size class in each treatment). After all spawning was complete in December 1996, each fish was measured, weighed and sampled (fin tissue) for DNA analysis. Eyed embryos were collected from two or three randomly chosen nests from each treatment in March, 1997. Parr and embryo collection was facilitated by the ability to lower the water levels in the raceways.

Unintentional fish movement occurred when the anadromous fish were placed in their sections prior to the covering of the enclosures with netting. Additionally, in one case, an anadromous female broke through the vexar partition, making future parr movement between sections possible. To prevent further disturbance to the fish, the

netting was not nailed down as originally planned.

Genetic Analysis

DNA was extracted from all potential parents and from 60 offspring from each of 2 or 3 nests per treatment. Each dissected embryo head or small sub-sample of fin tissue (approximately 50 mg) was digested in 100 µl of eyeball buffer (10 mM Tris, 50 mM KCl, 0.5% tween20) and proteinase K (0.1-0.4 µg) in a 500 µl tube and incubated for four hours to overnight at 45-55°C. Samples were vortexed 2-3 times during this digestion. Samples were heated at 94°C for 5-10 minutes to kill the proteinase K, frozen at -80°C (for times varying from overnight to several months), thawed, centrifuged at 14000 rpm (Eppendorf microcentrifuge) for 5 minutes, and then diluted 100X. Two µl of this DNA (approximate concentration 3-300 ng) was used for microsatellite analysis which was carried out using the hyper-variable loci developed and described by O'Reilly et al. (1996). Specifically, the tetranucleotide loci *Ssa171*, *Ssa197*, and *Ssa202* were examined on all samples. In addition, the dinucleotide locus *Ssa85* was examined in larger treatments or to distinguish between 2-3 potential fathers. In the few cases where these loci proved insufficient (14 of 1309 embryos genotyped, 4 from section 10, 1 from section 13, and 9 from section 7; see Figure 3-1 and Table 3-1 for section designations), additional loci were employed until paternity could be unambiguously established. In descending order, these dinucleotide loci were *Ssa12* (O'Reilly 1997), *Omy105*, and *Omy38* (Heath et al., accepted). To prevent any bias in the samples, microsatellite analysis was repeated on all individuals that had partial genotype information but for which parentage could not be assigned. No further attempts were made to genotype those embryos that had not amplified at any locus if the number of embryos from a nest that could be assigned parentage was greater than 50.

Data Analyses

Gene diversity at each locus in salmon from the Margaree River (sample sizes were 157, 166, 168, and 131 salmon for *Ssa202*, *Ssa197*, *Ssa171*, and *Ssa85*, respectively) was calculated as the heterozygosity expected under Hardy-Weinberg equilibrium, using TFPGA (Miller 1997). Paternity was determined by first identifying the maternal allele

at each locus (when possible) and then using the composite multilocus genotype to identify the father. In more complex treatments (e.g., when two females were present or when several males shared alleles at several loci), the program PROBMAX (Danzmann 1997) was used to determine parentage. This also allowed manipulation of the data set to identify potential mis-genotyping (see PROBMAX program manual).

In one treatment (section 11), 25 embryos could not be assigned fathers. After subtracting the maternal alleles, there were four putative paternal alleles at *Ssa202* and three at each of *Ssa171* and *Ssa197*. Alleles at *Ssa171* and *Ssa197* were clustered with each unique allele from *Ssa202* in which they co-occurred in the same embryo. Two clear groups emerged, each representing a presumably unsampled father. With two new putative paternal multilocus genotypes determined, the parentage analysis was rerun and paternity successfully assigned. In another treatment (section 13), all offspring were triploid, containing the maternal genotype and an additional allele at some or all loci. At those loci having only the maternal genotype, the paternal allele was assumed to be either one of the maternal alleles and paternity was determined treating either allele as possible.

Estimating $N_{e\ males}$

Following Lande and Barrowclough (1987), the effective number of males in each treatment was calculated as:

$$N_{em} = (N_m \overline{k_m} - 1) / [\overline{k_m} + (\sigma^2_{km} / \overline{k_m}) - 1]$$

where N_m is the actual number of males, k_m and σ^2_{km} represent the number of offspring and associated variance, respectively, produced by an individual in its lifetime. I used the estimated value of individual fertilization success as a proxy for lifetime success both from each nest individually as well as for the entire treatment, the latter by weighting each nest equally.

RESULTS

The number of anadromous fish and parr found in each section after spawning had been completed is given in Table 3-1. In cases where the number of anadromous males and females in each section differed from one, the anadromous fish had moved by leaping over the vexar barrier (with the exception of the female that punctured the barrier between sections 7 and 8). Genetic evidence later indicated that one of the anadromous males collected in section 11 had spawned in section 10. Four parr from section 8 were found to have spawned in section 7, thus the number of parr in the latter treatment present at the time of spawning might have ranged from 10 (number of parr identified as having spawned) to 23 (number of parr in both sections; Table 3-1). None of the females in the 3 uppermost sections (i.e., 4, 8, and 12) spawned, possibly as a result of the disturbance generated by the water inflow originating at the top of these sections. In addition, the uppermost sections also contained more parr at the conclusion of the experiment than there had been at the start. I attributed this to the movement of parr via the inflow from the water source of the hatchery raceways. These additional parr could only potentially have been involved in spawning in section 7 due to the hole in the partition between sections 7 and 8. In the end, I had results for fewer treatments than originally designed (4 treatments: 5, 10, 10, and 20 parr). However, I also had 4 additional treatments having parr:female:anadromous male ratios of 5:1:0, 7:2:1, 10-23:2:1 and 7:1:1-2 which provided interesting findings.

Allelic diversity in the wild Margaree River salmon was high for the four microsatellite loci examined (18, 16, 27, and 9 alleles at *Ssa202*, *Ssa197*, *Ssa171*, and *Ssa85*, respectively). Gene diversity (H_e) was also high (0.91, 0.90, 0.92, and 0.76 at *Ssa202*, *Ssa197*, *Ssa171*, and *Ssa85*, respectively).

As expected, most offspring possessed one or two alleles at each locus. However, the offspring from the single-pair treatment with 20 parr possessed two or three alleles at *Ssa202*, *Ssa197*, and *Ssa171*, and one or two alleles at *Ssa85*. In this treatment, almost all (166/167) offspring possessed both maternal alleles (the female was a homozygote at *Ssa85*) and often one additional allele at each locus. The lone exception was an embryo

lacking one of the maternal alleles at *Ssa197*. However, this individual was triploid at both *Ssa202* and *Ssa171*, thus this missing allele at *Ssa197* must have represented either a one-repeat mutation or only partial genomic triploidization. This finding was unexpected and the mechanism remains unclear. Previous occurrences of spontaneous triploids in fish are believed to have occurred by the fertilization of unreduced eggs (Cuellar and Uyeno 1972, Gold and Avise 1976, Thorgaard and Gall 1979), although their observed frequency was relatively low. It seems unlikely that the triploidy found in my study can be attributed to any form of handling stress. Artificial methods used to prevent the second meiotic division to create triploids are typically performed within 20 minutes of fertilization and often do not have complete success (reviewed by Ihssen et al. 1990). Hybrid Atlantic salmon X brown trout (*Salmo trutta*) females backcrossed with either an Atlantic salmon or brown trout male can also produce triploids (e.g., Johnson and Wright 1986, Dannewitz and Jansson 1996, Galbreath et al. 1997).

Effect of parr size on individual reproductive success

There was great variation in parr reproductive success among treatments (Figures 3-2 to 3-5) and, in most cases, there were also significant differences in individual reproductive success among nests within treatments. I found no evidence of an influence of parr body size on individual reproductive success in the single anadromous pair treatments (Figure 3-2a-d). In the treatment with five parr (Figure 2a), total parr success was low (mean of 4.7%) with only two parr identified as having fertilized eggs, although in one of the three nests, the largest individual was the only parr to spawn (the number of parr displayed in Figures 3-2 to 5-2 can be lower than the sample size for those treatments as a result of mortalities). Total parr success was higher in the two 10-parr treatments with seven and five parr identified as having been involved in spawning (average fertilization success of 55 and 26%, as represented by Figures 3-2b and 3-2c, respectively). In the 20-parr treatment, total parr success was also high (mean of 30%) and eleven parr were identified as having been involved in spawning (Figure 3-2d).

There was also little evidence of an influence of parr size on individual reproductive success in either of the treatments involving two anadromous females and

one anadromous male (Figure 3-3), although in one of the 4 nests in the 10-23 parr treatment, the two most successful parr were the largest parr (Figure 3-3i). In the treatment involving seven parr, six were identified as having been involved in spawning and mean total parr success was 76% (Figure 3-3a). In the 10-23 parr treatment (Figure 3-3b), ten parr were identified as having been involved in spawning and mean total parr success was 53%.

There was no apparent influence of parr size on individual reproductive success in the treatment that, at the time of salmon removal, had two anadromous males and one anadromous female (Figure 3-4; section 11). The specific lengths of the two putative contributors were unknown but, given the lengths of the parr present, both must have been from the 91-110 mm size class. In one nest, parr had fertilized all of the eggs, with six of the seven parr identified as having spawned (Figure 3-4i), while in the other nest examined, the anadromous male from section 10 had complete success (Figure 3-4ii).

There was a very strong relationship between individual parr size and individual reproductive success in the single treatment for which the anadromous male had died (Figure 3-5). In this treatment, all five parr were identified as having been involved in spawning with the largest parr fertilizing all the eggs in one nest (Figure 3-5i) and the second largest parr obtaining the highest fertilization success in the other nest sampled (Figure 3-5ii).

Parr Mortality and the Probability of Not Detecting Individual Paternity

In all single-pair anadromous salmon treatments, there was evidence of parr mortality between the onset of the experiment (October 31, 1996) and the removal of the salmon (December 9, 1996). In three of these single-pair treatments (sections 5, 6, and 10), the number of parr collected was one less than at the start of the experiment, and in the fourth (section 13) a decomposing parr was found. In the other sections where spawning occurred, there was no parr mortality in the section where the anadromous male had died (section 9) or in the section with two anadromous females, one anadromous male, and seven parr. Two parr were missing from the section with two anadromous males (section 11).

In only one treatment was there evidence that the missing parr had successfully fertilized eggs. Given the high allelic diversity at each locus used in this study, the probability of such an individual being reproductively successful and not detected (i.e., falsely assigned to another individual in that section) is very low. The identification of unaccounted contributors in section 11 and the initial inability to account for all paternity in section 7 until males from section 8 were included as potential fathers provides strong supporting evidence that no additional, unsampled, deceased males had spawned successfully in any of the sections.

Individual Parr Size and Probability of Spawning

Although there was no relationship between individual reproductive success and parr size within or among treatments, larger parr appeared to be involved more frequently in spawning than smaller parr (Figure 3-6). Combining results from all nests and dividing parr body sizes equally (i.e., $n = 23$ in each size class) among three size classes, large parr (96-120 mm) were identified as having been involved in spawning much more frequently than small parr (81-86 mm) ($p < 0.01$; G-test, Sokal and Rohlf 1981). Neither parr in the small nor large size classes differed in successful spawning events from those in the intermediate size class (87-95 mm) ($p = 0.37$ between small and intermediate size classes and $p = 0.08$ between intermediate and large size classes).

The mean relative individual reproductive success was similar for all size classes (10.5%, 10.6%, and 9.3% for small, medium and large size classes, respectively), where relative individual parr success = individual parr success / total parr success in a given treatment. The small size class was characterized by a higher variance in relative individual reproductive success than the large size class (0.044, 0.028, and 0.008 for small, medium and large size classes, respectively; because frequency data were used (to allow combining of data among treatments), variances were calculated using the arcsine of the frequencies (Sokal and Rohlf 1981)).

Effect of Intensity of Anadromous Male Competition and Effective Number of Males

Parr fertilization success appeared to decline with increased intensity of anadromous male competition (# anadromous males : # anadromous females) (Figure 3-7; the treatment with two anadromous males was excluded from the analysis because it was unclear when the second male had arrived during the experimental period). Parr success was higher in the two treatments with two anadromous females and one anadromous male than in the four treatments with one anadromous female and one anadromous male (63.8 vs 28.7%), although in one of the treatments with two anadromous females the number of parr may have been higher (10-23 parr) than in the other treatments.

To illustrate the potential influence mature male parr may have on effective population size, I calculated the effective number of males for each cross. There was no relationship between the effective number of males and the number of mature males (anadromous and parr) present in a treatment (Figure 3-8a; $r^2 = 0.003$, $p = 0.90$). However, given that the effective number of males there would have been had only one anadromous male and no parr been present is one, mature male parr reproductive contributions increased the effective number of males to more than one in all treatments (Figure 3-8b, Table 3-2). This proportional increase in effective number of males relative to the number of anadromous males present appears to be related to the intensity of anadromous male competition. The increase in effective number of males as compared to the actual number of anadromous males present was marginal in the single anadromous pair treatment with five parr ($N_{e\,\sigma} = 1.10$) but greater in the other single anadromous pair treatments ($N_{e\,\sigma} = 1.75$, 3.67, and 2.01) and generally greater still in the treatments involving two anadromous females and one anadromous male ($N_{e\,\sigma} = 5.06$ and 3.19; Figure 8b, Table 3-2). In the treatment with five parr but no anadromous male, the effective number of males was greater than 2 (Figure 8b, Table 3-2). Of note is that, because different parr are often involved in each nest in a treatment, the overall effective number of males calculated by weighting each nest equally can be greater than the mean of the effective number of males from each nest (Table 3-2). This would result in my

estimates of $N_{e\sigma}$ being systematically biased downward. This bias is likely to be minimal as this effect will have an increasingly diminishing influence with each nest sampled; the overall increase in $N_{e\sigma}$ is only larger than that in each individual cross in only half the crosses (Table 3-2).

DISCUSSION

Effects of Mature Male Parr and Anadromous Male Competition on Effective Number of Males

To my knowledge, the present study is the first to estimate the effective number of males from individual fertilization success in fish. While my results should not be taken as absolute, because I did not sample all nests and I used a proxy for lifetime reproductive success, they do illustrate how mature male parr can greatly increase the effective number of males when the latter is estimated from anadromous individuals alone. This result will be of particular importance to small Atlantic salmon populations given that anadromous females typically outnumber anadromous males during spawning. Genetic contribution from these non-migratory parr will also serve to maintain genetic variation within populations and genetic differentiation among populations.

The total number of males present does not appear to influence the effective number of males. Of potentially greater importance to the effective number of males is the intensity of anadromous male competition. As the number of anadromous females per anadromous male increases, total parr reproductive success and, consequently, the effective number of males both increase. The increase in total parr reproductive success could be the result of the anadromous male being unable to chase away parr from multiple females simultaneously. Alternatively, the anadromous male may become less able to fertilize as many eggs at each reproductive bout as sperm is depleted through repeated spawning (Mjølnerød et al. 1998). Irrespective of the cause, this finding suggests that the influence of mature male parr on effective population size will be greatest when population sizes are at their lowest and when the potential for loss of genetic variation is highest.

Some caution should be exercised when applying the results from this and other single anadromous pair treatment experiments to natural populations. Single anadromous pair treatment experiments, albeit with variation within and among studies, typically find total parr fertilization success to be approximately 30% (Hutchings and Myers 1988 - 20%, Morán et al. 1996 - 51%, Thomaz et al. 1997 - 30%, present study - 31%).

However, the degree to which such studies are representative of natural situations is difficult to assess. During spawning, anadromous females typically spawn over a few days while anadromous males can spawn for several weeks (Fleming 1996). The result is that while anadromous salmon sex ratios are typically female-biased, the operational sex ratio on the spawning grounds may often be heavily male-biased (Fleming 1996). The results from my study and a previous experiment (Jordan and Youngson 1992 - 11% mean total parr success) suggest that increased anadromous male competition will lead to a reduction in total parr reproductive success. Thus, perhaps in contrast to my single anadromous pair treatment experiments for which parr success always resulted in an increase - and often a very large increase - in the effective number of males relative to the number of anadromous males present, the effective number of males in multiple anadromous pair matings may be significantly less.

Alternatively, parr success may well be greater in the wild than in experimental conditions, even with increased anadromous male competition. When spawning in faster currents, the parr's advantage of closer proximity to the female's vent may be amplified due to the increased diluting effect the current will have on the sperm (Jones 1959). Similarly, heterogeneous substrate should provide more hiding locations for parr than exist in many controlled experiments, potentially increasing the access by parr to females. Martinez et al. (2000) found that mature male parr can often obtain the majority of the fertilization success. Two studies which were not able to identify the life history type of each male also noted that secondary male contribution can be high (Thompson et al. 1998 - 29%, Morán and García-Vázquez 1998 - 72%). Thus, the factors influencing parr reproductive success in the wild, and its influence on effective population size, require further attention.

Effect of Parr Size on Individual Reproductive Success

Our results suggest that, when competing with an anadromous male, individual parr body size does not influence individual parr reproductive success. The general lack of such an association was not anticipated. Male parr compete for proximity to the female prior to spawning and the largest parr is often the closest (Myers and Hutchings 1987). Thus, at

low parr densities, I had expected there to be a fertilization advantage to being large. Two factors might explain the lack of such a relationship in my study. The first could be related to the importance of individual fertilization success in the first nest laid to the previous overall finding of a significant relationship between parr size and individual fertilization success at low parr density (Thomaz et al. 1997 - n = 6 parr). Thomaz et al. (1997) only found a significant association between parr size and individual reproductive success in nests in which parr had spawned for the first time. It is unclear whether there is a continuous turn-over of parr between spawning events when parr densities are low. If not, parr will rarely be spawning for the first time. Thus, my finding of no association between parr size and individual fertilization success may be typical under some natural situations.

The second factor which could account for the independence of individual parr reproductive success and parr size in my study may be related to the considerably greater range in parr size used in Thomaz et al.'s (1997) experiment (11-65g vs 5.9-20.6g) and their greater representation of the extremes in size classes. My range in parr size and the relative proportions of the size classes reflect those observed in the Margaree River. This range is probably representative of most Canadian populations for which parr growth rates are lower than those in southern Europe (contrast parr lengths in France (e.g., Baglinière and Maisse 1985) with those in Atlantic Canada (e.g., Myers et al. 1986)). Thus, the influence of parr size on individual fertilization success may be lower in Canadian populations due to their smaller range in body size.

The lack of an association between parr size and individual reproductive success at higher parr densities (10 and 20 parr) appears to be robust. At these densities, total parr contribution was high (mean 27 to 55% versus 5% for the treatment with five parr) and similar findings were obtained in three separate treatments. The lack of a relationship between individual parr size and reproductive success might be expected at higher parr densities given that, relative to low levels of mate competition, the dominant parr must presumably chase away more subdominant (smaller) parr to maintain increased access to the female. The difficulty of maintaining this favourable position would be

expected to increase with larger numbers of parr as the dominant parr expends continually greater effort attempting to maintain his position to a point at which body size has no influence on fertilization success (Hutchings and Myers 1994). My findings provide some support for this hypothesis.

Although larger parr were involved in more spawning events than smaller parr (Figure 3-6), both size classes had similar mean relative individual reproductive success. Smaller parr, when involved in spawning, frequently had higher relative reproductive success than the larger parr that spawned. Consequently, smaller parr had much higher variance (0.044) in relative individual reproductive success than the largest parr (0.008). While there is no clear explanation for this finding, it could be related to a greater survival cost of reproduction experienced by smaller parr.

The cost of reproduction for a parr can be placed into three categories: (i) the cost of maturing (energy diverted from somatic to gonadal development; e.g., Thorpe, 1994), (ii) the risk of mortality during spawning (e.g., Hutchings and Myers 1987, present study), and (iii) the actual energy expended during spawning (e.g., Jonsson et al. 1997, for anadromous Atlantic salmon). The increased annual mortality attributed to parr maturation has been estimated at 44% in one Newfoundland population (Myers 1984). However, it is unclear whether this increased mortality is due primarily to maturation (i) or to spawning (ii and iii). Hutchings (1994) found that post-reproductive survival was positively associated with body size in one Newfoundland population of brook trout (*Salvelinus fontinalis*). Investigating the difference in investment in spermatogenesis, Gage et al. (1995) found that parr, as a group, invested relatively more per unit body mass than anadromous males, although it is unclear whether this trend exists among parr of different sizes. Smaller parr may not need to be as reproductively successful as larger parr to obtain equal fitness if the former mature earlier in life (Hutchings and Myers 1994). However, if the cost of reproduction is much greater for small parr than large parr, smaller parr may need to achieve higher reproductive success than larger parr.

In summary, I found no effect of parr body size on individual parr fertilization success. Parr body size does, however, appear to determine whether an individual parr

fertilizes any eggs or not. Using my estimates of individual male reproductive success, I illustrated quantitatively the influence mature male parr fertilization success has on the effective number of males. Because total parr success appears to be inversely related to the intensity of anadromous male competition, mature male parr will likely be most influential on effective population size when the number of anadromous males in a population is low.

Table 3-1. Number of anadromous males (# ♂♂), anadromous females (# ♀♀) and mature male parr (# parr) in each section after spawning in all sections had been completed.

Section	# ♂♂	# ♀♀	# parr	comments
1	1	0	3	♀ left prior to spawning
2	1	0	12	♀ left prior to spawning
3	1	2	7	♀ arrived prior to spawning
4	1	2	20	no spawning - upstream section
5	1	1	9	1 parr missing
6	1	1	4	1 parr missing
7	1	2	10	♀ arrived prior to spawning
8	1	0	13	♀ left prior to spawning
9	1	1	5	♂ dead prior to spawning
10	0	1	9	1 parr missing; ♂ left after spawning
11	2	1	5	2 parr missing; unclear when 2nd ♂ arrived
12	1	1	?	no spawning - upstream section
13	1	1	20	1 parr dead; offspring triploid

Table 3-2. Number of anadromous males (# ♂♂), anadromous females (# ♀♀) and mature male parr (# parr) in each section during spawning and the effective number of males ($N_{e\,\sigma}$) calculated for each nest (i-iii) and overall (all). Mean fertilization success of parr, as a group, was calculated by weighting each nest equally.

Section	# ♂♂	# ♀♀	# parr	$N_{e\,\sigma i}$	$N_{e\,\sigma ii}$	$N_{e\,\sigma iii}$	$N_{e\,\sigma all}$	$\bar{x}$ parr success (%)
6	1	1	5	1.07	1.07	1.15	1.1	4.7
10	1	1	10	2.33	1.33	-	1.75	25.9
5	1	1	10	1.88	2.95	2.89	3.67	55
13	1	1	20	1.21	2.92	2.28	2.01	29.9
3	1	2	7	3.96	1.34	2.17	5.06	75.8
7	1	2	10-23	2.01	2.15	2.95	3.19	52.5
9	0	1	5	1	1.67	-	2.38	100
11	1-2	1	7	3.67	1	-	3.16	50

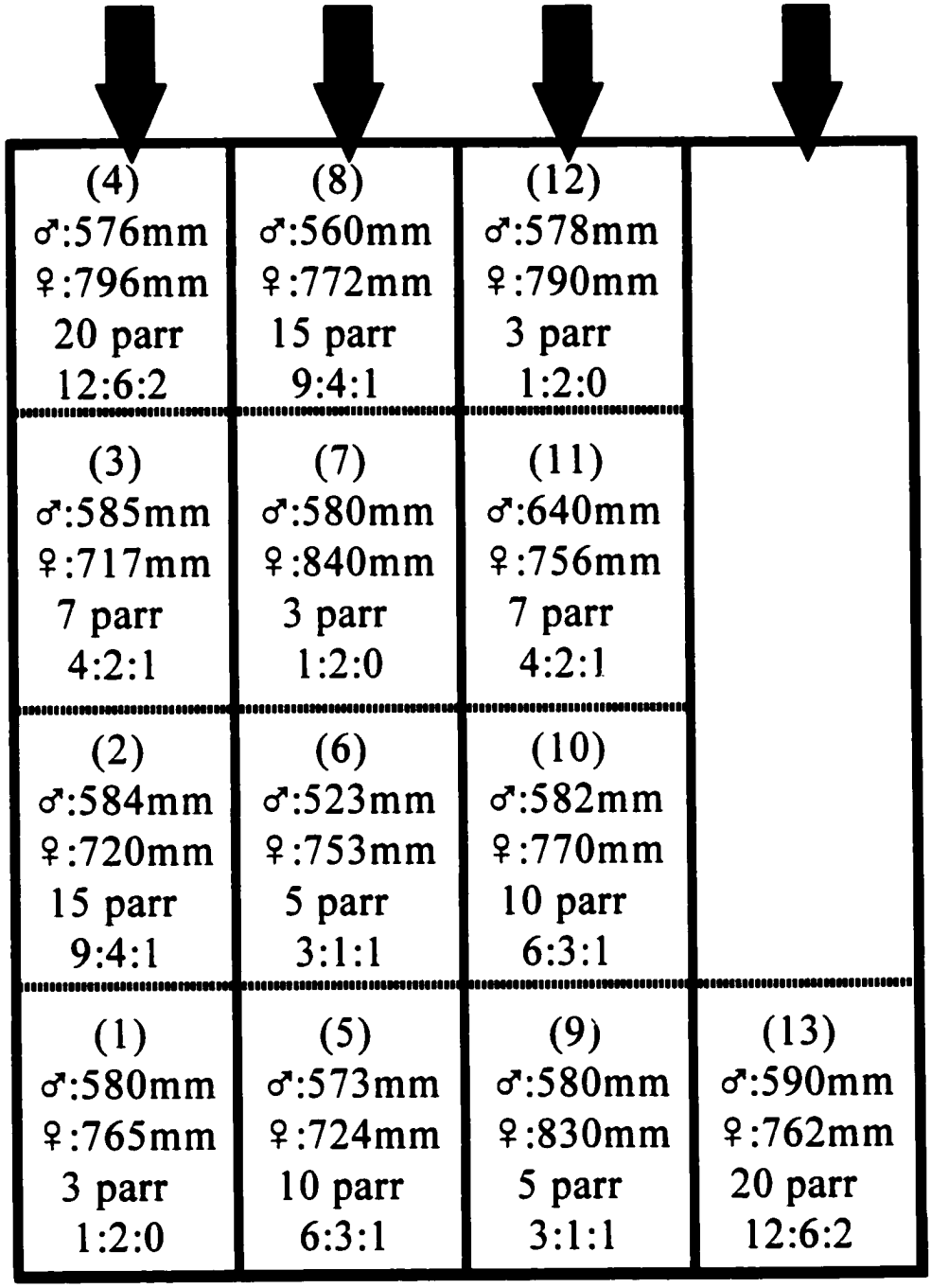

Figure 3-1. Single pair treatment experimental design with the anadromous male (♂) and female (♀) lengths and the number of mature male parr (parr) in each section. Ratio represents the number of parr from each size class (81-90 mm : 91-110 mm : 111-123 mm). Numbers in parentheses indicate section number, and arrows indicate location of water inflow.

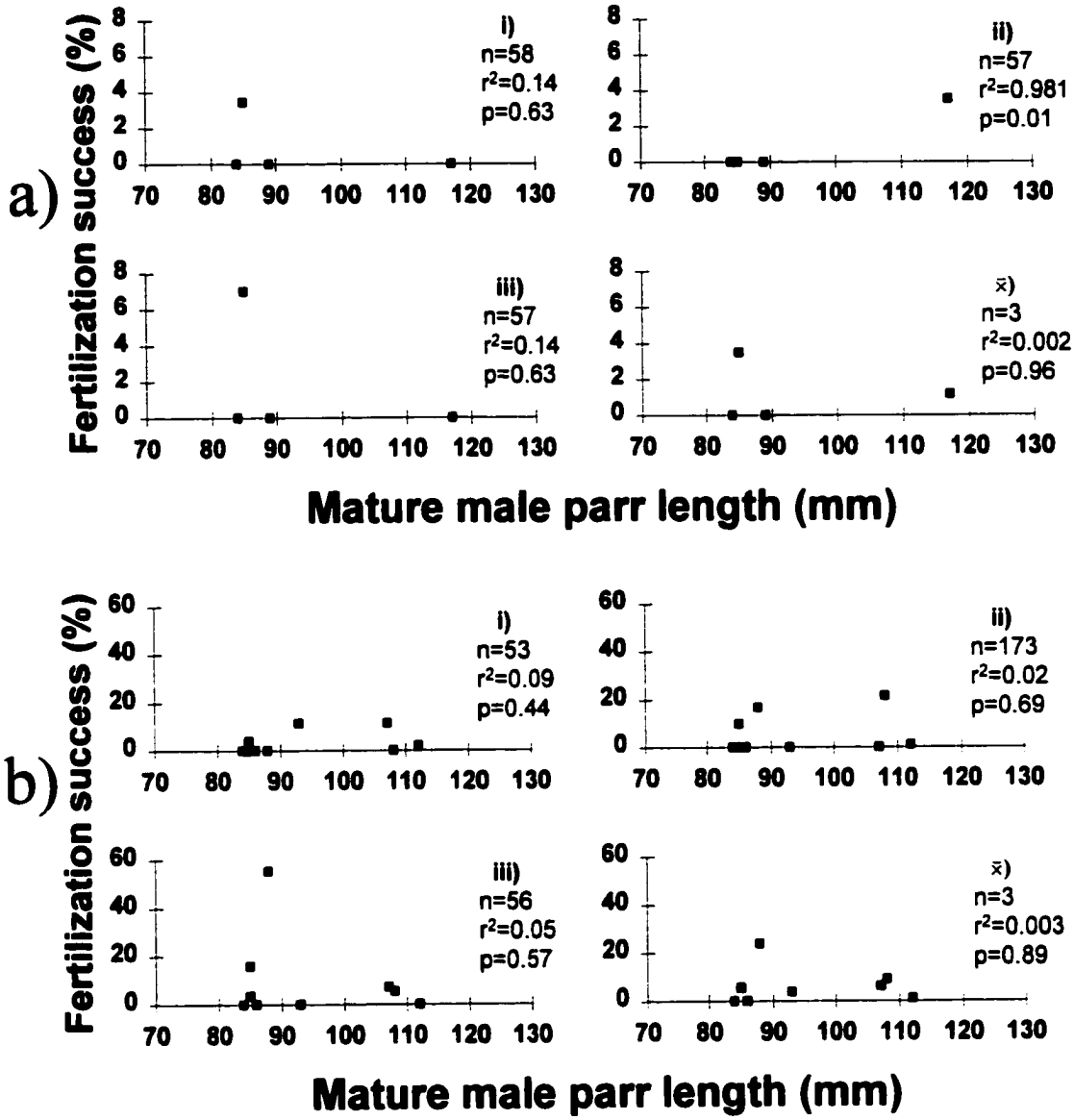

Figure 3-2. Individual parr fertilization success versus parr length for the single anadromous pair crosses and a) 5 parr, b) and c) 10 parr, and d) 20 parr. For the individual nests (i-iii), *n* refers to the number of embryos upon which the frequencies were determined; for the mean of the nests ($\bar{x}$), *n* refers to the number of nests upon which the mean was calculated. The r^2 and associated *p*-values refer to the linear regressions between parr fertilization success and parr body size.

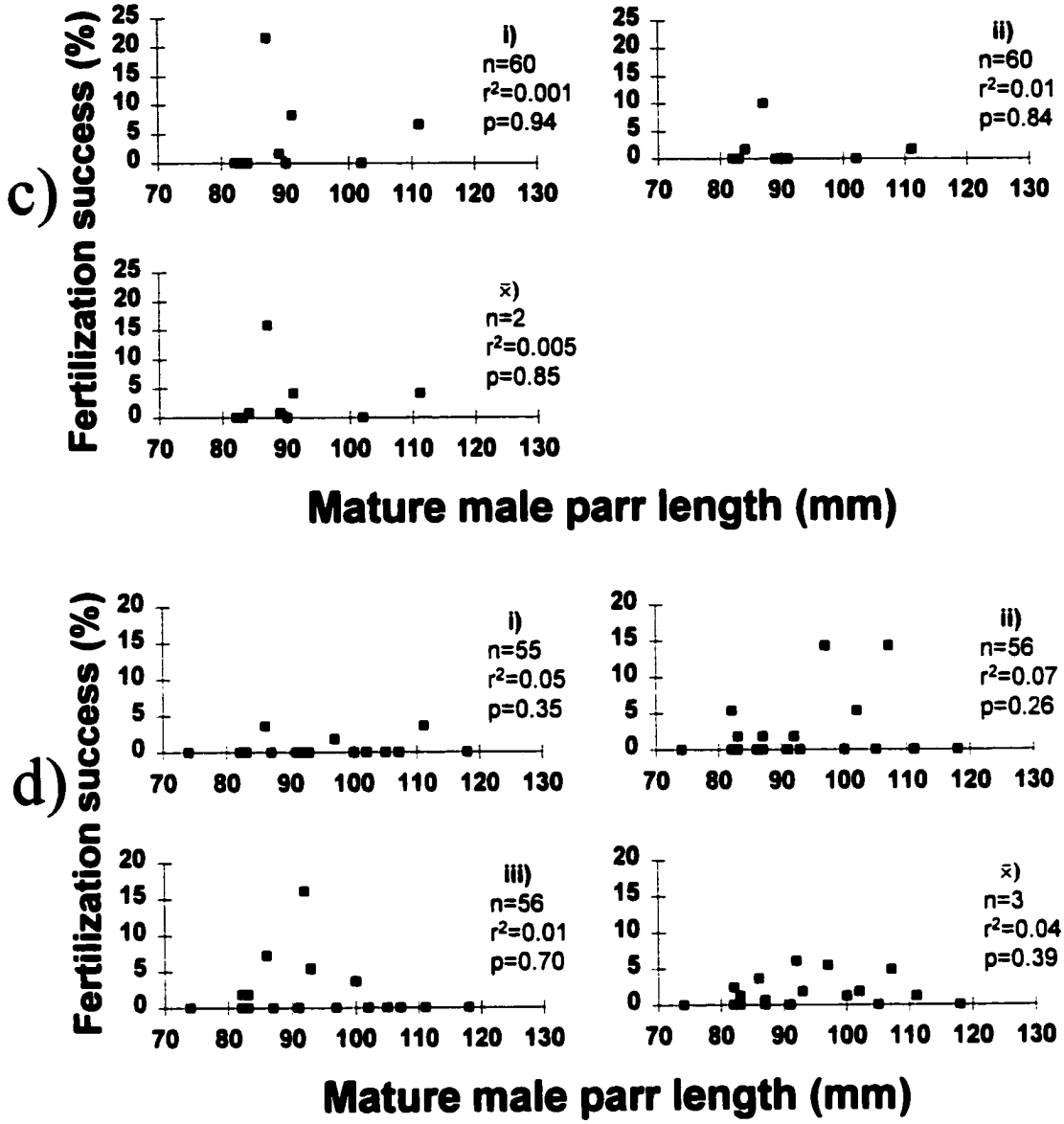

Figure 3-2. Continued.

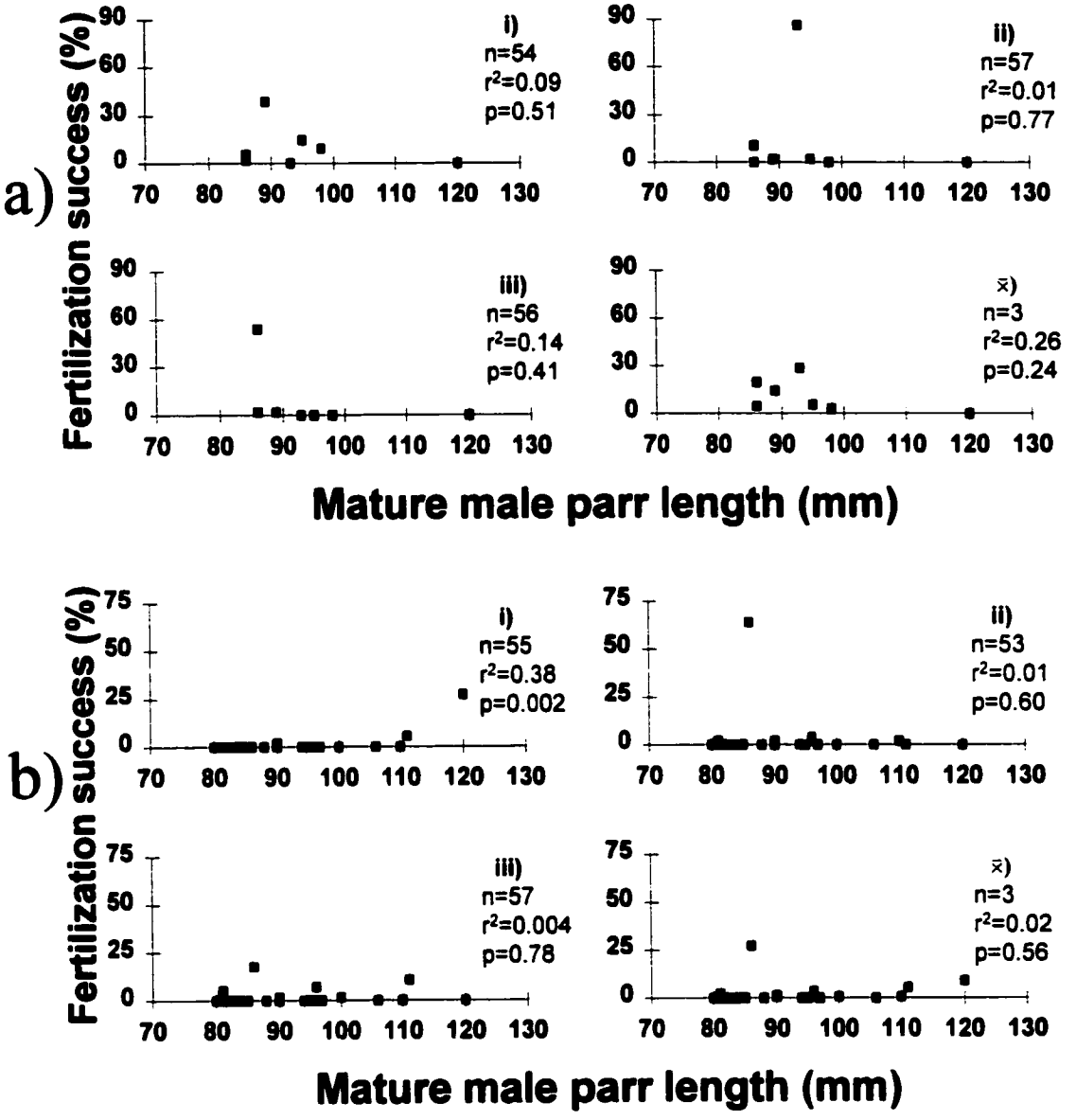

Figure 3-3. Individual parr fertilization success versus parr length for the crosses involving one anadromous male, two anadromous females, and a) 7 parr and b) 10-23 parr. For the individual nests (i-iii), *n* refers to the number of embryos upon which the frequencies were determined; for the mean of the nests ($\bar{x}$), *n* refers to the number of nests upon which the mean was calculated. The r^2 and associated *p*-values refer to the linear regressions between parr fertilization success and parr body size.

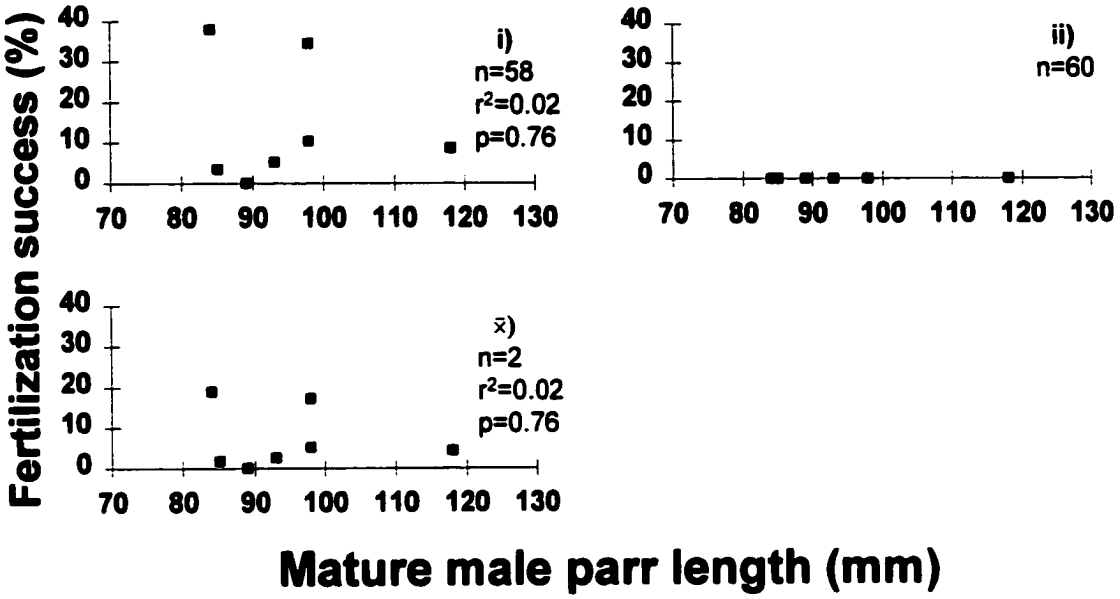

Figure 3-4. Individual parr fertilization success versus parr length for the crosses involving two anadromous males, one anadromous female, and 7 parr. For the individual nests (i-ii), *n* refers to the number of embryos upon which the frequencies were determined; for the mean of the nests ($\bar{x}$), *n* refers to the number of nests upon which the mean was calculated. The r^2 and associated *p*-values refer to the linear regressions between parr fertilization success and parr body size.

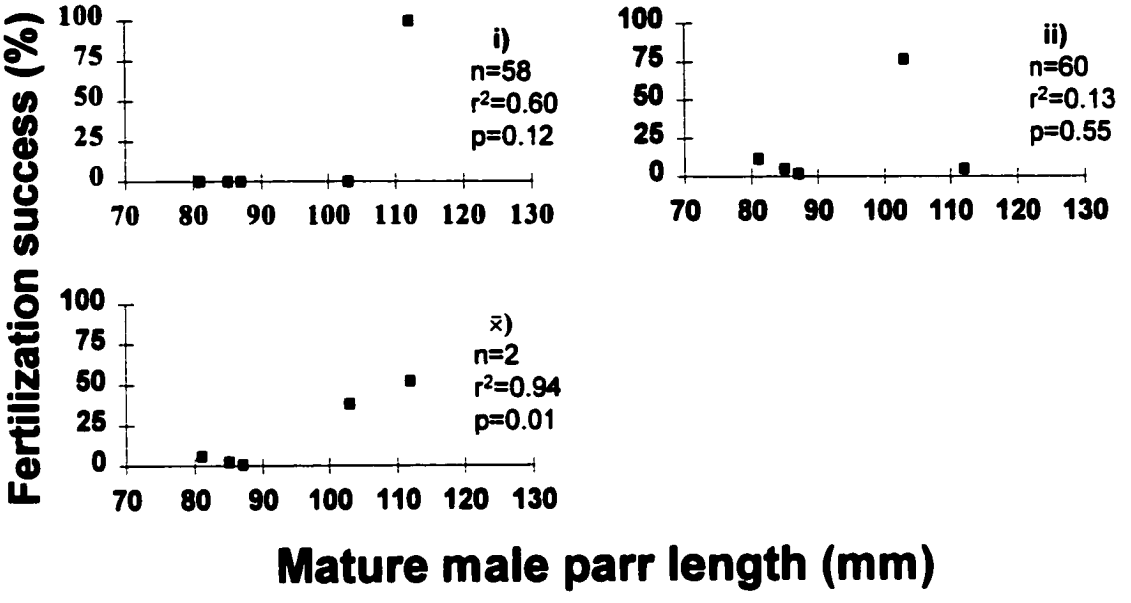

Figure 3-5. Individual parr fertilization success versus parr length for the cross involving no anadromous males, one anadromous females and 5 parr. For the individual nests (i-ii), *n* refers to the number of embryos upon which the frequencies were determined; for the mean of the nests ($\bar{x}$), *n* refers to the number of nests upon which the mean was calculated. The r^2 and associated *p*-values refer to the linear regressions between parr fertilization success and parr body size.

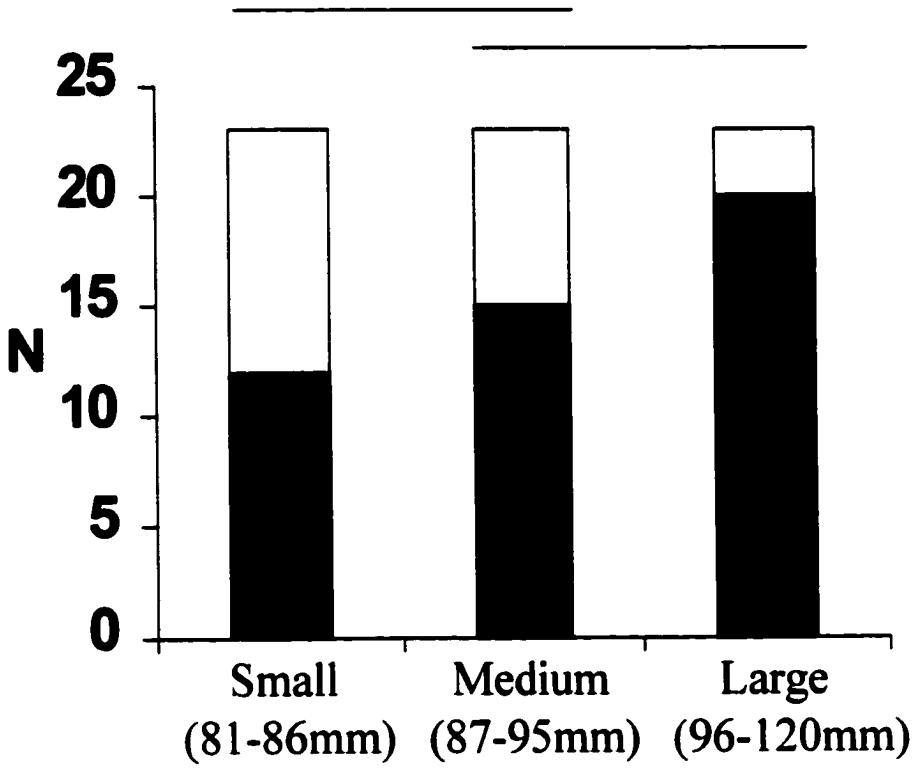

Figure 3-6. Number of parr of different size classes that did (O) and did not successfully fertilize at least 1 egg (Q). Bars connect size classes that did not differ significantly (G-test).

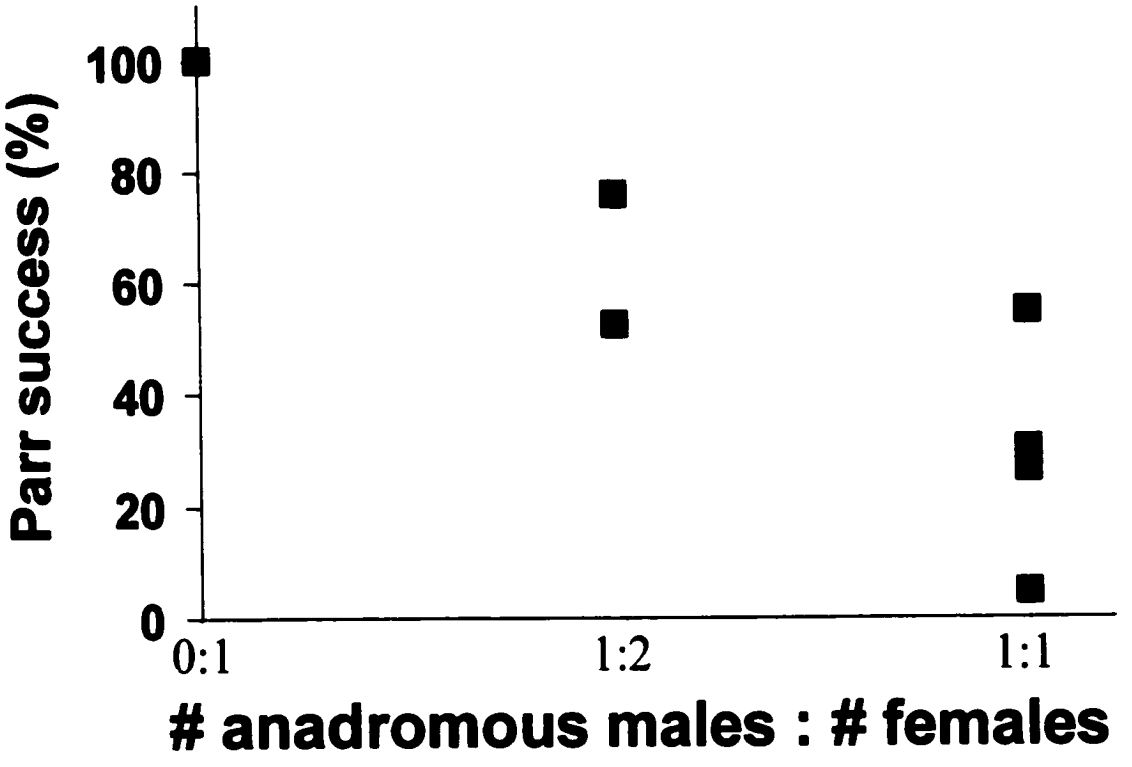

Figure 3-7. Percent parr fertilization success versus intensity of anadromous male competition; the regression was significant with the inclusion of the treatment with no anadromous male ($r^2 = 0.75$, $p = 0.012$), but not with its exclusion ($r^2 = 51$, $p = 0.108$).

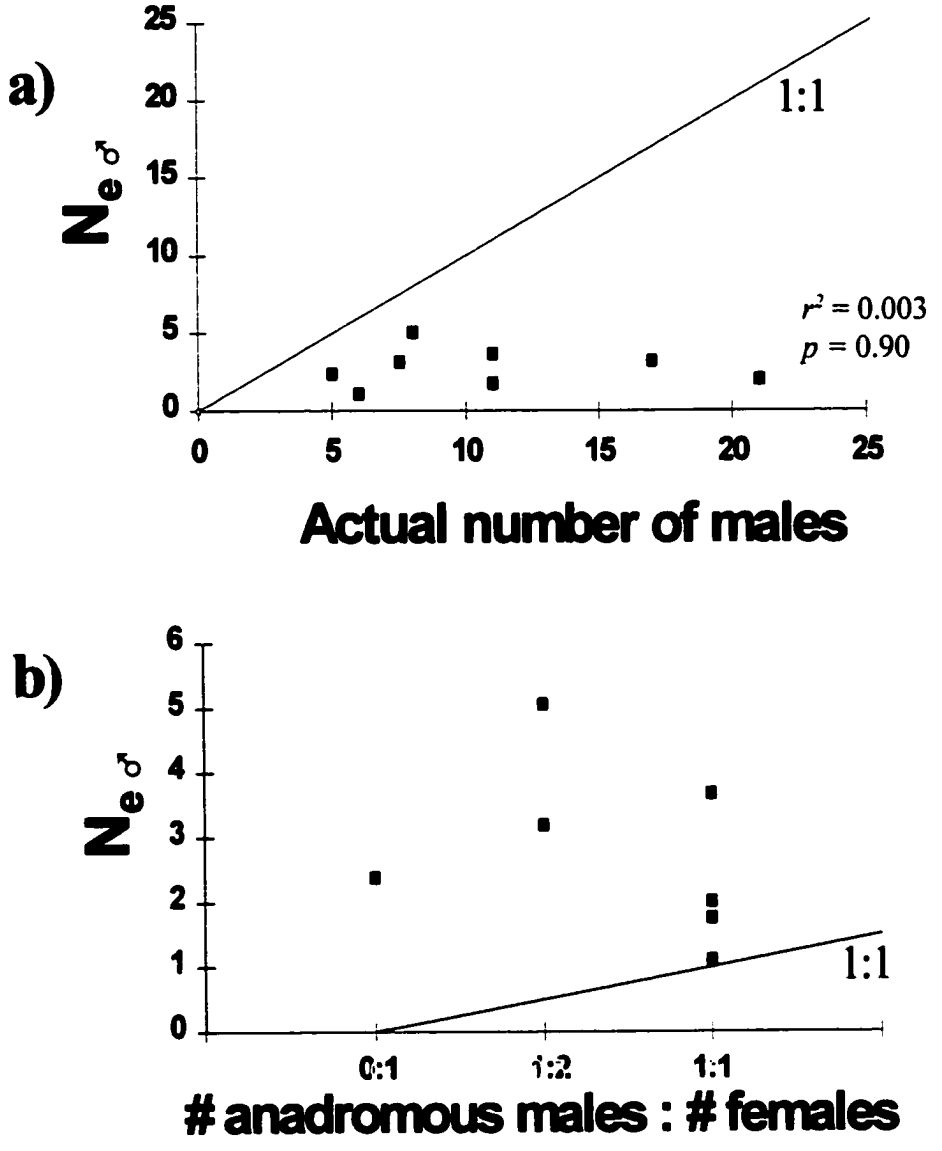

Figure 3-8. Effective number of males per cross ($N_{e\,♂}$) versus a) number of mature males (anadromous and parr) and b) number of anadromous males : number of anadromous females. The r^2 and associated p-value refer to the linear regression between effective and actual number of males. The line on each graph represents the 1:1 expectation between a) effective and actual number of males and b) effective number of males and actual number of anadromous males.

CHAPTER 4.

Individual variation in Atlantic salmon fertilization success: implications for effective population size

INTRODUCTION

Effective population size, or N_e, is of considerable interest to conservation biologists because of its negative association with the rate of loss of genetic variation (Wright 1931). Mating systems can strongly influence N_e, notably by affecting the likelihood that an individual will reproduce (Nunney 1991), by influencing the variance in individual reproductive success within one or both sexes (Nunney 1993), and by affecting the number of mates per individual (Sugg and Chesser 1994). Therefore, in addition to estimating the number of adults of reproductive age of both sexes, reliable estimates of N_e require accurate estimates of the variance in individual reproductive success for both sexes within the context of an organism's typical mating structure. Increased risks of extinction faced by many populations and species underscores the need for greater empirical understanding of the influence that individual reproductive success and mating structure have on N_e.

Individuals of many species employ different tactics to obtain reproductive success. Fishes have often been used to study such alternative reproductive strategies (reviewed by Taborsky 1994). Salmonids, commonly exhibiting an anadromous life history (a freshwater component early in life followed by a period of rapid growth at sea and eventual return to the natal river to spawn), have been of particular interest in this field. Atlantic salmon (*Salmo salar*) is one such salmonid for which there is great interest on the influence of alternative reproductive strategies on N_e.

Atlantic salmon male parr (smaller individuals who have not migrated to sea) can mature and compete with the larger anadromous males for access to anadromous females during spawning and do fertilize some eggs (Hutchings and Myers 1988, Jordan and Youngson 1992, Morán et al. 1996, Thomaz et al. 1997, Jones and Hutchings In press). It have been suggested that mature male parr fertilization success can increase a female's

number of mates as well as potentially increasing effective population size by increasing the effective number of males and by affecting the variance in reproductive success (L'Abée-Lund 1989, Jones and Hutchings In press). However, previous studies on Atlantic salmon reproductive success have been either limited primarily to single anadromous pair crosses (Hutchings and Myers 1988, Morán et al. 1996, Thomaz et al. 1997, Jones and Hutchings In press) or were unable to assign individual reproductive success due to the limitation of the genetic markers at the time (Jordan and Youngson 1992). Such experiments, by their design, were unable to determine the influence of increased competition from anadromous males on parr mating behaviour or fertilization success. Furthermore, they were unable to quantify either the actual number of mates of either sex or the variance in individual reproductive success.

Estimates of the number of anadromous salmon of reproductive age within single Atlantic salmon populations are often available. However, estimating N_e remains difficult because many factors can affect mating structure and create variability in individual reproductive success in both sexes. The sex ratio in anadromous salmon is typically female-biased. However, because an individual female will spawn over 5-6 days, while an anadromous male can potentially spawn throughout the entire several-week spawning period, the operational sex ratio on the spawning grounds is typically male-biased (reviewed by Fleming 1996). For females, variation in individual size, and consequently fecundity (e.g., Thorpe et al. 1984), and variation in pre-emergence survival (e.g., Pauwels and Haines 1994) can create variation in individual reproductive success. Suitable spawning locations are often limited and a female risks lower offspring survival either by being relegated to less suitable spawning locations or by having nests dug up by subsequent spawners (Fleming et al. 1996). The potential for multiple mates also exists for both sexes. Given the extended spawning potential of anadromous males, individual males could spawn with many females. More than one anadromous male may spawn with a single female at any one time (e.g., Mjølnerød et al. 1998) and anadromous females generally spawn several times (Jones 1959).

The alternative reproductive strategy that exists within male Atlantic salmon can

also lead to increased variance in individual reproductive success and have further implications for a population's effective size. Several factors can influence reproductive success in male Atlantic salmon. Total parr fertilization success within nests is dependent on the number of parr and on the order of nest construction (Hutchings and Myers 1988, Thomaz et al. 1997). Individual parr fertilization success may also depend on parr size (Thomaz et al. 1997), although this may only be true at low parr densities (Jones and Hutchings In press). Similarly, when spawning with a single female, the body size of competing anadromous males can also be an important determinant of individual fertilization (Mjølnerød et al. 1998). A primary limitation of these studies, however, is that determinants of male fertilization success were assessed under conditions in which males had access to only one female. Thus, the degree to which these correlates of reproductive success are influenced by more natural spawning conditions, e.g., the presence of multiple females, multiple anadromous males, and multiple parr, is not known.

As population sizes decline, quantifying the mating structure and variation in individual reproductive success, as well as factors associated with this variation is critical. In addition, understanding the influence alternative reproductive strategies have on effective population size is of central importance to the conservation of genetic diversity in many species. I use Atlantic salmon as a model species to address the following objectives: (1) to quantify individual fertilization success, to identify the factors associated with individual fertilization success, and to describe the mating structure of anadromous males and females and mature male parr in a semi-natural environment; and (2) to quantify the influence that variation in individual fertilization success and the influence alternative reproductive strategies have on effective population size.

MATERIALS AND METHODS

Field Experiment

Experiments were undertaken in outdoor flow-through raceways (14.6 m X 1.2 m cement channels with controlled water flow) at the Margaree Fish Hatchery, Nova Scotia (46°21'N, 60°58'W), in the autumn of 1996. Substrate suitable for spawning was added to the raceways to a depth of 30 cm. Mature male parr were electrofished from nearby tributaries of the Margaree River one week prior to the experiment. Grilse males (i.e., males that spent 1 winter at sea before returning to spawn) were obtained from the fish trap on Lake O'Law River, the largest of Margaree River's tributaries. Multi-sea-winter (MSW) males and females were obtained by seining the Margaree River. All fish were in spawning condition but had not yet been involved in spawning at the time of their collection.

I established two replicates of experimental crosses each having 4 MSW females, 2 MSW males, 2 grilse males and 20 mature male parr of different sizes (range 68-123 mm). The parr were divided into 4 size classes: 69-80 mm (mean of 75.3 ± 3.2 mm SD), 81-90 mm (84.9 ± 2.7 mm), 91-110 mm (97.9 ± 5.7 mm), and 111-123 mm (116.9 ± 3.7 mm). Parr were placed in the raceways prior to the addition of the anadromous salmon (body size data, by replicate, are presented in Table 4-1). The raceways were covered with netting anchored by cinder blocks. I measured the length and weight of each fish and collected a fin sample from each fish for microsatellite DNA analysis after spawning had been completed in December, 1996.

Eyed embryos were collected from 8 and 10 nests from replicates A and B, respectively, in March, 1997. While the number of nests each female will make depends on habitat quality and the degree of competition from other females (Fleming 1996), similar sized females (approximately 4 kg) in Europe created an average of approximately 6 nests. I thus sampled an estimated 37% of all the nests constructed. Parr and embryo collection was facilitated by the ability to lower the water levels in the raceways. To sample nests, markers were placed every 1.3 m along the length of the raceways. Beginning at the centre of the raceway along the transect of the downstream

marker, 10 to 15 cm of substrate were dug away. The area cleared expanded outwards from the centre point until a nest was found. This procedure was then repeated at the next upstream marker transect. In the B raceway, nests were not found near 3 transects, necessitating a search below the first (downstream) transect to increase the number of nests sampled.

Genetic Analysis

DNA was extracted from all potential parents and from 60 offspring from each of 6 nests per raceway. To identify the major contributors to the remaining nests in each raceway, 10 embryos from each of these remaining nests (nests 7-10 in raceway A, nests 7-8 in raceway B) were also examined. Each dissected embryo head or small sub-sample of fin tissue (approximately 50 mg) was digested in 100 µl of eyeball buffer (10 mM Tris, 50 mM KCl, 0.5% tween20) and proteinase K (0.1-0.4 µg) in a 500 µl tube and incubated between 4 and 16 hours at 45-55°C. Samples were vortexed 2-3 times during this digestion. Samples were heated at 94°C for 5-10 minutes to kill the proteinase K, frozen at -80°C (for times varying from overnight to several months), thawed, centrifuged at 14000 rpm (Eppendorf microcentrifuge) for 5 minutes and then diluted 100X. Two µl of this DNA (approximate concentration 3-300 ng) were used for microsatellite analysis. The tetranucleotide microsatellite loci *Ssa171*, *Ssa197*, and *Ssa202* and the dinucleotide locus *Ssa85* were run on all samples following the methods described by O'Reilly et al. (1996). In the cases where these loci proved insufficient in determining parentage (141 of 755 embryos genotyped, 35 of 392 from raceway A and 106 of 363 from raceway B; see Table 4-1 for replicate designations), additional loci were examined until paternity could be unambiguously established. In descending order, these dinucleotide loci were *Ssa12* (O'Reilly 1997), *Omy105*, and *Omy38* (Heath et al. accepted). To eliminate bias, microsatellite analyses were repeated and/or extended to all individuals that had partial genotype information but for which parentage could not initially be assigned unambiguously. No further attempts were made to genotype those embryos which had not amplified at any locus if the number of embryos from a nest that could be assigned parentage was greater than 50. Thus all embryos for which any microsatellite

information exists were unambiguously assigned parentage. For the nests in which only 10 embryos were examined, only one attempt to genotype embryos was made; all embryos with any genotype information in these nests had sufficient data to unambiguously assign parentage.

Data Analyses

Gene diversity at each locus in salmon from the Margaree River (sample sizes were 157, 166, 168, and 131 salmon for *Ssa202*, *Ssa197*, *Ssa171*, and *Ssa85*, respectively) was calculated as the heterozygosity expected under Hardy-Weinberg equilibrium using TFPGA (Miller 1997). Parentage was determined by the program PROBMAX (Danzmann 1997) which assigns progeny to parents from a mixture of potentially contributing parents when parental genotypes are known. This program also allows manipulation of the data set to identify potential mis-genotyping, such as failure to recognize a heterozygote due to stuttering or mis-genotyping by one allele length (see PROBMAX program manual).

To determine the power of these microsatellite loci to assign parentage with increased sample sizes, all embryos with complete 4-locus genotype information from each embryo data set (for raceways A and B) were combined. Parentage was then assigned by PROBMAX for each embryo data set, using the corresponding parental data set. The parental data sets were then combined, approximately doubling the number of potential parents, and the analysis rerun.

Estimating N_e

Following Lande and Barrowclough (1987), the effective number of males was calculated as:

$$N_{em} = (N_m\overline{k_m} - 1) / [\overline{k_m} + (\sigma^2{}_{km} / \overline{k_m}) - 1]$$

where N_m is the actual number of males, and k_m and $\sigma^2{}_{km}$ represent the number of offspring and associated variance, respectively, produced by an individual in its lifetime. The equivalent method was used to calculate the effective number of females. I used the determined value of individual fertilization success as my measure of lifetime success

both from each nest individually as well as for the entire raceway by weighting each nest equally.

The effective population size for each raceway was calculated after Wright (1938) as:

$$N_e = 4N_{em}N_{ef} / (N_{em} + N_{ef})$$

where N_{em} and N_{ef} are the effective number of males and females, respectively.

RESULTS

As previously reported (Jones and Hutchings In press), there was substantial allelic diversity among the wild Margaree River salmon at the four microsatellite loci examined (18, 16, 27, and 9 alleles at *Ssa202*, *Ssa197*, *Ssa171*, and *Ssa85*, respectively). Gene diversity (H_e) was similarly high (0.91, 0.90, 0.92, and 0.76 at *Ssa202*, *Ssa197*, *Ssa171*, and *Ssa85*, respectively). These levels of variation resulted in a high success rate in parental determination for each embryo with just these 4 loci (357 of 392 embryos from raceway A and 279 of 363 from raceway B). Most initially unassigned embryos had insufficient information to distinguish between 2, and occasionally 3, potential fathers. When only those embryos with complete 4-locus genotype information were used in the analysis, the number of complete parental assignments were 311 of 341 embryos from raceway A (16 with 2, and 14 with 3 potential fathers) and 262 of 336 embryos from raceway B (74 with 2 potential fathers). When the potential parental contribution was artificially increased by combining the parental data sets, the number of times these four loci were unable to distinguish between the true father and other potential fathers of a given embryo increased by 14 in raceway A (to 20 with 2, 23 with 3, and 1 with 4 potential fathers) and by 24 in raceway B (to 71 with 2, and 27 with 3 potential fathers). In all cases, additional information from other loci allowed unambiguous parental assignment.

Effect of Size on Male Reproductive Success

Overall, size was clearly an important determinant of fertilization success. The total anadromous male mean fertilization success was higher than the total parr mean fertilization success per nest (62.9% and 77.0% for the 4 anadromous males versus 37.1% and 23.0% for the 20 parr in raceways A and B, respectively, averaged over nests 1 to 6; Tables 2 and 3). Mean individual fertilization success was thus 15.7% and 19.3% for the anadromous males and 1.9% and 1.2% for the parr in raceways A and B, respectively (averaged over nests 1 to 6; similar results were obtained when the data from all nests were included). While mean anadromous male fertilization success was generally high, there were some nests in which parr obtained substantial success.

Despite the apparent importance of size among all males, there was little evidence of such an overall association within life history type. Individual mature male parr size was independent of individual fertilization success within (Table 4-2; all r^2 were less than 0.2 and their associated p-values were all greater than 0.1) and among nests in each raceway (Figure 4-1). Similarly, there was no apparent relationship between anadromous male size and individual fertilization success among nests within raceways (Figure 4-2).

Although there was no evidence of an association between individual fertilization success and parr size when all parr were included in the analysis, there was some evidence of such a relationship among only those parr identified as having spawned. In raceway A, when all 10 nests were weighted equally, there was a significant relationship between parr size and the number of nests in which a parr was identified as having spawned ($r^2 = 0.51$, $p = 0.004$; $n = 14$ parr; Figure 4-3) and between parr size and mean individual fertilization success ($r^2 = 0.43$, $p = 0.011$; $n = 14$ parr; Figure 4-3), although the latter association was not significant when the analysis was restricted to the 6 nests for which sample sizes exceeded 50 ($r^2 = 0.18$, $p = 0.17$; $n = 12$ parr for parr size and number of times an individual was identified as having spawned and $r^2 = 0.06$, $p = 0.42$; $n = 12$ parr for parr size and mean fertilization success). In raceway B in which parr were only identified as having spawned in the nests in which sample sizes exceeded 50, there was a significant relationship between parr sizes and the number of nests a parr was identified as having spawned ($r^2 = 0.77$, $p = 0.049$; $n = 5$ parr; Figure 4-3) but not parr size and mean individual fertilization success ($r^2 = 0.58$, $p = 0.13$; $n = 5$ parr; Figure 4-3).

Spawning Associations

The incidence of successful multiple anadromous spawnings within nests was low. In the 3 nests in which more than one anadromous male had spawned with the same anadromous female (nests A2, A3 and B1; Table 4-3), fertilization success of the second anadromous male was less than 4%. Among nests in which more than one female had spawned, (nests A1, A6, B2 and B3; Table 4-3), the second female contributed less than 4% of the eggs. In 2 of these 4 instances (nests B2 and B3), the same anadromous male was involved in spawning with both females.

In contrast to the low number of multiple anadromous partners within nests, multiple partnering at locations throughout the raceways appeared to be common (Table 4-3, Figure 4-4). For example, in raceway A, $♀_{ii}$ and $♀_{iii}$ spawned with anadromous males in 4 and 5 nests, respectively, and both had spawned with 3 different anadromous males. Females in raceway B also had multiple anadromous male partners, although to a lesser degree (Table 4-3, Figure 4-4). Anadromous males also spawned with multiple partners (Table 4-3, Figure 4-4); in raceway B, $♂_{iii}$ was identified as having spawned in 6 nests and spawned with all 4 anadromous females. Similar to the anadromous salmon, individual parr also appeared to participate in spawning with different females and along the entire length of the raceways (Table 4-2; Figure 4-4). In addition to spawning with multiple partners, the anadromous salmon of both sexes and the mature male parr also spawned along the entire length of the raceways (Figure 4-4).

Effective Population Size

Multiple anadromous males spawning simultaneously with the same anadromous female had little influence on the effective number of males ($N_{e\,♂}$) per nest, increasing it 3-8% above the value expected had only one anadromous male been involved in spawning at that nest (Table 4-4). Similarly, the occurrence of embryos from multiple females in a nest also only resulted in an increase in the effective number of females ($N_{e\,♀}$) per nest by 3-8% over the value expected had only one anadromous female contributed to that nest (Table 4-4). Both occurrences were also relatively rare, further diminishing their effect on N_e.

The influence of mature male parr fertilization success on $N_{e\,♂}$ per nest varied considerably but tended to be greatest when total parr fertilization success was high. For example, when total parr success was 100% in a nest in raceway A, rather than an expected $N_{e\,♂}$ value of 1 had the female spawned with a single anadromous male, $N_{e\,♂}$ per nest increased to 4.7 (nest A10, calculation not shown) and 7.7 (Table 4-4), although in raceway B, $N_{e\,♂}$ was only 1.8 at the nest with 100% parr success (Table 4-4). Low levels of total parr fertilization success (2-4%) had little influence on $N_{e\,♂}$ with $N_{e\,♂}$ per nest increasing only 3-8% due to the parr fertilization success. At moderate levels of total parr

fertilization success (30-45%), $N_{e\,♂}$ per nest was slightly greater than 2.

Restricting the analysis to the 6 nests with the largest sample sizes from each raceway, mean increases in $N_{e\,♂}$ per nest were approximately 2.3 and 1.3 times the value expected had only one anadromous male been involved in spawning at each nest in raceways A and B, respectively. These increases were mostly the result of parr fertilization success. If the nests in which parr obtained 100% success had been an artifact of the raceway structure and were excluded from the analysis, mean $N_{e\,♂}$ in both raceways declined to 1.18 times the values expected had only one anadromous male been involved in spawning at each nest.

When the variance in individual reproductive success was considered over the entire raceway, $N_{e\,♂}$ was 6.02 and 2.41 in raceways A and B, respectively, rather than an expected $N_{e\,♂}$ of 4 had only the anadromous males spawned. The $N_{e\,♀}$ was 2.59 and 2.66 in raceways A and B, respectively, rather than an expected $N_{e\,♀}$ of 4. The overall N_e was 7.24 and 5.06 in raceways A and B, respectively, a decrease from an expected N_e of 8 had only the 4 anadromous males and the 4 anadromous females spawned and had all spawned with the variance and means in individual fertilization equal as expected under idealized conditions (Hartl and Clark 1989).

If the nests in which parr had 100% fertilization success were excluded from the analyses, the preceding values all decrease. The $N_{e\,♂}$ decrease to 4.26 and 1.76 in raceways A and B, respectively, when nests A6 and B5 are excluded. The $N_{e\,♀}$ decrease to 2.45 and 2.37 in raceways A and B, respectively. The overall N_e declines to 6.22 and 4.04 in raceways A and B, respectively. If the total parr contribution is artificially reduced to 0% (by assigning all parr fertilization success to the anadromous male that dominated at each nest), the $N_{e\,♂}$ declines to 3.63 and 1.48 at raceways A and B, respectively, resulting in N_e values of 5.85 and 3.64. This represents a decline in N_e of only 9% over their preceding values.

DISCUSSION

Effect of Body Size on Male Reproductive Success

Although I documented high variation in individual fertilization success, the considerably larger anadromous males obtained much greater success than parr. This result is consistent with all previous studies of alternative reproductive success in Atlantic salmon (Hutchings and Myers 1988, Jordan and Youngson 1992, Morán et al. 1996, Thomaz et al. 1997, Jones and Hutchings In press). Parr need not have as high fertilization success as anadromous males to obtain similar fitness because they reproduce at a younger age and avoid the high mortality associated with anadromy (Hutchings and Myers 1994).

Although I found mean individual parr fertilization success to be approximately 13 times lower than that of the mean individual anadromous male fertilization success, I may still have overestimated individual parr fertilization success. In natural situations, the ratio of mature male parr to anadromous males appears to be higher than that in my experiment (e.g., L'Abée-Lund 1989), which, at similar total parr fertilization success levels, would result in lower mean individual parr fertilization success. My test of the potential power of these genetics markers to distinguish among greater numbers of potential parents suggests that successful parentage assignment can be achieved with greater numbers of potential contributors. The most frequent cases of unassigned parentage in my simulation involved the inability to distinguish between 2 or 3 potential fathers and generally involved the same individuals in each raceway. Pre-screening of individuals to be used in experiments could allow for the exclusion of those individuals sharing alleles at multiple loci, preventing such analytical difficulties. Alternatively, as demonstrated in this experiment (e.g., with 2 males in raceway B which required an additional 3 loci to unambiguously determine paternity), additional loci can be used to distinguish between potential parents. It is thus possible in future studies to increase the number of parr present to more natural ratios.

The influence of increased anadromous male competition on total parr success is difficult to ascertain. Single anadromous pair cross experiments, albeit with variation within and among studies, have typically found total parr fertilization success per nest to

be approximately 30% (Hutchings and Myers 1988 - 20%, Morán et al. 1996 - 51%, Thomaz et al. 1997 - 30%, Jones and Hutchings In press - 31%), while the only multiple-pair cross to date revealed considerably lower total parr success (Jordan and Youngson 1992 - 11% mean total parr success). The results from my experiment suggest higher total parr success (30%) in multiple anadromous pair crosses. However, if the 3 nests in which parr obtained 100% success were an artifact of the raceway structure (e.g., nests constructed immediately adjacent to the walls) and are excluded, total parr success would be considerably lower (16%), supporting the hypothesis that increased anadromous male competition results in lower total parr fertilization success. I recognize that my findings remain somewhat inconclusive as the raceway walls could function in a manner similar to large boulders found in natural systems. Thus, the results obtained in nests adjacent to the raceway walls may indeed reflect natural conditions.

During spawning, mature male parr are arranged in a hierarchical manner with the largest male nearest the female (Myers and Hutchings 1987). At low parr densities (6 parr), one study found a relationship between individual parr size and reproductive success when parr were competing with one anadromous male for fertilization opportunities with one anadromous female (Thomaz et al. 1997). The cost of maintaining this improved access to the female is expected to increase with larger numbers of competitors to a point at which body size may have no influence on fertilization success (Hutchings and Myers 1994); Jones and Hutchings (In press) found some evidence supporting this. In the present experiment, only 2 of 18 nests showed evidence of more than two parr successfully spawning and many parr were found not to have fertilized any eggs at all. It is thus not surprising that no relationship between parr size and individual reproductive success was found either within or among nests. Larger parr might be expected to spawn more frequently and thus have lower variance in mean reproductive success (Jones and Hutchings In press). Although the low detected success rate precludes a testing of this hypothesis in my experiment, the apparent relationship between parr size and number of nests in which an individual spawned, among those parr who were detected to have spawned, provides some support for it.

In contrast to the number of studies that have examined the factors influencing individual parr reproductive success, less attention has been directed to examining the factors influencing individual reproductive success among anadromous males. I found no evidence of an influence of size on anadromous male reproductive success. The high individual reproductive success by a smaller male in raceway B contrasts somewhat with Mjølnerød et al.'s (1998) finding that size was often an important determinant of dominance and individual fertilization success. Jones (1959), however, noted that the dominance of an anadromous male in a group "is certainly not dependent on size" and suggested that dominance may be related to an individual's readiness to spawn.

Spawning Associations

Both anadromous males and females spawned with multiple mates. Females clearly spawn with different males in successive spawning events and males do spawn with different females. The results from this study may even underestimate the number of potential spawning partners because the maximum number of potential anadromous mates was limited to 4. Anadromous females lay many nests (e.g., Jones (1959) - up to 8, Fleming (1996) - 5 to 7) and can spawn with multiple males each time (e.g., Mjølnerød et al. 1998). Mjølnerød et al. (1998) described using one anadromous male in 31 different spawning trials. Although information regarding this male's involvement in his earlier trials (1-27) was not presented, he was still obtaining some fertilization success as late as the 29th event while another male was dominating fertilization success as late as his 15th trial.

I observed little evidence of multiple anadromous males being involved in spawning within nests. Among the few cases I detected, the second anadromous male achieved very little fertilization success. Although single-pair matings are common, matings involving multiple males are reportedly of a higher frequency than observed in my experiment (13-63%, Fleming 1996). Further, when multiple male fertilization events occur, the second male has been reported to obtain slightly higher fertilization success than that reported here (up to 16%, Mjølnerød et al. 1998). The occurrence of embryos from multiple females in the same nest suggests that some degree of nest

superimposition occurred in my experiment, although the extent of complete nest displacement cannot be assessed.

Female Atlantic salmon place their eggs in multiple nests. Barlaup et al. (1994) suggested that this is not a result of patchy high quality substrate but a result of a "bet-hedging" strategy. My finding of multiple nests per female over the length of the raceway in an environment where all substrate was suitable for spawning supports this contention. Some females have up to 4 redds, each redd potentially containing several nests (Fleming et al. 1996) and can move over 500 m between them (Baglinière et al. 1990). This behaviour could also reflect a "bet-hedging" strategy (Fleming 1996).

Effective Population Size

The results of my experiment suggest that variance in anadromous male individual fertilization success has the greatest overall influence on N_e while parr individual fertilization success is of only moderate influence. The effective number of males, both within nests and raceways, was lower than the number of males identified as having any fertilization success and was much lower than the number of males available for spawning (24 males were present in each raceway, the overall $N_{e\,\sigma}$ was 6.02 and 2.41 in raceway A and B, respectively).

Because estimates of the number of anadromous males often exist while usually little is known about the number of mature male parr present at spawning, the influence of mature male parr fertilization success, in addition to that of the anadromous males, is of great interest. Within individual nests, the influence of parr fertilization success can increase the effective number of males several times that which would be expected had only one anadromous male spawned with the female. From such a finding alone or by extrapolating from previous single-pair cross experiments, one might conclude that parr fertilization success would profoundly affect N_e. However, due to their greater overall fertilization success, anadromous males have a greater potential influence on N_e than parr. This potential is realized because there is great variation in individual anadromous male fertilization success. Indeed, variance in both anadromous male and female success resulted in a decrease in N_e over the value expected had there been no variance in

reproductive success by up to 46% and 26%, respectively, while parr success may increase N_e by as little as 9% (Table 4-4). I suggest that a critical parameter to quantify in the wild is variance in anadromous salmon reproductive success, and parr fertilization success, as a determinant of N_e, is likely to be of secondary import.

Assumptions made in my calculations of N_e can result in some biases. For all calculations of N_e, I weighted each nest equally. This could result in an overestimation of N_e for two reasons. Firstly, the number of eggs per nest, spawned by a single female, tends to decline with each subsequent nest constructed by that female (Fleming et al. 1996). If some males are more successful in spawning with females when females first spawn, there will be a higher variance in individual male fertilization rate resulting in a lower $N_{e\,\sigma}$. Secondly, nests can be lost (Barlaup et al. 1994) and loss may depend on factors such as spawning time, or the ability of females to obtain desirable spawning locations (Fleming et al. 1996). Nest loss will result in an increase in the variance of individual female reproductive success resulting in a further decrease in $N_{e\,♀}$. While these factors may bias my calculations of N_e, my overall conclusions remain robust.

In reviewing the factors that influence the ratio of effective to actual population sizes in nature, Frankham (1995) concluded that variance in family size and unequal sex ratios were of prime importance. However, of greater importance was the effect of fluctuations in population size; this was not addressed in my study. Over the short term, N_e is the harmonic mean of the annual effective population size and is thus most influenced by the smallest annual N_e (Wright 1938). When populations are at their lowest abundance, fewer anadromous males will be present on the spawning grounds. Simultaneously, the number of mature male parr per female will remain constant or possibly increase; when parr densities are lower, growth rates increase and faster-growing individuals tend to have a higher probability of maturation (e.g., Thorpe 1986). Thus, at low population sizes, mature male parr may have their highest fertilization success and may have their greatest within-generation influence on N_e.

My findings have many general implications to natural populations of other species. The different findings in my two replicates emphasize not only the clear need for

replication in such experiments but also how, even in populations of stable size, annual differences in N_e will likely exist due to inter-annual variation in individual reproductive success. Again, because N_e is the harmonic mean of the annual effective population size and is thus most influenced by the smallest annual N_e, this can be of great concern to threatened or endangered species for which population sizes are already small. When quantification of N_e is desirable to aid in the conservation of natural populations, quantification of the variance in individual reproductive success, and the potential degree of inter-annual variability in this estimate, is essential.

Table 4-1. Lengths of anadromous males ($\sigma^{\prime}_{i}$ - $\sigma^{\prime}_{iv}$), anadromous females (φ_{i} - φ_{iv}), and number of parr from each size class in each replicate raceway (Rep.).

	Anadromous salmon lengths (cm)								**# parr from each size (mm) class**			
Rep.	$\sigma^{\prime}_{i}$	$\sigma^{\prime}_{ii}$	$\sigma^{\prime}_{iii}$	$\sigma^{\prime}_{iv}$	φ_{i}	φ_{ii}	φ_{iii}	φ_{iv}	**70-80**	**81-90**	**91-110**	**111-122**
A	80	78	63	52	82	81	78	74	8	7	4	1
B	90	78	60	59	90	84	82	77	8	7	4	1

Table 4-2. Individual parr fertilization success in each nest. Mean individual parr success is given for nests 1-6 ($\bar{x}_{1\text{-}6}$) and for all nests ($\bar{x}_{all}$) as are the sample size (n) and total parr success (Total (%)) for each nest. Samples sizes for the means refer to the number of nests.

		Individual parr fertilization success (%) in nest:											
Parr[1]	**length[3]**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	$\bar{x}_{1\text{-}6}$	$\bar{x}_{all}$
a2	77									10		**0**	**1**
a4	79									20		**0**	**2**
a5	80						15.3					**2.6**	**1.5**
a7	82						1.7					**0.3**	**0.2**
a8	83						11.9					**2**	**1.2**
a9	83						3.4					**0.6**	**0.3**
a11	88						13.8					**2.3**	**1.4**
a12	89						5.1				12.5	**0.9**	**1.8**
a13	89						25.4					**4.2**	**2.5**
a14	90						8.5				37.5	**1.4**	**4.6**
a15	91				1.7	74.6						**12.7**	**7.6**
a17	99						8.5					**1.4**	**0.9**
a18	107		1.7	44.6				44.4			12.5	**7.7**	**10.3**
a19	107						6.8				37.5	**1.1**	**4.4**
b6	78					33.3				n/a	n/a	**5.6**	**4.2**
b7	79				1.9		1.8			n/a	n/a	**0.6**	**0.5**
b11	87			13.3						n/a	n/a	**2.2**	**1.7**
b13	89		1.8		3.8					n/a	n/a	**0.9**	**0.7**
b17	106				11.3	66.7	1.8			n/a	n/a	**13.3**	**10**
A - *n*		60	58	56	60	59	59	9	9	10	8	**6**	**10**
A - Total (%)		**0**	**1.7**	**44.6**	**1.7**	**74.6**	**100**	**44.4**	**0**	**30**	**100**	**37.1**	**39.7**
B - *n*		59	56	60	53	60	56	9	10	n/a	n/a	**6**	**8**
B - Total (%)		**0**	**1.8**	**13.3**	**17**	**100**	**3.6**	**0**	**0**	**n/**	**n/a**	**22.6**	**17**

[1]Only parr identified as having fertilization success are included in table; no fertilization success was detected for parr in Raceway A of 74, 77, 81, 85, 93 and 122 mm and in Raceway B of 70, 73, 75, 77, 78, 82, 83, 84 88, 91, 95[2], 100[2], 109, 114, and 118 mm.

[2]Lengths approximate because parr decomposing at time of collection.

[3]Length frequencies differ from those presented in Table 4-1 as a result of parr growth over the course of the experiment.

Table 4-3. Individual anadromous female (anad. ♀) involved, individual anadromous male fertilization success (anad. ♂ success), total parr fertilization success and the number of embryos for which parentage was assigned (*n*) for each nest. When embryos from more than one female were found in a nest, the nest was subdivided based on the anadromous female contribution.

Nest	n	anad. ♀	anad. ♂ success	total parr success
$A1_{ii}$	59	$♀_{Aii}$	$♂_{Aii}$ -100%	0%
$A1_{iii}$	1	$♀_{Aiii}$	$♂_{Ai}$ -100%	0%
A2	58	$♀_{Aiii}$	$♂_{Ai}$ - 94.8%; $♂_{Aiii}$ - 3.4%	1.7%
A3	56	$♀_{Aiii}$	$♂_{Aiv}$ - 53.6%; $♂_{Aiii}$ - 1.8%	44.6%
A4	60	$♀_{Aii}$	$♂_{Ai}$ - 98.3%	1.7%
A5	59	$♀_{Aii}$	$♂_{Aiii}$ - 25.4%	74.6%
$A6_{vi}$	58	$♀_{Aiv}$	0%	100%
$A6_{ii}$	1	$♀_{Aii}$	0%	100%
A7	9	$♀_{Aiii}$	$♂_{Aiv}$ - 55.6%	44.4%
A8	9	$♀_{Aii}$	$♂_{Ai}$ - 100%	0%
A9	10	$♀_{Aiii}$	$♂_{Aiii}$ - 70%	30%
A10	8	$♀_{Ai}$	0%	100%
B1	59	$♀_{Biv}$	$♂_{Bi}$ - 98.3%; $♂_{Bii}$ - 1.7%	0%
$B2_{i}$	54	$♀_{Bi}$	$♂_{Biii}$ - 100%	0%
$B2_{ii}$	2	$♀_{Bii}$	$♂_{Biii}$ - 50%	50%
$B3_{i}$	58	$♀_{Bi}$	$♂_{Biii}$ - 87.9%	12.1%
$B3_{ii}$	2	$♀_{Bii}$	$♂_{Biii}$ - 50%	50%
B4	53	$♀_{Bi}$	$♂_{Biii}$ - 83%	17%
B5	60	$♀_{Biii}$	0%	100%
B6	56	$♀_{Biii}$	$♂_{Biii}$ - 96.4%	3.6%
B7	9	$♀_{Biv}$	$♂_{Bi}$ - 100%	0%
B8	10	$♀_{Biv}$	$♂_{Biii}$ - 100%	0%

Table 4-4. Actual ($N_{a\,♂}$) and effective number of males ($N_{e\,♂}$), actual ($N_{a\,♀}$) and effective females ($N_{e\,♀}$), and effective population size (N_e) calculated for each nest. Mean effective numbers ($\bar{x}$) were calculated as the mean of the $N_{e\,♂}$ and $N_{e\,♀}$ from each nest in a raceway; N_e was calculated from these resulting means. The overall effective numbers (overall) were calculated by using the overall individual fertilization success values from each raceway (weighting each nest equally at $n = 50$). Similar calculations were made excluding the nests in which parr obtained 100% fertilization success ($A_{not\,6}$ and $B_{not\,5}$). The influence of parr on effective sizes was removed by assigning all parr fertilization success to the dominant male at that nest (no_parr).

Nest	$N_{a\,♂}$	$N_{e\,♂}$	$N_{a\,♀}$	$N_{e\,♀}$	N_e
A1	2	1.03	2	1.03	2.07
A2	3	1.11	1	1	2.11
A3	3	2.1	1	1	2.71
A4	2	1.03	1	1	2.03
A5	2	1.63	1	1	2.48
A6	10	7.65	2	1.04	3.66
B1	2	1.04	1	1	2.03
B2	2	1.04	2	1.08	2.11
B3	2	1.31	2	1.07	2.35
B4	4	1.43	1	1	2.36
B5	2	1.82	1	1	2.58
B6	3	1.08	1	1	2.07
A $\bar{x}$	3.67	2.43	1.33	1.01	2.85
$A_{overall}$	3.67	6.02	1.33	2.59	7.24
$A_{not\,6}$	2.4	4.26	1.2	2.45	6.22
A_{no_parr}	-	3.63	-	2.45	5.85
B $\bar{x}$	2.5	1.29	1.33	1.03	2.29
$B_{overall}$	2.5	2.41	1.33	2.66	5.06
$B_{not\,5}$	2.6	1.76	1.4	2.37	4.04
B_{no_parr}	-	1.48	-	2.37	3.64

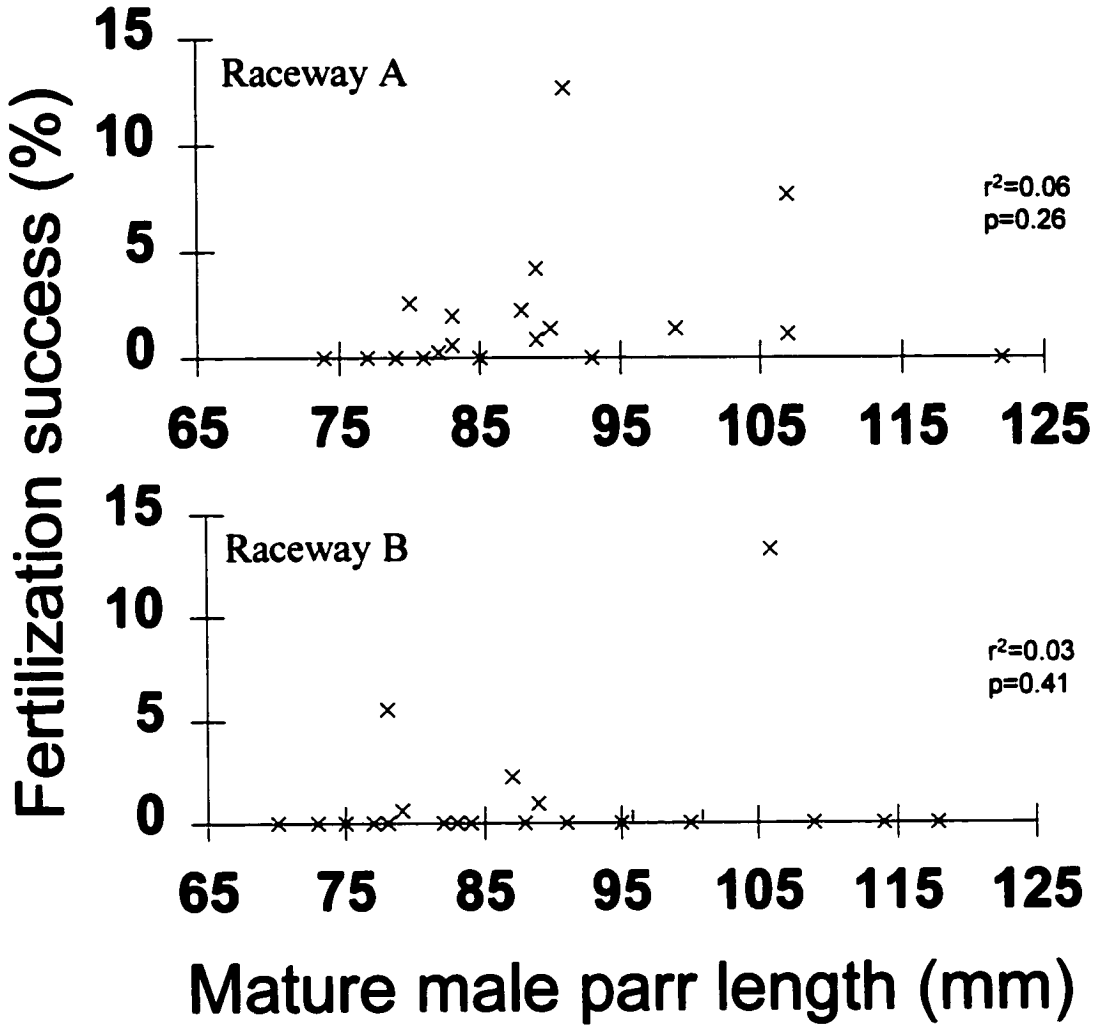

Figure 4-1. Individual parr fertilization success averaged over 6 nests (X) versus parr length for each raceway (A and B). Length frequencies differ from those presented in Table 4-1 as a result of parr growth over the course of the experiment. In raceway B, X[1] parr lengths were from decomposed parr and are approximate. The r^2 and associated *p*-values refer to the linear regressions between individual parr fertilization success and parr body size.

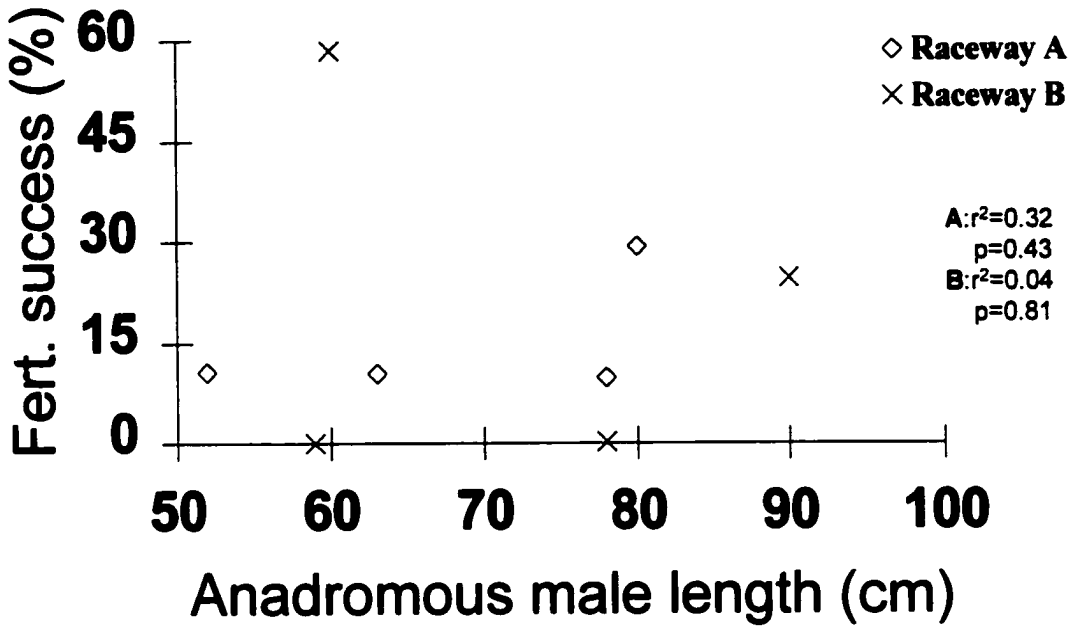

Figure 4-2. Individual anadromous male fertilization success versus anadromous male length averaged over 6 nests for raceway A (◇) and raceway B (×). The r^2 and associated *p*-values refer to the linear regressions between individual anadromous male fertilization success and anadromous male body size.

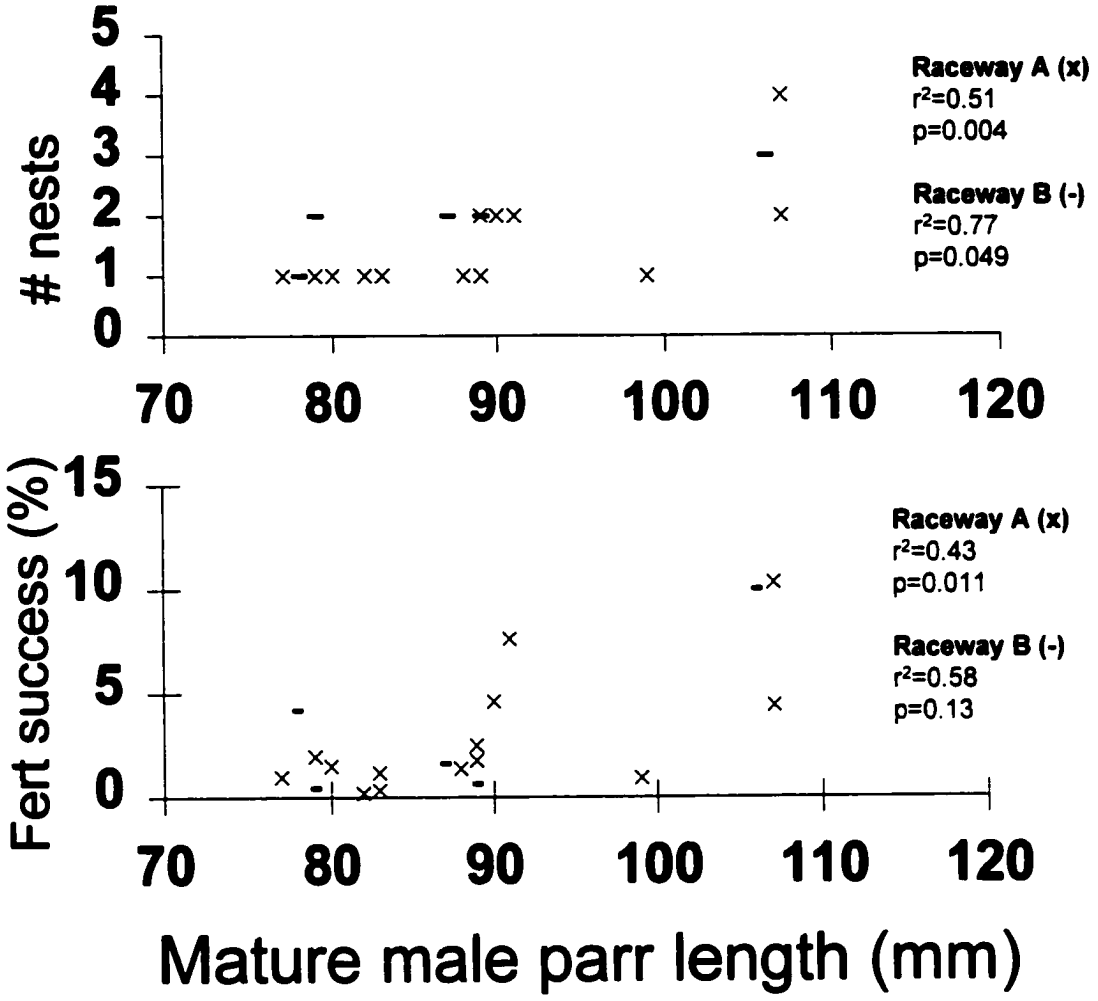

Figure 4-3. Number of nests in which an individual mature male parr was identified as having spawned (# nests) and mean individual parr fertilization success versus mature male parr length for those parr who were identified as having spawned in any of the 10 nests in raceways A (X) and 8 nests in raceway B (-). The r^2 and associated p-values refer to the linear regressions between the respective y variables and mature male parr length.

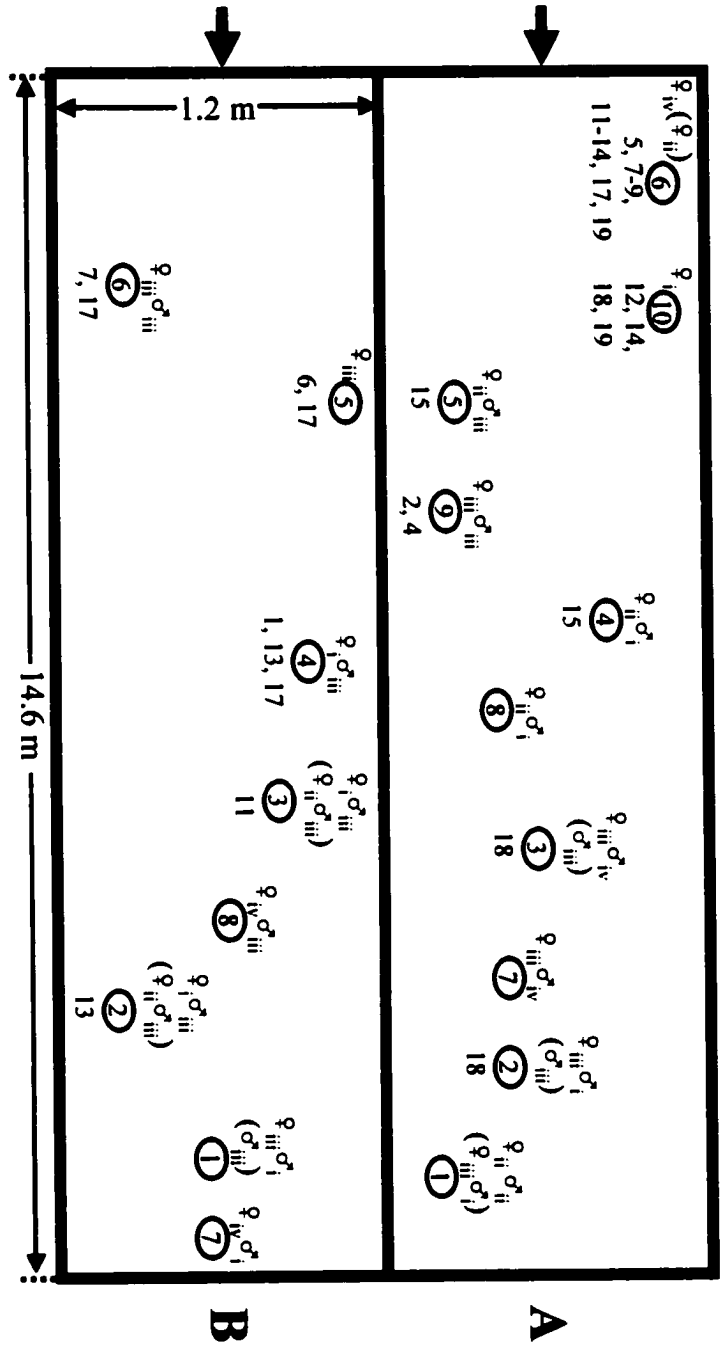

Figure 4-4. Anadromous males ($\sigma^{\prime}_{i}$ - $\sigma^{\prime}_{iv}$), anadromous females (φ_{i} - φ_{iv}) and parr (1-20) involved in spawning at each nest in both raceways. Number in ellipses refer to the nest numbers designated in Tables 2 to 4. Anadromous fish with minimal contribution (1-2 embryos) at a nest are indicated in parentheses. Arrows indicate location of water inflow. Note that the raceway lengths and widths are not to scale and that the nest sizes are exaggerated.

CHAPTER 5.

Temporal and spatial microsatellite variation in Atlantic salmon populations in eastern Canada: implications for conservation and management

INTRODUCTION

Habitat fragmentation, environmental degradation and species exploitation have all contributed significantly to population extinctions. Having both a freshwater and marine component to their life, anadromous species such as Atlantic salmon (*Salmo salar*) are especially sensitive to exploitation and habitat alterations, including physical barriers to migration. Consequently, anadromous species have had a lengthy history of population declines and extirpations. As a result of their commercial and recreational value, Atlantic salmon have also been the recipients of well-intentioned efforts to supplement and reintroduce small and extirpated populations. Reintroduction programmes are often labour-intensive and expensive; appropriate source populations need to be identified and sufficient numbers of individuals translocated. This effort and expense may be justifiable if the programmes are successful and the purported benefits are realized.

As reintroduction programmes are increasingly being used in conservation management of a variety of species, it would be useful to examine a previous attempt to reintroduce Atlantic salmon. Beginning in the early and mid 1800s, Atlantic salmon rivers in the Inner Bay of Fundy (Inner Bay) were often used as a means of transporting timber to the sea, frequently with sawmills and associated dams at their base. These complete barriers to seaward migration caused the extirpation of Atlantic salmon in many of these rivers. When these obstructions are removed, re-establishment can occur either by natural recolonization or by artificial reintroduction. Given the absence of reintroduction programmes in most Inner Bay rivers, current Atlantic salmon populations exist as a result of natural recolonization.

The Point Wolfe River, whose barrier was not removed until 1985, is one Inner Bay river for which a reintroduction programme was implemented. Reintroduction was deemed more desirable than allowing re-establishment by natural straying partially

because of concerns of low numbers of founders and associated genetic effects, the possibility that strays (i.e., salmon returning to the Point Wolfe River rather than their river of origin) would be less desirable, and the lengthy time required to establish the expected population size (Alexander and Galbraith 1982); it is unclear whether such benefits to reintroduction truly existed or whether they justified the effort of this program.

Since that time, the number of anadromous adults in the Inner Bay of Fundy has declined almost two orders of magnitude, from an estimated 40,000 to fewer than 500 (L. Marshall, Department of Fisheries and Oceans, pers. com.). When the results of reintroduction programmes are not those anticipated, it is critical to determine the reasons for their lack of success (Campbell 1980). Furthermore, it would be of interest to conservation biologists to understand the effect of low population sizes in the wild on subsequent population genetic diversity.

Many Atlantic salmon undergo a long migration to offshore Greenland and remain at sea for two or more winters (Multi Sea Winter or MSW salmon) while others do not migrate as far and remain at sea for only one winter (grilse). Atlantic salmon from most rivers in the Inner Bay are thought to be grilse, and subsequently repeat spawners with very few MSW salmon. Salmon from the Inner Bay also appear to have different trends of abundance than salmon from other regions (e.g., Huntsman 1931) and to smoltify at younger ages (e.g., White and Huntsman 1938, Jessop 1986). Such differences have led some to hypothesise that the Inner Bay salmon represent a separate grouping. Understanding the evolutionary relationship among individuals from different rivers and regions can be difficult in anadromous species. On the one hand, there is a high potential for gene flow among rivers. But this potential is mitigated, to various degrees, by the propensity of salmon to home to their natal river (Stabell 1984), allowing for differentiation among rivers and adaptation to local environments within rivers (e.g., Taylor 1991). The current low population sizes in most rivers in the Inner Bay have prompted efforts to have these populations officially recognized as being endangered, including the establishment of a live gene bank programme. It is thus not only of general interest but essential to the implementation of an appropriate future conservation

programme to understand the evolutionary relationship of Inner Bay Atlantic salmon to other Maritime populations.

The effective size of many salmonid populations is small (i.e., less than a few hundred individuals), potentially resulting in large variation in allele frequencies. Thus, to determine the biological significance of genetic structuring of populations in a given region, it is essential to assess the temporal stability of the observed variation. This becomes even more important when populations have declined because greater drift in allele frequencies might be expected among generations than when population sizes are larger. Historical stocking practices can lead to introgression of foreign alleles and the genetic divergence of stocked populations from their historical genetic composition (e.g., see discussion in Nielsen et al. 1999). Thus, it is also of value to examine historical relationships among populations which have been manipulated because it is these relationships that are desirable to re-establish. The advent of the aquaculture industry in the Bay of Fundy may further confound the genetic relationships among populations because escapees can alter the genetic composition of native salmon populations (e.g., Clifford et al. 1998). It is further necessary to examine temporal genetic variation to assess the utility of actively reintroducing salmon (as opposed to allowing natural recolonization) and to quantify the effects of low population sizes on genetic diversity. Recent developments with genetic techniques (e.g., microsatellite analysis) allows for the use of historical samples (e.g., fish scales) for analysis (reviewed in O'Reilly and Wright 1995), making it possible to examine historical relationships among populations.

The objectives of this study were three-fold. I first tested whether a reintroduction programme would be preferable to a natural recoloziation process from a genetic diversity point of view. This was done by comparing the genetic variation of Atlantic salmon actively reintroduced to a river to the genetic variation of salmon in a naturally recolonized river. I also compared the genetic similarity between the reintroduced population to its source population. Secondly, I quantified the effects of declining numbers of returning anadromous salmon on the genetic diversity of juvenile parr. Thirdly, I quantified the genetic diversity among Atlantic salmon populations in the Inner

Bay and the degree of differentiation between Inner Bay salmon populations and populations in other Maritime rivers.

METHODS

Populations in the Inner Bay of Fundy

I quantified microsatellite variation among individuals from 6 rivers in the Inner Bay and 2 elsewhere in the Canadian Maritimes (Figure 5-1; Table 5-1). For the purpose of analysis, individuals were grouped into "collections" based on sampling location, life history type (parr or anadromous) and year group. Year groups were defined as the year of return for adults and the year of birth for parr. When parr were collected from several locations in one river in a given year, sub-location designations were maintained (Table 5-2). In two instances of low sample sizes, parr of different ages among which genetic variation did not differ were combined (parr from the Point Wolfe River 1982 and 1984 year groups and the 1980-1982 year groups from the Gaspereau River; Table 5-2). In six instances, only fin clips of parr of mixed, undetermined ages were collected (5 locations in the Big Salmon River and parr from the Margaree River); these were treated as single samples for each of these rivers.

Point Wolfe River

Point Wolfe River has been dammed intermittently since 1823 for log driving and to power a sawmill. Early dams were probably not complete barriers to salmon migration and were often washed out, e.g., over 500 salmon were caught in a weir in the tidal flats at the river mouth in 1849 (Perley 1851). The dams were later constructed in a sturdier fashion. Due to these dams, Atlantic salmon were probably extirpated from the Point Wolfe River by the time Fundy National Park was created in 1948. Prior to the removal of all dams in 1985, 42 000 fall fingerlings had been stocked into Point Wolfe River annually from 1982 to 1985 (Granger and Priest 1988). These fish were the progeny of Atlantic salmon in Big Salmon River, located 70 km west of Point Wolfe River. It was predicted that, by 1988, more than 1300 salmon would return to the river annually to spawn (Alexander and Galbraith 1982). This expectation was never realized. The estimated number of returning salmon peaked at 200 in 1985 and has since declined to zero (Jones and Clay 1995; Figure 5-2; A. Caissie, Parks Canada, pers. com.).

Upper Salmon (Alma) River

This river has a history of anthropogenic influences similar to that of Point Wolfe River. However, after the collapse of its last remaining dam in 1954, Atlantic salmon recolonized the river without intervention. The number increased rapidly with as many as 1200 anadromous salmon estimated to have returned to the river in 1967, but returns subsequently declined to near zero in the late 1980s and early 1990s (Jones and Clay 1995; Figure 5-2). No recorded stocking of salmon has taken place in this river.

Big Salmon River

Big Salmon River, another river integral to the logging industry, was dammed in the mid 1800s and in the 1920s. In the early 1930s, a fish passage was built; in 1963, the dam was removed (Jessop 1986). This river has been stocked with non-native salmon intermittently from 1938 to 1969 (Jessop 1986). Known origins of stocked fish include Miramichi River (1963-1966) and Restigouche River (1968 and 1969; Jessop 1976). Smolts from Big Salmon River hatchery broodstock were raised in sea cages and approximately 200 adults were released into Big Salmon River to spawn in each of 1994 and 1995. As a result of this action, parr densities and adult returns increased for a period of time (Amiro and Jefferson 1998)

Petitcodiac River

The Petitcodiac has the largest drainage size of any river in the Inner Bay (Table 5-1). Following the construction of a causeway in 1967 (which included an inadequate fish ladder), salmon numbers declined and the species was eventually extirpated. I included samples from this river because of its potential historical importance as a source population for other Inner Bay populations. Samples were limited to collections made during the population decline. Stocking that has occurred in this river includes salmon from the Miramichi River and River Philip between 1942 and 1946 (Elson 1962). Undocumented stocking in the 1980s may have occurred using progeny of Big Salmon River hatchery broodstock (P. Amiro, Department of Fisheries and Oceans, pers. com.).

Stewiacke River

This river has the second largest drainage size of Inner Bay rivers (Table 5-1) and is the largest river in the Minas Basin (Figure 5-1). A small number of salmon from a Miramichi source may have been stocked in the Stewiacke in the mid 1960s (P. Amiro, pers. comm.). Since 1970, the only salmon stocked have been derived from Stewiacke River collections. Like other Inner Bay rivers, the number of anadromous salmon returning and the number of parr present in this river have declined since 1990 with few adults observed in recent assessment surveys (P. Amiro, pers. com.).

Gaspereau River

Possibly unlike other Inner Bay populations, Gaspereau River salmon are believed to spawn after more than one winter at sea following a migration to Greenland (Amiro and Jefferson 1996). Due to a hydro-electric dam and an ineffectual fish ladder, this population has been heavily augmented by hatchery fish derived from returns to the river. My collections were broodstock fish. Thus, despite low sample sizes, they are representative of the genetic variation present in this river, at least for the years during which the fish were sampled.

Additional Study Populations

To assess the extent of differentiation between Inner Bay populations and other Maritime Canadian populations, and to provide a reference scale for the differentiation within the Inner Bay, I included collections from 2 other rivers, namely the Margaree River (Cape Breton) and the Hammond River (drains into Kennebecasis Bay which is itself an embayment of the Saint John River estuary, an outer Bay of Fundy river) (Figure 5-1). The Hammond River was stocked with Saint John River source salmon until the late 1970s, after which progeny from the early return component of the Hammond River anadromous fish returns have been stocked (T. Pettigrew, pers. com.). The mill dam located on the Hammond River until the early 1900s was 15 km upstream, below which suitable habitat for spawning exists (T. Pettigrew, pers. com.).

Genetic Analyses

DNA was extracted from dried scales and EtOH-preserved fin samples. Anadromous salmon scales (n = 1-3) were digested in 200 µl of eyeball buffer (10 mM Tris, 50 mM KCl, 0.5% tween20) and proteinase K (0.1-0.4 µg) in a 1500 µl tube. Parr scales (n = 3-10) and fin tissue (approximately 50 mg) were digested in 100 µl of eyeball buffer and proteinase K (0.1-0.4 µg) in a 500 µl tube. Samples were incubated between 2 and 16 hours at 45-55°C and were vortexed 2-3 times during this digestion. Samples were heated at 94°C for 5-10 minutes to kill the proteinase K, centrifuged at 14000 rpm (Eppendorf microcentrifuge) for 5 minutes and diluted if necessary. Two µl of this DNA (approximate concentration 3-300 ng) were aliquoted into microtitre plates and used for microsatellite analysis. The tetranucleotide microsatellite loci *Ssa171*, *Ssa197*, and *Ssa202* and the dinucleotide loci *Ssa85* (O'Reilly et al. 1996) and *Ssa12* (O'Reilly 1997) were analyzed on all samples, following methods modified from O'Reilly et al. (1996).

Modifications were required because the DNA quality obtained from scales was poor, resulting in many artifact bands for most loci. Consequently, tetraplexing as described by O'Reilly et al. (1996) was only possible for EtOH preserved tissue samples. When the DNA source was dried scales, the initial examination of microsatellite variation was conducted using 3 PCRs, one with *Ssa171* and *Ssa197*, one with *Ssa202* and *Ssa85*, and one with *Ssa12*. If incomplete information was obtained for any sample, each locus was re-examined separately; an alternate set of primers for *Ssa202* (*Ssa202*'a: CATGTGTTAATGTTGCGTG, *Ssa202*'b: GGTAACTGGCTCAACTCAC, 56°C annealing temperature; P.T. O'Reilly, pers. com.), which had a product 162 bp smaller, was used in the second and subsequent attempts to amplify this locus. All PCRs were conducted in 96-well microtitre plates. Eight individuals of known genotype were included in each PCR in the last row of each plate. These individuals were selected on the basis of their allele sizes at *Ssa171* to create an allelic ladder spanning the allelic size range with regular rungs; such a ladder is necessary to accurately score this locus and was placed after every eighth sample on each gel. Gels were manually scored and verified by a second researcher. Poor DNA quality, as well as older isotope and/or old developer

chemicals, occasionally resulted in weak bands. Information was omitted for individuals homozygous at a locus on a poorer quality autoradiograph and for which no subsequent analysis improved the result. This most affected results at *Ssa171* and, to a lesser degree, at *Ssa202* and *Ssa197*. Similarly, autoradiographs for which the allelic ladder did not work were not used.

Statistical Analyses

Within-collection heterogeneity

Observed heterozygosity, gene diversity, mean number of alleles, conformity to Hardy-Weinberg expectations, and pairwise linkage disequilibrium were analysed with GENEPOP version 3.2a (Raymond and Rousset 1995) and pop100gene version 1.1.02 (Piry and Bouget, available at http://www.ensam.inra.fr/URLB/pop100gene/pop100gene.html). Conformity to Hardy-Weinberg expectations was tested using the exact test of Guo and Thompson (1992). When the number of alleles at a locus in a collection was less than 5, the complete enumeration (Louis and Dempster 1987) was used. For loci with more than 4 alleles in a collection, a Markov chain method was used to estimate the exact *p*-value (Guo and Thompson 1992). To allow comparison of the number of alleles at each locus in each collection despite unequal sample sizes, sample sizes were equalized to a low value. I developed a Visual Basic macro in Microsoft Excel (version 2000) to determine the mean number of unique alleles/locus in 1000 random samples of 40 alleles (equivalent to n = 20) at each locus in each collection; for each locus in each collection, i) 40 alleles were sampled with replacement, ii) the number of unique alleles counted, iii) i and ii repeated 1000 times, iv) the mean of the 1000 sampling events calculated.

Among-collection Heterogeneity

Heterogeneity among collections was quantified by θ and Φ_{ST}; two metrics were used to test whether Inner Bay salmon were members of a unique phylogenetic lineage. Weir and Cockerham's (1984) weighted analysis of variance was used to estimate θ (θ is analogous to F_{ST} but is independent of sample size, number of alleles at each locus and number of subpopulations sampled) and an analogous method was used to calculate Φ_{ST} (Michalakis

and Excoffier 1996), a measure which incorporates allele size and which may be more sensitive to divergent phylogenetic histories. Analyses were conducted at two levels: among collections within rivers and among rivers. For within-river collections, all year groups and/or sub-locations were used; for among-river comparisons, all collections from each river were pooled. Both analyses were repeated by excluding collections that deviated from Hardy-Weinberg expectations or that had more than one pair of loci in significant linkage disequilibrium. All of these calculations were made with GENEPOP. Significance of these values was tested using a χ^2 statistic (Workman and Niswander 1970, modified by Jordan et al. 1992). The number of alleles used to calculate this χ^2 statistic was the lowest number found at any locus in any of the populations included in the test. This resulted in a slight underestimate of the number of alleles per locus, thus making the test a conservative one.

To partition genetic variation among collections, I constructed dendrograms with two estimates of genetic distance, using the neighbour-joining method (Saitou and Nei 1987). First, I used a modified Cavalli-Sforza and Edwards (1967) chord distance (D_A; Nei et al. 1983), the underlying model of which assumes pure genetic drift. I also used D_{SW} distance (Shriver et al. 1995), the underlying model of which assumes a stepwise mutation model and uses the assumed phylogenetically informative value of the differences in allele sizes by including the number of mutational steps between alleles into the distance estimate (Shriver et al. 1995). Distance measures which incorporate variance in allele size may better reflect phylogenetic relationships and genetic distances among populations if either time since divergence or mutation rates is high (Shriver et al. 1995). Bootstrapped confidence values on branches were obtained by resampling loci within collections 1000 times. The genetic distances and dendrograms were calculated with NJBAFD (Takezaki, available at ftp://ftp.nig.ac.jp/pub/Bio/njbafd/). Dendrograms were visualized with TREEVIEW (Page 1996). Pairwise collection differences were calculated using genotypic proportions with GENEPOP. It was necessary to use this more conservative test (as opposed to allelic count differences) due to a large number of deviations from Hardy-Weinberg proportions.

Within-population Bottlenecks

The genetic data from each collection were used to ascertain whether there was evidence of a recent population bottleneck, using the programme BOTTLENECK (Cornuet and Luikart 1996). The principle used is that, after a reduction in a population's effective size, both the number of alleles and gene diversity (H_e) decline but the number of alleles declines at a faster rate than H_e. Using the observed number of alleles and the sample size at each locus, the expected H_e is calculated based on different models of locus evolution. In populations at mutation-drift equilibrium, loci should have approximately equal probability of displaying H_e excess or deficit; in populations that have recently experienced a population bottleneck, the observed H_e will be higher than that expected for the given number of alleles present in the population and the model of locus evolution. Thus, an excess for H_e implies a population bottleneck. The infinite allele model (IAM) mutation model was used in this analyses; Cornuet and Luikart (1996) found less power and occasional spurious results at loci with non-continuous allele distributions using an alternative model, the stepwise mutation model (SMM). Similarly, O'Connell et al. (1997) found microsatellite data for rainbow trout (*Onocorhynchus mykiss*) in Lake Ontario to best fit the IAM. I used 1000 iterations for all analyses.

RESULTS

Within-collection Variation

The number of alleles at each locus was high, ranging from 8 to 33 (Figures 3-7). Gene diversity (H_e) at each locus was also high, ranging from 0.513 to 0.873 (Table 5-3). A greater number of deviations from Hardy-Weinberg proportions was found than would have been expected by chance (39 out of 279 tests at $p < 0.05$; see Table 5-3); 9 remained significant after conducting a Bonferroni correction for multiple testing (Rice 1989) (Table 5-3). Over all loci, 15 of the 56 collections deviated from Hardy-Weinberg proportions (Fisher's exact test, at $p < 0.05$; see Table 5-3) and 7 remained significant after correcting for multiple testing (Table 5-3). There was also a greater number of instances of linkage disequilibrium than would have been expected by chance (91 out of 516 tests at $p < 0.05$; see Table 5-3); 30 remained significant after correcting for multiple testing (Table 5-3). The distribution of heterozygote deficiencies was similar to the number of heterozygote surpluses (19 were deficiencies and 20 were surpluses), suggesting that these findings were due to some form of non-random mating and not scoring difficulties.

There was a positive relationship between the number of alleles found at each locus and sample size (Table 5-3, Figure 5-8: $r^2 = 0.20, p = 0.0005$ at *Ssa12*; $r^2 = 0.39, p < 0.0001$ at *Ssa171*; $r^2 = 0.15, p = 0.003$ at *Ssa197*; $r^2 = 0.39, p < 0.0001$ at *Ssa202*; $r^2 = 0.17, p = 0.002$ at *Ssa85*). However, most collections had fewer alleles than expected based on the bootstrapped values of all Inner Bay individuals (Table 5-3, Figure 5-8), indicating some degree of substructuring. Furthermore, some collections had many fewer alleles than expected (e.g., all Upper Point Wolfe River collections), indicating a likely population bottleneck (Table 5-3, Figure 5-8); the Margaree River collections had the greatest number of alleles (Table 5-3). While the sample sizes for the Gaspereau River collections were too small to assess number of alleles individually, the pooled results for all the adult collections indicate that salmon from this river, as well as salmon from the Petitcodiac River near the time of its extirpation, clearly have fewer alleles than are typically found in Inner Bay rivers (Table 5-4). There tended to be a positive relationship

between gene diversity and bootstrapped number of alleles for each collection, with a much stronger relationship at the loci with many alleles than those with few (Figure 5-9: $r^2 = 0.04, p = 0.23$ at *Ssa12*; $r^2 = 0.64, p < 0.0001$ at *Ssa171*; $r^2 = 0.77, p < 0.0001$ at *Ssa197*; $r^2 = 0.62, p < 0.0001$ at *Ssa202*; $r^2 = 0.41, p < 0.0001$ at *Ssa85*; $r^2 = 0.73, p <$ 0.0001 mean of all loci). Declines in genetic diversity (H_e and number of alleles) in the Point Wolfe, Big Salmon and Upper Salmon rivers did not become apparent until the 1992 year class and only in some locations (Table 5-3, Figure 5-10). Despite this, there does appear to be a positive relationship between genetic diversity and the observed number of anadromous salmon in these two rivers (Figure 5-11; $r^2 = 0.302, p = 0.0052$ for H_e and $r^2 = 0.19, p = 0.068$ for number of alleles). The results of the bottleneck analyses would suggest that most collections had recently experienced a bottleneck (Table 5-3); 54 of 56 tests indicated H_e excess ($p < 0.001$, sign test) and this result was significant when probabilities were combined using Fisher's (1954 as described in Sokal and Rolf 1981) method (p <0.001).

Genetic Relationships Among Collections

The neighbour-joining analysis of the modified Cavalli-Sforza and Edward's (D_A) genetic distance showed that, within the same rivers for some rivers, different year groups tended to cluster together while others did not (Figure 5-12). High bootstrap values supported the clustering of the Gaspereau River adult year groups with that of the parr. Collections from the two more geographically distant rivers, the Margaree and Hammond rivers, clustered together, with sub-clustering based on river origin. The collections from the Stewiacke and Petitcodiac rivers clustered together but without sub-structuring based on river origin. The collections from the Point Wolfe, Upper Salmon, and Big Salmon rivers were only weakly sub-structured based on river origin and there tended to be low bootstrap support for this structuring. Similar but weaker structuring was found when the D_{SW} genetic distances were used (Figure 5-13). Of note was the observation that collections from the more geographically divergent collections did not become more genetically divergent with the different weighting of allele sizes.

The θ and Φ_{ST} analyses supported the observations made in the clustering

analyses. Within rivers, the inclusion of collections that deviated from Hardy-Weinberg and/or had loci in linkage disequilibrium, inflated, at times to a great degree, the estimations of heterogeneity among collections (with the exception of the θ values taken within the Stewiacke River; Table 5-5). A clear example of this is within the Point Wolfe River. Including all collections, a highly significant θ of 0.0414 was observed. However, after removing the deviating collections, the θ observed was a non-significant 0.0043 (Table 5-5). In most cases, after removal of the deviating collections, θ values within rivers were small. However, within the Gaspereau River and, to a lesser extent, the Big Salmon River, higher, albeit non-significant, θ values were still observed (Table 5-5). Analyses at the higher hierarchical level (among rivers) indicate that the heterogeneity among rivers increases with spatial scale, at least when the influence of the divergent Gaspereau collections is excluded; among the Point Wolfe, Big Salmon and Upper Salmon rivers (θ = 0.0058), within the Inner Bay (0.0104), all of the Bay of Fundy (0.0125), and finally all rivers (0.0137). The trends observed with the Φ_{ST} analyses were similar but clearly absent were the great increases that would have been expected as the spatial scale increased if the Inner Bay rivers had originated from a separate phylogenetic group (Table 5-5).

DISCUSSION

Effects of Population Bottlenecks

Anadromous salmon in Inner Bay rivers have declined dramatically since the 1980s. Low population size can affect genetic variation within and among populations in a number of ways. In addition to an actual loss of genetic variation, deviations from genotypic expectations and correlation of alleles at physically unlinked loci can occur as a result of few parents producing the fish included in a collection. Similarly, high divergence among year groups due to increased genetic drift can be expected. To varying degrees, I found evidence of all of these effects of population bottlenecks in some of my collections. It is essential in the interpretation of the results to consider the possible effects of low population sizes when attempting to understand the underlying population structure.

Assuming that the loss of genetic variation occurred within the last ten generations, loss of genetic variation is clearly evident in many collections in this study. The most severely impacted were the salmon from the upper section of the Point Wolfe River; the 1996 year group had a mean of 5.13 alleles per locus (standardized for sample size) and gene diversity of 0.694 (the most variable collection was the Margaree River adults with a mean of 10.17 alleles per locus and H_e of 0.827). While there was a strong relationship between gene diversity (H_e) and number of alleles per locus, the latter was much more sensitive to population declines than the former (Figure 5-9), as would be expected from theoretical models (e.g., Nei et al. 1975). For example, the 1996 upper Point Wolfe River collections had 50.4% of the number of alleles as the Margaree adult collections but 83.9% of the gene diversity. Similarly, the maximum range in gene diversity in the Upper Salmon and Point Wolfe rivers was only 15.5% (0.688-0.814; Figure 5-11) while that for the number of alleles was 44.4% (5.13-9.23; Figure 5-11). Because the number of alleles is sensitive to sample size (Figure 5-8), it is essential to standardize sample sizes (e.g., by resampling - this study; rarefaction - Hurlbert 1971 as used in Comps et al. (2001)) when it is not possible to obtain equal sample sizes.

With very few anadromous salmon returning to the rivers in the Inner Bay, the

amount of genetic variation preserved in some locations may actually be somewhat surprising. For example, in the Point Wolfe and Upper Salmon rivers, juveniles in some locations in the 1990s are likely the progeny of one or few females. Given that anadromous salmon returns are typically female-biased (e.g., Jessop 1986), in many cases it may have been unlikely that an anadromous male was present during spawning. Females would thus be spawning with mature male parr alone (e.g., Jones and Hutchings In press), something that has been demonstrated experimentally (Hutchings and Myers 1985). Successful spawning by mature male parr can increase effective population size (e.g., Jones and Hutchings In press and In revision). However, the mature male parr in these rivers appear to be a maximum of 5 years old (Jones, unpublished data). Thus, as the parr in each location become more and more related, the "buffering" capacity of these parr will diminish rapidly if few anadromous female continue to return each year.

In addition to a loss of genetic variation, greater instances of loci deviating from Hardy-Weinberg expectations, and more occurrences of physically unlinked loci displaying evidence of linkage disequilibrium than expected by chance, also suggest that some populations have experienced a bottleneck. These measures have low power to detect non-random mating (Charkraborty and Leimar 1987, Thompson et al. 1988). The number of significant deviations from random expectations reflects the severity of the bottlenecks observed in these populations.

The life-history stage of the individuals included in each collection appears to be associated with these deviations. Collections consisting of parr had a much more frequent incidence of deviations from Hardy-Weinberg expectations, more evidence of linkage disequilibrium, and possibly lower genetic diversity than did collections consisting of adults. Others have made similar observations (e.g., Banks et al. 2000). Collections consisting of parr may be problematic because there can be an over-representation of individuals from limited family groups. This problem will be lessened when larger numbers of anadromous fish spawned the parr sampled and when the parr are sampled over an extended portion of the river. It is not clear from the data in this study the extent to which this should be of general concern; the collections of parr were biased

toward more recent years after population bottlenecks whereas the adult collections tended to be from years preceding population declines. However, the collection of parr from the Margaree River, the one river where parr and adults were sampled at the same time and that has a large population size, had one instance of loci in linkage disequilibrium and did have lower gene diversity and slightly lower number of alleles than the adult collection. It is clearly important to consider the source of collections in population studies and to be aware of possible shortcomings.

Further evidence that many collections in this study experienced bottlenecks is that collections with reduced genetic variation also appeared to be more genetically divergent from other collections. Increased genetic variance among populations is expected when populations experience bottlenecks (e.g., Carson 1990), and can be clearly illustrated with the upper Point Wolfe River collections. Most Point Wolfe River collections clustered with Big Salmon and Upper Salmon River collections, with small genetic distances and low bootstrap support for the differences. However, the upper Point Wolfe River collections were among the most divergent collections with large genetic distances and high bootstrap support for the differentiation. The genetic divergence of the Gaspereau collections is probably similarly explained and is likely an artifact of a stocking programme that collected fewer than 20 individuals as broodstock each year. Supplementation with so few broodstock can result in a decrease in effective population size (Ryman and Laikre 1991), which could lead to rapid loss in genetic diversity and drift in genetic variation.

Results of the analyses to detect recent bottlenecks in populations (Cornuet and Luikart 1996) also suggest that most populations had experienced a recent bottleneck. While it is possible or even likely that this is the case, it is difficult to assess the power of this analysis as compared to simple reduction in alleles or deviations from Hardy-Weinberg expectations. One possible means of addressing this issue may be to use the genetic information to determine the genetic relationship among individuals in a collection (e.g., unrelated, full sib, half sib, etc). Collections from populations which have recently experienced a bottleneck would be more likely to contain related

individuals than collections from populations which have not.

Efficacy of Reintroduction Programmes

The reintroduction of Atlantic salmon to the Point Wolfe River was ultimately a failure; anadromous salmon have not been observed in recent years and parr can no longer be found (A. Caissie, Fundy National Park, pers. com.). However, the number of anadromous salmon returning to the river did initially increase and the subsequent decline occurred at a time when the numbers of salmon in all Inner Bay rivers were declining.

My results suggest that, from a genetic diversity perspective, active reintroduction of Atlantic salmon to the Point Wolfe River, while attaining its goals initially, was unnecessary. The reintroduction effort represented an attempt to introduce salmon from a source presumed to be appropriate (Big Salmon River) in great enough numbers to maintain similar genetic diversity of this new population. My results indicate that this occurred: collections of Point Wolfe River salmon after the reintroduction clustered closely with Big Salmon River collections. Furthermore, the gene diversity and number of alleles present in early Point Wolfe River collections were similar to those from the Big Salmon River.

Despite the success in achieving these goals, it remains questionable as to whether such efforts were necessary. The neighbouring Upper Salmon River collections also had similar gene diversity and number of alleles and also was genetically similar to both the Big Salmon and Point Wolfe River collections. It is likely that the number of salmon returning to the Point Wolfe River increased much more rapidly as a result of the stocking than the numbers increased in the Upper Salmon River in the early years following the loss of their respective dams. Furthermore, recolonization of the Upper Salmon River occurred in part during the blockage of the Petitcodiac River (mid 1960s), which may have resulted in a greater straying rate to the Upper Salmon River than would normally have occurred.

My results suggest that the Big Salmon River salmon may not have been the most suitable source for the reintroduction into the Point Wolfe River. The adults collected in 1970 had three departures from Hardy-Weinberg expectations, all three being

heterozygote deficiencies, and evidence of linkage disequilibrium at unlinked loci. Such observation could indicate a mixture of populations. For example, the Restigouche River salmon that were stocked into the Big Salmon River in 1968 and 1969 (Jessop 1976) could produce such an result. Hatchery stocked salmon have lower returns than native salmon (Stabell 1984). However, if stocked in sufficient numbers, as occurred, some returns would be expected. The 1970 collection clustered with later collections, possibly suggesting that some of the Restigouche salmon were reproductively successful. The clustering of the 1967 Big Salmon River collection with those of the Petitcodiac and Stewiacke Rivers may indicate that Big Salmon River salmon were once more closely related to salmon from those rivers, however, this evidence is weak due to low sample sizes of these collections, especially at *Ssa171* (Table 5-3).

Despite the possible needlessness of the reintroduction effort in the Pt. Wolfe River as compared to the natural recolinization of the Upper Salmon River, reintroductions can still be useful and may, at times, be necessary. Recolonization of a river by natural straying can be a long process and may result is severe inbreeding if few adults initially stray into the river. Furthermore, salmon may be more likely to stray into a river if parr are already present (e.g., Johannesson 1987). Therefore, the need to use artificial planting of salmon as a means of reintroduction may be dependent on the probability that sufficient numbers of strays enter the river in question. Because most straying appears to occur among proximate rivers (Elo 1993), this likelihood can be assessed on the basis of neighbouring population sizes. Had population sizes in the Inner Bay, especially in the Upper Salmon and Big Salmon Rivers, remained at the levels found in the 1960s to early 1980s, the Point Wolfe River likely would have been recolonized sufficiently by strays. Conversely, there is currently little likelihood of the rivers in the Inner Bay being recolonized naturally because so few salmon remain. Thus, if salmon are to return to these rivers, intervention will be necessary. An appropriate strategy for Inner Bay salmon may be to revitalize selected rivers, perhaps based on habitat area and quality, and allow others to be recolonized over time with less intervention.

Phylogenetic Divergence of Inner Bay Salmon

My microsatellite provide no evidence to suggest that Inner Bay populations represent a unique phylogenetic origin, based on their genetic similarity to both more geographically distant populations (Hammond and Margaree rivers); the genetic distances between the geographically more distant populations and the Inner Bay collections are of a magnitude one might expect under an isolation by distance model and the degree of the difference does not increase with D_{SW} as compared to D_A (or Φ_{ST} as compared to θ) as would be expected if they had been separated for a long period of time (e.g., Slatkin 1995). Using such an approach, Tessier and Bernatchez (2000) suggest that salmon in Lake Saint-Jean evolved from two lineages.

Calculations of F_{ST} or analogous metrics using variable genetic markers such as microsatellites can provide seemly low estimates of population differentiation for several reasons (e.g., Hedrick 1999). F_{ST} is calculated as $(H_T - H_S) / H_T$ (where H_T is the expected heterozygosity of an individual in an equivalent random mating total population and H_S is the mean of the expected heterozygosity of individuals in equivalent random mating subpopulations; Hartl and Clark 1989). With $H_S = 0.771$ in this study (Table 5-3), the greatest obtainable value for F_{ST}, if an infinite number of populations was studied and each population had alleles not observed in any other population (i.e., $H_T \approx 1$), is 0.229. As the number of populations being compared decreases, so does the expected maximum value of F_{ST} (because $H_T < 1$). Because the divergence of the populations in this study are most likely relatively recent (since the last glaciation event < 10,000 years ago), differences among the populations would be attributable to allele frequency drift, further reducing the maximum value of F_{ST} to be expected.

While reasons exist to expect lower values for F_{ST} with microsatellites, other studies with Atlantic salmon have found higher values than the current study. McConnell et al. (1997) reported overall θ values of 0.054 for Atlantic salmon in Atlantic Canada. Similarly, Fontaine et al. (1997) reported a pairwise mean Φ_{ST} value of 0.075 for Atlantic salmon from Quebec. These values are 4-6 times greater than those found in this study. Several reasons could account for this. Firstly, the same suite of loci were not used in

these studies, although the overall mean expected heterozygosities were similar. Secondly, these studies included populations over a much greater geographical range, thus greater differentiation may be expected. Finally, sampling can have an effect. McConnell et al. (1997) had a mean sample size slightly greater than 32 individuals per location and Fontaine et al. (1997) had a mean of slightly less than 25 individuals per population; mean sample sizes for the group analysis excluding the Gaspereau and all collections deviating from Hardy-Weinberg and linkage equilibrium expectations was over 173 individuals per group. Also, McConnell et al.'s (1997) collections consisted mostly of parr and all of Fontaine et al.'s (1997) collections were parr. Low sample sizes when using variable loci can result in exaggerated perceived differentiation; indeed, when conducting a group comparison where some populations were pooled, Fontaine et al. (1997) reported a much lower Φ_{ST} value of 0.013 and McConnell et al (1997) similarly reported a diminished θ value of 0.034. Thus, despite the seemingly low values obtained, my results are similar to other studies over broader geographical range and indicate some degree of population structuring within the Inner Bay and structuring between the Inner Bay and other populations.

One limitation with inferring phylogenetic uniqueness, or lack thereof, from microsatellite data alone is the large number of introductions, both known and undocumented, of salmon from other locations. Early stocking practices often involved transferring salmon among hatcheries within regions and even over large geographical distances (e.g., MacCrimmon and Gots 1979). A comparison of pre-introduction collections, if they exist, may be necessary to resolve this issue. Alternatively, or in addition to microsatellite analyses, other methods more amenable to phylogenetic analysis, e.g., sequence analysis of mtDNA, would be desirable. For example, mtDNA analyses of another North American salmonid, the brook trout (*Salvelinus fontinalis*), have suggested that several glacial refugia effected the divergence of six lineages (Danzmann et al, 1998). Similarly, recent work with Atlantic salmon has suggested that there are two divergent lineages of Atlantic salmon in Europe (Verspoor et al. 1999). Tessier and Bernatchez (2000) identified two divergent lineages in Québec, and it is

possible that more divergent lineages of Atlantic salmon exist in North America.

In spite of the lack of evidence supporting a unique phylogenetic origin for Inner Bay salmon, it is essential to consider the potential uniqueness of the resource. Evolution of optimal life history strategies can occur rapidly (e.g., Reznick et al. 1990, Hendry and Kinnison 1999). Non-Inner Bay salmon with a three- to four-year freshwater parr stage and a MSW anadromous stage transplanted into an Inner Bay river changed to a typical Inner Bay two- to three-year parr stage and grilse anadromous stage (White and Huntsman 1938). However, attainment of an optimal life history almost certainly requires tens of generations (e.g., Reznick et al. 1997), at a minimum. With many external forces clearly affecting the persistence of Inner Bay salmon, the salmon most adapted to the Inner Bay environment should be used in any reintroduction efforts. Because of the apparent reproductive isolation of Inner Bay salmon and the likelihood that they have been so since the last glaciation event, hundreds or even thousands of generations have passed for selection favouring the most fit life history strategies to have occurred. Thus, Inner Bay salmon are likely genetically the most adapted salmon for the Inner Bay environment.

Conclusions

The use of collections of different year groups in combination with collections from different rivers provides mild at best evidence of structuring of salmon from some rivers but not all. The effects of small population size are evident in the pattern of genetic variation and must be carefully considered when drawing inferences of population differentiation. My findings suggest salmon from each river represent populations with some degree of reproductive isolation and that, under certain circumstances, reintroduction efforts into small rivers may be unnecessary. In addition, my results are not consistent with the hypothesis that salmon from the Inner Bay of Fundy represent a divergent phylogenetic lineage.

Table 5-1. Rivers (abbreviations) and their locations, drainage areas and removal date of last barrier to salmon migration.

River	Location	drainage area (km^2)	barrier removed
Pt. Wolfe (PW)	Inner Bay-Chignecto Bay	130	1985
Upper Salmon (US)	Inner Bay-Chignecto Bay	174	1954
Big Salmon (BS)	Inner Bay-North	332	early 1930s
Petitcodiac (PE)	Inner Bay-Chignecto Bay	3000	in place
Stewiacke (ST)	Inner Bay-Minas Basin	2700	1930s
Gaspereau (GA)	Inner Bay-Minas Basin	508	in place; fish ladder
Hammond (HA)	outer BoF	453	1900s[1]
Margaree (MA)	Cape Breton	1170	

[1]Dam was 15 km upstream and habitat below is suitable for spawning.

Table 5-2. River and within-river location, collection type (parr (p), anadromous (a) or young-of-the-year (y)), year group, collection abbreviation, collection number, and sample sizes of Atlantic salmon examined for microsatellite variation.

River	Sub-location	Type	Year group	Abbrev.	Collection	*n*
Point Wolfe		p	82	PWp82&4	1	23
Point Wolfe		p	84	PWp82&4	1	6
Point Wolfe		p	88	PWp88	2	13
Point Wolfe		p	91			6
Point Wolfe	at Bennett Bk	p	92			7
Point Wolfe	Upper	p	92	PW.Up92	3	61
Point Wolfe	at Key Hole	p	92	PW.Kp92	4	29
Point Wolfe	Lower	p	92			9
Point Wolfe	Upper	p	93	PW.Up93	5	81
Point Wolfe	at Bennett Bk	p	93	PW.Bp93	6	59
Point Wolfe	at Key Hole	p	93	PW.Kp93	7	44
Point Wolfe	Lower	p	93	PW.Lp93	8	15
Point Wolfe	at Bennett Bk	p	94	PW.Bp94	9	51
Point Wolfe	at Key Hole	p	94	PW.Kp94	10	60
Point Wolfe	Upper	p	94			4
Point Wolfe	Upper	y	96	PW.Up96	11	77
Point Wolfe	at Bennett Bk	y	96	PW.Bp96	12	97
Point Wolfe	at Key Hole	y	96	PW.Kp96	13	27
Point Wolfe	Lower	y	96			4
Big Salmon		a	67	BSa67	14	45
Big Salmon		a	70	BSa70	15	58
Big Salmon		a	74	BSa74	16	18
Big Salmon		a	85	BSa85	17	15
Big Salmon		a	88	BSa88	18	31
Big Salmon		a	89	BSa89	19	44
Big Salmon		a	90	BSa90	20	17
Big Salmon		p	89	BSp89	21	24

Table 5-2. Continued.

River	Sub-location	Type	Year group	Abbrev.	Collection	*n*
Big Salmon		p	90	BSp90	22	36
Big Salmon	AB	p	93-95	BS.Ap93-5	23	44
Big Salmon	CB	p	93-95	BS.Cp93-5	24	32
Big Salmon	CP	p	93-95			7
Big Salmon	MB	p	93-95	BS.Mp93-5	25	29
Big Salmon	SD	p	93-95	BS.Sp93-5	26	50
Petitcodiac		a	83	PEa83	27	35
Petitcodiac		a	84	PEa84	28	21
Upper Salmon		a	74	USa74	29	37
Upper Salmon		a	83	USa83	30	26
Upper Salmon		a	84	USa84	31	39
Upper Salmon		a	86			5
Upper Salmon		a	88			3
Upper Salmon		a	89	USa89	32	15
Upper Salmon		a	90			3
Upper Salmon		p	81			9
Upper Salmon		p	82	USp82	33	46
Upper Salmon		p	84			9
Upper Salmon		p	85	USp85	34	50
Upper Salmon		p	90			1
Upper Salmon		p	91			6
Upper Salmon		p	92	USp92	35	15
Upper Salmon		p	93	USp93	36	31
Upper Salmon		p	94			8
Upper Salmon	at Black Hole	y	96	US.Bp96	37	37
Upper Salmon	at Forks	y	96	US.Fp96	38	100
Upper Salmon	at Pumphouse	y	96	US.Pp96	39	19

Table 5-2. Continued.

River	Sub-location	Type	Year group	Abbrev.	Collection	*n*
Stewiacke		a	78	STa78	40	48
Stewiacke		a	83	STa83	41	52
Stewiacke		a	84	STa84	42	49
Stewiacke		a	88	STa88	43	27
Stewiacke		a	89	STa89	44	13
Stewiacke		a	90	STa90	45	15
Stewiacke		a	91	STa91	46	22
Gaspereau		a	83	GAa83	47	14
Gaspereau		a	84	GAa84	48	12
Gaspereau		a	85	GAa85	49	20
Gaspereau		a	86	GAa86	50	15
Gaspereau		p	80	GAp80-2	51	2
Gaspereau		p	81	GAp80-2	51	12
Gaspereau		p	82	GAp80-2	51	3
Hammond	1	p	93-95			10
Hammond	1	y	96	HR.1p96	52	21
Hammond	2	y	96	HR.2p96	53	31
Hammond	3	y	96	HR.3p96	54	51
Margaree		a	96	MAa96	55	34
Margaree		p	92-94	MAp92-4	56	134

Table 5-3. Sample size (n), actual (A) and bootstrapped (A_b) number of alleles, observed heterozygosity (H_o), and gene diversity (H_e) for each collection at each locus and mean values over all loci, number of loci pairs in linkage disequilibrium (at $p < 0.05$)/number of test conducted (tests that remained significant after multiple testing are indicated in bold), and two-tailed p-values and direction of H_e excess (+) or deficiency (-) of Bottleneck analyses. Collection abbreviations are defined in Table 5-2.

Collection	*Ssa12*					*Ssa171*					*Ssa197*					*Ssa202*				
	n	A	A_b	H_o	H_e	n	A	A_b	H_o	H_e	n	A	A_b	H_o	H_e	n	A	A_b	H_o	H_e
PWp82&4	27	6	4.71	0.481	0.481	14	10	8.97	0.929	0.89	25	11	9.03	0.92	0.88	18	11	n/a	1	0.863
PWp88	12	4	n/a	0.5	0.536	8	8	n/a	0.875	0.86	12	7	n/a	0.917	0.86	12	12	n/a	0.833	0.870
PW.Up92	61	4	3.84	0.525	0.52	61	11	8.62	**0.902*****	0.82	61	7	5.68	0.689	0.67	61	10	6.91	**0.787*****	0.774
PW.Kp92	29	5	4.19	0.448	0.491	28	16	12.46	1	0.91	29	11	8.8	0.828	0.85	29	13	11.04	0.931	0.900
PW.Up93	74	4	3.90	0.622	0.602	62	14	9.07	0.871*	0.85	77	6	4.27	0.727*	0.66	74	12	8.92	0.919***	0.844
PW.Bp93	58	4	3.58	0.552	0.529	45	18	12.12	0.933	0.89	56	12	8.75	0.875*	0.84	43	11	9.98	0.953	0.895
PW.Kp93	38	4	3.43	0.579	0.505	32	17	13.15	0.844*	0.91	39	12	8.84	0.718	0.8	32	14	11.04	0.906*	0.892
PW.Lp93	15	3	n/a	0.533	0.508	14	12	n/a	0.857	0.9	15	8	n/a	0.933	0.85	14	11	n/a	1	0.876
PW.Bp94	50	5	4.10	0.52	0.545	44	16	12.68	0.909	0.91	49	11	8.13	0.796	0.82	44	14	10.82	0.864*	0.886
PW.Kp94	55	3	2.99	0.564	0.523	41	14	11.34	0.927*	0.91	56	12	8.73	0.821	0.8	49	16	11.27	0.857	0.891
PW.Up96	75	4	3.88	0.747	0.655	62	8	6.53	0.839*	0.71	72	5	4.02	0.611	0.61	74	11	7.68	0.838*	0.812
PW.Bp96	97	6	4.21	0.454	0.465	37	14	10.99	0.973	0.88	96	12	8.51	0.75	0.79	79	12	9.51	0.924***	0.876
PW.Kp96	27	3	2.91	0.296	0.322	26	11	10.14	0.885	0.9	27	8	6.72	0.741	0.75	27	10	8.39	0.926	0.844
BSa67	45	4	3.36	0.489	0.494	0	0	n/a	n/a	n/a	38	10	8.5	0.921	0.85	44	15	12.30	0.864	0.892
BSa70	57	5	3.40	0.579	0.484	40	20	12.89	0.825	0.9	42	9	7.13	0.667	0.79	42	15	12.39	**0.595*****	0.914
BSa74	17	4	n/a	0.412	0.357	10	n/a	8.53	0.8	0.86	18	8	n/a	0.944	0.81	14	11	n/a	0.929	0.915
BSa85	12	4	n/a	0.667	0.576	9	n/a	9.25	0.889	0.88	14	7	n/a	0.714	0.85	12	10	n/a	0.917	0.884

Table 5.3. Continued.

Collection	*Ssa12*					*Ssa171*					*Ssa197*					*Ssa202*				
	n	*A*	A_b	H_o	H_e	*n*	*A*	A_b	H_o	H_e	*n*	*A*	A_b	H_o	H_e	*n*	*A*	A_b	H_o	H_e
BSa88	31	5	3.77	0.452	0.546	25	14	11.01	0.84	0.89	31	10	8.68	0.871	0.86	30	15	11.62	0.9	0.886
BSa89	42	3	2.95	0.5	0.444	37	16	10.92	0.865	0.88	44	10	8.88	0.795	0.86	44	17	11.83	0.818*	0.873
BSa90	15	4	n/a	0.467	0.628	15	9	n/a	0.933	0.88	17	9	n/a	0.824	0.86	14	11	n/a	0.786	0.905
BSp89	17	3	n/a	0.471	0.383	17	11	n/a	0.941	0.87	24	10	8.22	0.917	0.87	20	12	10.20	0.85	0.858
BSp90	35	4	3.42	0.514	0.491	29	12	8.83	0.828	0.81	36	12	9.08	0.861	0.84	32	17	12.27	0.844	0.903
BS.Ap93-5	41	4	3.80	0.415	0.378	16	9	n/a	0.813	0.84	44	12	9.30	0.705*	0.85	35	14	10.80	0.857	0.894
BS.Cp93-5	32	4	3.55	0.375	0.411	27	12	10.66	0.926	0.91	32	9	7.66	0.875	0.84	30	14	11.55	0.967	0.905
BS.Mp93-5	29	3	2.87	0.379	0.447	25	13	10.92	0.96	0.9	29	9	8.04	0.862	0.84	26	14	11.32	1	0.872
BS.Sp93-5	49	5	4.18	0.327**	0.489	45	19	12.61	0.889	0.91	48	11	8.48	0.875	0.85	45	15	11.16	0.778**	0.897
PEa83	34	4	3.41	0.529	0.576	18	11	n/a	1	0.91	35	11	8.76	0.686	0.84	35	10	8.71	0.914	0.872
PEa84	19	3	n/a	0.789	0.676	10	11	n/a	0.9	0.93	19	10	n/a	0.842	0.88	16	9	n/a	0.875	0.865
USa74	30	4	3.48	0.667	0.572	27	12	10.01	0.963	0.9	29	8	7.00	0.793	0.78	29	15	12.60	0.862	0.927
USa83	25	5	4.08	0.6	0.616	22	15	11.51	0.864	0.91	25	10	9.04	0.8	0.87	22	11	9.71	0.909	0.883
USa84	34	3	2.99	0.412	0.448	20	15	12.45	0.95	0.93	34	10	9.07	0.765	0.88	21	12	10.42	0.952	0.893
USa89	15	4	3.74	0.6	0.674	14	9	n/a	0.929	0.91	15	9	n/a	0.867	0.85	15	10	n/a	0.867	0.885
USp82	44	3	3.00	0.614	0.585	24	11	9.64	0.917	0.89	46	11	9.83	0.957	0.87	29	13	10.39	0.862	0.871
USp85	48	3	2.99	0.625	0.559	22	17	13.66	0.864	0.92	43	12	10.13	0.953	0.89	31	15	11.98	0.839	0.894
USp92	14	3	n/a	0.5	0.603	13	13	n/a	0.923	0.93	14	11	n/a	0.857	0.89	15	12	n/a	0.867*	0.922
USp93	31	3	3.00	0.548	0.543	30	12	10.19	0.9	0.89	30	12	9.84	0.867	0.86	30	14	11.72	0.867	0.892
US.Bp96	37	3	2.64	0.189	0.222	37	10	8.54	0.946***	0.84	37	9	7.26	0.811*	0.76	37	11	8.55	**0.892*****	0.874
US.Fp96	99	4	3.44	0.404	0.429	62	16	12.63	1.000***	0.92	96	10	7.96	0.833	0.82	100	15	10.39	**0.890*****	0.849
US.Pp96	19	3	n/a	0.632	0.494	17	12	n/a	0.765	0.9	16	10	n/a	0.875	0.88	5	8	n/a	0.8	0.933

Table 5.3. Continued.

Collection	*Ssa12*					*Ssa171*					*Ssa197*					*Ssa202*				
	n	***A***	A_b	H_o	H_e	***n***	***A***	A_b	H_o	H_e	***n***	***A***	A_b	H_o	H_e	***n***	***A***	A_b	H_o	H_e
STa78	48	3	2.97	0.5	0.561	7	10	n/a	0.857	0.95	42	13	9.67	0.833	0.85	46	10	8.53	0.826	0.861
STa83	52	3	2.98	0.519	0.583	5	7	n/a	0.8	0.91	49	15	10.83	**0.714*****	0.86	46	13	10.78	0.87	0.896
STa84	46	4	3.35	0.565	0.599	21	12	10.57	0.857	0.91	45	12	9.83	0.933	0.83	41	12	9.99	0.854	0.875
STa88	27	3	2.90	0.556	0.561	27	13	11.26	**0.852*****	0.9	27	11	8.89	**1.000*****	0.82	27	10	8.75	0.889***	0.871
STa89	12	3	n/a	0.5	0.649	6	7	n/a	1	0.92	13	8	n/a	0.923	0.86	12	8	n/a	0.917	0.88
STa90	15	3	n/a	0.667	0.536	15	11	n/a	0.933	0.89	15	11	n/a	0.867	0.89	13	8	n/a	0.846	0.868
STa91	22	3	3.00	0.545	0.644	6	7	n/a	0.833	0.89	21	10	8.50	0.714	0.83	21	11	9.42	0.857	0.87
GAa83	14	3	n/a	0.714	0.537	9	9	n/a	0.889	0.92	14	8	n/a	0.857	0.88	13	6	n/a	0.846	0.834
GAa84	12	2	n/a	0.167	0.159	8	6	n/a	1	0.82	12	7	n/a	0.75	0.86	11	4	n/a	0.273	0.398
GAa85	20	3	2.98	0.45	0.554	11	7	n/a	0.909	0.81	20	9	8.36	0.8	0.88	20	8	6.98	0.65	0.721
GAa86	15	2	n/a	0.333	0.37	9	8	n/a	1	0.87	15	9	n/a	0.933	0.89	11	8	n/a	0.727	0.848
GAp80-2	17	3	n/a	0.176	0.266	15	8	n/a	1	0.87	17	10	n/a	0.824	0.87	17	9	n/a	1	0.872
HR.1p96	21	3	2.85	0.667	0.557	15	14	n/a	0.800*	0.92	20	9	7.75	0.95	0.84	20	10	9.35	0.9	0.896
HR.2p96	31	4	3.50	0.581	0.63	26	14	11.53	0.962	0.9	31	9	7.45	0.742	0.8	29	12	10.23	0.69	0.886
HR.3p96	40	5	3.80	0.500**	0.613	8	11	n/a	1	0.94	44	9	7.61	0.864	0.81	42	12	10.00	0.762	0.839
MAa96	29	5	3.98	0.621	0.567	34	20	15.51	0.882	0.95	34	14	11.75	0.941	0.91	34	15	11.53	0.941	0.899
MAp92-4	85	6	4.14	0.612	0.548	132	24	14.73	0.924	0.92	134	16	11.81	0.888*	0.9	123	18	12.25	0.943	0.908
Mean		3.75		0.512	0.513		12.1		0.869	0.87		10		0.832	0.84		11.9		0.858	0.868

* $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, *** $p \leq 0.001$ for deviations from Hardy-Weinberg genotypic proportions.

Table 5-3. Continued.

Collection	*Ssa85*					Mean: all loci				LD	Bottleneck
	n	*A*	A_b	H_o	H_e	*A*	A_b	H_o	H_e	# sig	*p*-values
PWp82&4	26	7	5.94	0.500**	0.739	9	n/a	0.766	0.77	1/10	0.625 +
PWp88	11	7	n/a	0.727	0.766	7.6	n/a	0.77	0.778	0/10	0.219 -
PW.Up92	61	6	4.09	0.705	0.657	7.6	5.83	**0.721****	0.688	3/10	0.031 +
PW.Kp92	29	7	6.22	0.828	0.795	10.4	8.54	0.807	0.79	0/10	0.063 +
PW.Up93	76	9	5.46	0.724	0.737	9	6.32	**0.772****	0.739	7/10, **3**	0.031 +
PW.Bp93	50	9	6.95	0.76	0.77	10.8	8.28	0.815	0.784	0/10	0.031 +
PW.Kp93	37	8	6.78	0.811	0.76	11	8.65	0.772*	0.774	1/10	0.031 +
PW.Lp93	15	7	n/a	0.8	0.772	8.2	n/a	0.825	0.782	0/10	0.031 +
PW.Bp94	48	9	6.79	0.688	0.759	11	8.50	0.755*	0.784	0/10	0.031 +
PW.Kp94	47	8	6.74	0.723	0.76	10.6	8.21	0.778*	0.775	3/10, **1**	0.031 +
PW.Up96	77	4	3.53	0.818**	0.677	6.4	5.13	**0.771****	0.694	5/10, **1**	0.031 +
PW.Bp96	96	8	7.30	0.76	0.786	10.4	8.10	0.772**	0.761	7/10, **4**	0.063 +
PW.Kp96	27	7	6.52	0.741*	0.783	7.8	6.93	0.718*	0.719	5/10, **1**	0.063 +
BSa67	43	9	7.53	0.721	0.721	7.6	n/a	n/a	n/a	0/6	0.063 +
BSa70	51	11	7.39	**0.431****	0.748	12	8.64	**0.619****	0.767	1/10	0.156 +
BSa74	16	7	n/a	0.75	0.798	7.8	n/a	0.767	0.749	1/10	0.625 +
BSa85	15	7	n/a	0.867	0.779	7.6	n/a	0.811	0.792	0/9	0.219 +

Table 5.3. Continued.

Collection		*Ssa85*				Mean: all loci				LD	Bottleneck
	n	*A*	A_b	H_o	H_e	*A*	A_b	H_o	H_e	# sig	*p*-values
BSa88	31	7	5.53	0.645*	0.667	10.2	8.12	0.742	0.769	0/10	0.094 +
BSa89	44	8	7.07	0.818	0.735	10.8	8.33	0.759	0.759	1/10, 1	0.032 +
BSa90	16	8	n/a	0.75	0.788	8.2	n/a	0.752	0.812	0/10	0.032 +
BSp89	24	7	5.86	0.542	0.624	8.6	n/a	0.744	0.719	0/10	0.813 +
BSp90	36	8	6.78	0.722	0.775	10.6	8.07	0.754	0.763	0/10	0.063 +
BS.Ap93-5	43	7	6.10	0.698	0.708	9.2	n/a	0.697	0.734	4/10, 2	0.063 +
BS.Cp93-5	31	7	6.58	0.806	0.792	9.2	8.00	0.79	0.771	1/10	0.063 +
BS.Mp93-5	29	7	6.39	0.793	0.747	9.2	7.91	0.799	0.761	4/10, 1	0.031 +
BS.Sp93-5	49	9	7.56	0.694	0.755	11.8	8.80	0.712**	0.779	3/10, 1	0.063 +
PEa83	35	6	5.81	0.714	0.758	8.4	n/a	0.769	0.792	1/10	0.031 +
PEa84	20	6	5.59	0.8	0.797	7.8	n/a	0.841	0.828	0/10	0.031 +
USa74	35	9	7.81	0.857	0.77	9.6	8.18	0.828	0.789	0/10	0.031 +
USa83	25	8	6.67	0.84	0.748	9.8	8.21	0.803	0.805	0/10	0.031 +
USa84	36	8	6.80	0.694	0.731	9.6	8.35	0.755	0.776	1/10	0.031 +
USa89	15	6	n/a	0.8	0.701	7.6	n/a	0.812	0.803	0/10	0.031 +
USp82	41	9	7.54	0.829	0.753	9.4	8.08	0.836	0.794	1/10	0.031 +
USp85	47	8	7.38	0.766	0.81	11	9.23	0.809	0.814	0/10	0.031 +
USp92	15	6	n/a	0.667	0.662	9	n/a	0.763	0.802	0/6	0.063 +
USp93	30	8	7.10	0.733	0.814	9.8	8.37	0.783	0.8	0/10	0.031 +
US.Bp96	37	11	8.89	0.892**	0.856	8.8	7.18	**0.746*****	0.711	8/10, 2	0.094 +
US.Fp96	100	10	7.79	0.830*	0.806	11	8.44	**0.791*****	0.764	8/10, 3	0.031 +
US.Pp96	19	8	n/a	0.895	0.829	8.2	n/a	0.793	0.806	0/5	0.625 +

Table 5-3. Continued.

Collection		*Ssa85*				Mean: all loci				LD	Bottleneck *p*-
	n	*A*	A_b	H_o	H_e	*A*	A_b	H_o	H_e	# sig	IAM
STa78	47	8	7.15	0.702	0.764	8.8	n/a	0.744	0.797	0/6	0.031 +
STa83	52	9	6.73	0.673	0.739	9.4	n/a	0.715***	0.798	1/6	0.063 +
STa84	47	9	7.39	0.766*	0.796	9.8	8.23	0.795	0.802	1/10	0.031 +
STa88	27	7	6.13	0.778	0.739	8.8	7.59	**0.815****	0.779	8/10, **6**	0.031 +
STa89	12	8	n/a	0.583	0.888	6.8	n/a	0.785	0.84	0/3	0.031 +
STa90	14	8	n/a	0.857	0.772	8.2	n/a	0.834	0.789	0/10	0.063 +
STa91	21	8	6.41	0.524	0.699	7.8	n/a	0.695	0.788	0/6	0.094 +
GAa83	14	6	n/a	0.643	0.804	6.4	n/a	0.79	0.795	3/10	0.031 +
GAa84	12	7	n/a	0.75	0.797	5.2	n/a	0.588	0.607	0/8	1 -
GAa85	20	8	7.36	1	0.841	7	n/a	0.762	0.76	0/10	0.063 +
GAa86	14	7	n/a	0.929	0.849	6.8	n/a	0.785	0.766	0/7	0.031 +
GAp80-2	17	5	n/a	0.765	0.777	7	n/a	0.753	0.731	1/10	0.094 +
HR.1p96	20	7	6.64	0.85	0.813	8.6	n/a	0.833	0.803	1/10, **1**	0.031 +
HR.2p96	31	8	6.59	0.677	0.754	9.4	7.86	0.73	0.794	2/10, **1**	0.031 +
HR.3p96	48	8	6.42	0.583*	0.679	9	n/a	0.742**	0.776	1/6, **1**	0.031 +
MAa96	27	9	8.08	0.778	0.812	12.6	10.17	0.833	0.827	0/8	0.031 +
MAp92-4	104	9	7.53	0.731	0.753	14.6	10.1	0.82	0.806	1/10, **1**	0.031 +
Mean		7.714		0.745	0.763			0.763	0.771		

* $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, *** $p \leq 0.001$ for deviations from Hardy-Weinberg genotypic proportions.

Table 5-4. Bootstrapped number of alleles at each locus and total and mean values over all loci for collections pooled by river and life history stage (a - anadromous and p - parr). River abbreviations are defined in Table 5-1.

Collection	*Ssa12*	*Ssa171*	*Ssa197*	*Ssa202*	*Ssa85*	Total	Mean
PW.p	4.24	12.62	8.72	11.34	7.07	43.98	8.80
BS.a	3.74	12.74	9.10	12.74	7.58	45.90	9.18
BS.p	3.80	11.99	9.30	12.60	7.59	45.27	9.05
PE.a	3.29	11.61	9.33	8.88	5.77	38.87	7.77
US.a	3.69	12.43	9.04	12.62	7.52	45.29	9.06
US.p	3.46	14.60	9.85	12.54	8.57	49.01	9.80
ST.a	3.08	12.78	10.72	10.17	7.30	44.04	8.81
GA.a	2.82	9.69	8.60	8.24	7.10	36.44	7.29
HR.p	3.53	14.00	8.02	10.60	6.97	43.12	8.62
MA.a	3.98	15.51	11.75	11.53	8.08	50.85	10.17
MA.p	4.14	14.73	11.81	12.25	7.53	50.46	10.09
All Inner Bay	4.10	14.14	10.03	12.87	7.93	49.08	9.82
All collections	4.04	14.95	10.26	12.99	8.05	50.30	10.06

Table 5-5. θ and Φ_{ST} values among year groups/sub-location within rivers and among groups of rivers with all collections and excluding collections that deviated from Hardy-Weinberg expectations and/or had loci in linkage disequilibrium; *n* refers to the number of year groups/sub-locations within rivers or the number of rivers (number of year groups/sub-location) within grouping.

River/grouping	All collections			Non H-W, LD deviating collections		
	n	θ	Φ_{ST}	*n*	θ	Φ_{ST}
Pt. Wolfe (PW)	13	0.0414***	0.0339***	6	0.0043	0.007
Upper Salmon (US)	11	0.0246***	0.0351***	9	0.0074	0.0167
Big Salmon (BS)	13	0.0126	0.0218***	9	0.0113	0.0182*
Petitcodiac (PE)	2	0.0001	-0.001	2	0.0001	-0.0008
Stewiacke (ST)	7	0.008	0.0144	5	0.0081	-0.0028
Gaspereau (GA)	5	0.0213	0.0357	4	0.0183	0.055
Hammond (HA)	3	0.0121	0.0325**	2	0.002	0.0071
Margaree (MA)	2	0.002	-0.0017	2	0.002	-0.0017
PW, BS, US	3 (37)	0.0122***	0.0182***	3 (24)	0.0058**	0.0058**
Inner Bay	6 (51)	0.0212***	0.0222***	6 (34)	0.0194***	0.0145***
Inner Bay no GA	5 (46)	0.0156***	0.0188***	5 (30)	0.0104***	0.0085***
BoF	7 (54)	0.0231***	0.0244***	7 (36)	0.0207***	0.0163***
BoF no GA	6 (49)	0.0179***	0.0212***	6 (32)	0.0125***	0.0109***
all	8 (56)	0.0228***	0.0224***	8 (38)	0.0203***	0.0138***
all no GA	7 (51)	0.0184***	0.0199***	7 (34)	0.0137***	0.0101***

* $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, *** $p \leq 0.001$

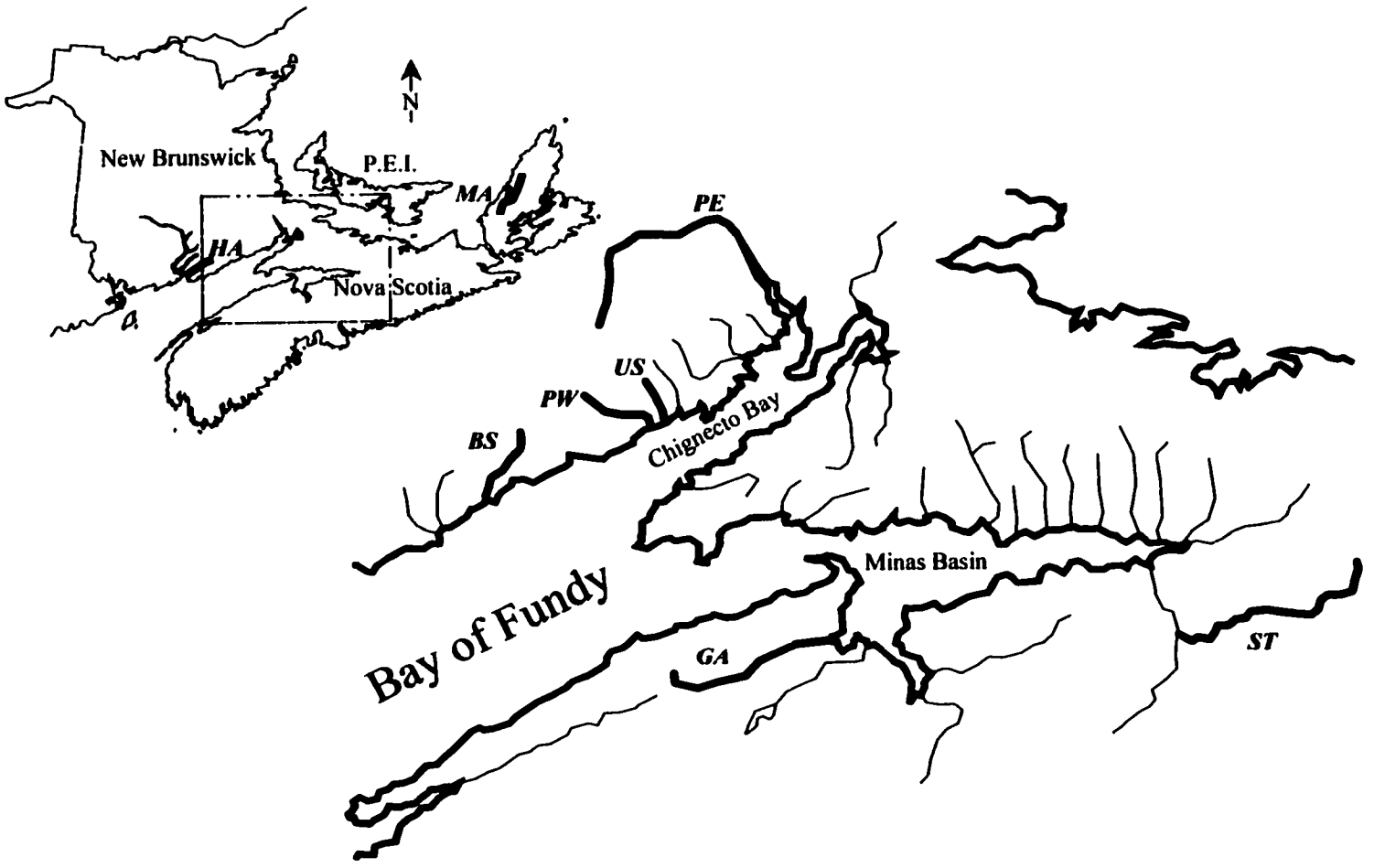

Figure 5-1. Map of Canadian Maritimes (inset at upper left) and Inner Bay of Fundy (area shown indicated by box in inset). Rivers sampled are in bold (abbreviations in italics are defined in Table 5-1).

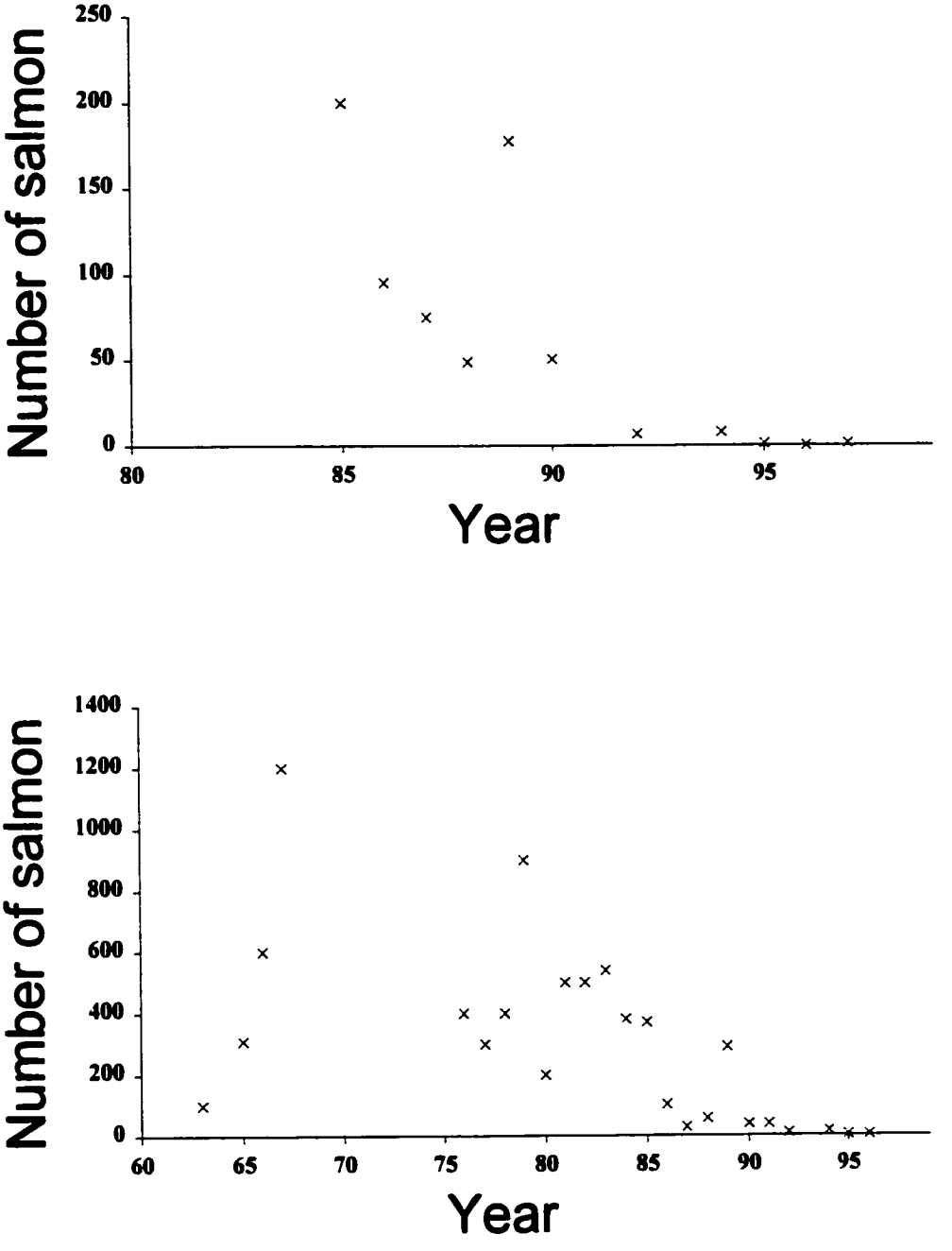

Figure 5-2. Estimated number of returning anadromous salmon in the Point Wolfe (upper graph) and Upper Salmon rivers.

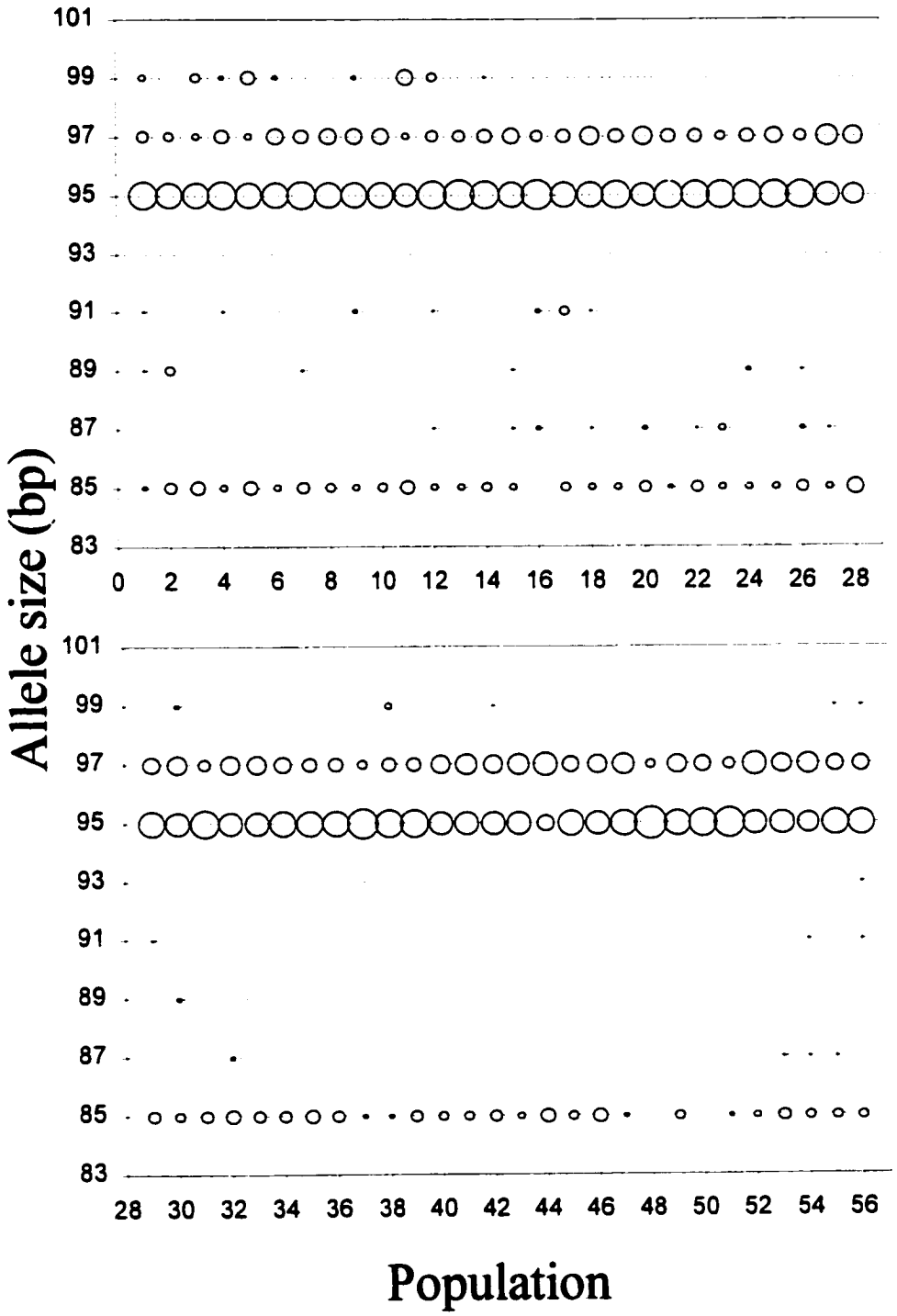

Figure 5-3. Allele frequencies at *Ssa12* for each collection. Relative allele frequency for each collection is indicated by the size of the circle; collection numbers are as defined in Table 5-2 (Pt. Wolfe: 1-13; Big Salmon: 14-26; Petitcodiac: 27-28; Upper Salmon: 29-39; Stewiacke: 40-46; Gaspereau: 47-51; Hammond: 52-54; Margaree: 55-56).

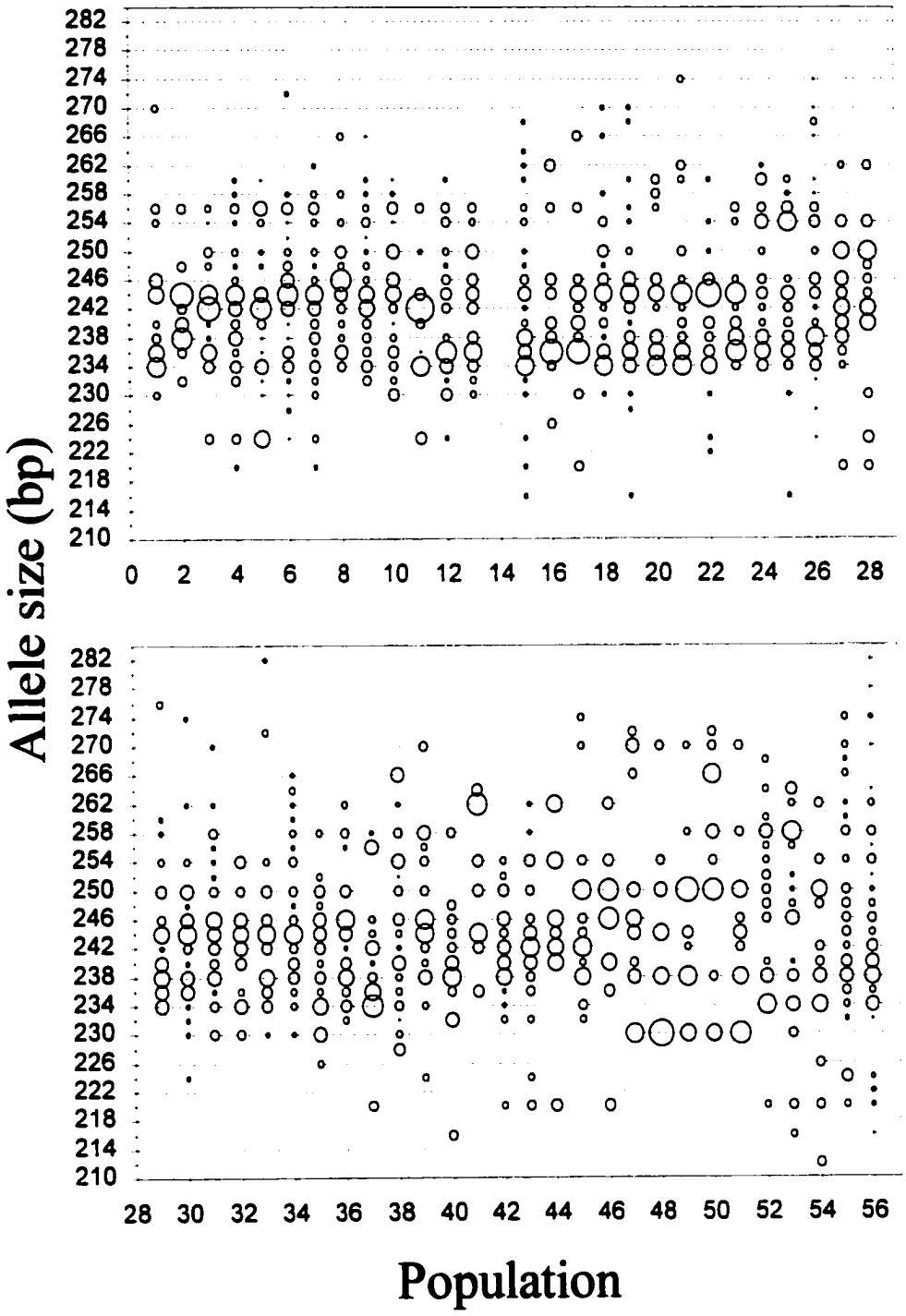

Figure 5-4. Allele frequencies at *Ssa171* for each collection. Relative allele frequency for each collection is indicated by the size of the circle; collection numbers are as defined in Table 5-2 (Pt. Wolfe: 1-13; Big Salmon: 14-26; Petitcodiac: 27-28; Upper Salmon: 29-39; Stewiacke: 40-46; Gaspereau: 47-51; Hammond: 52-54; Margaree: 55-56).

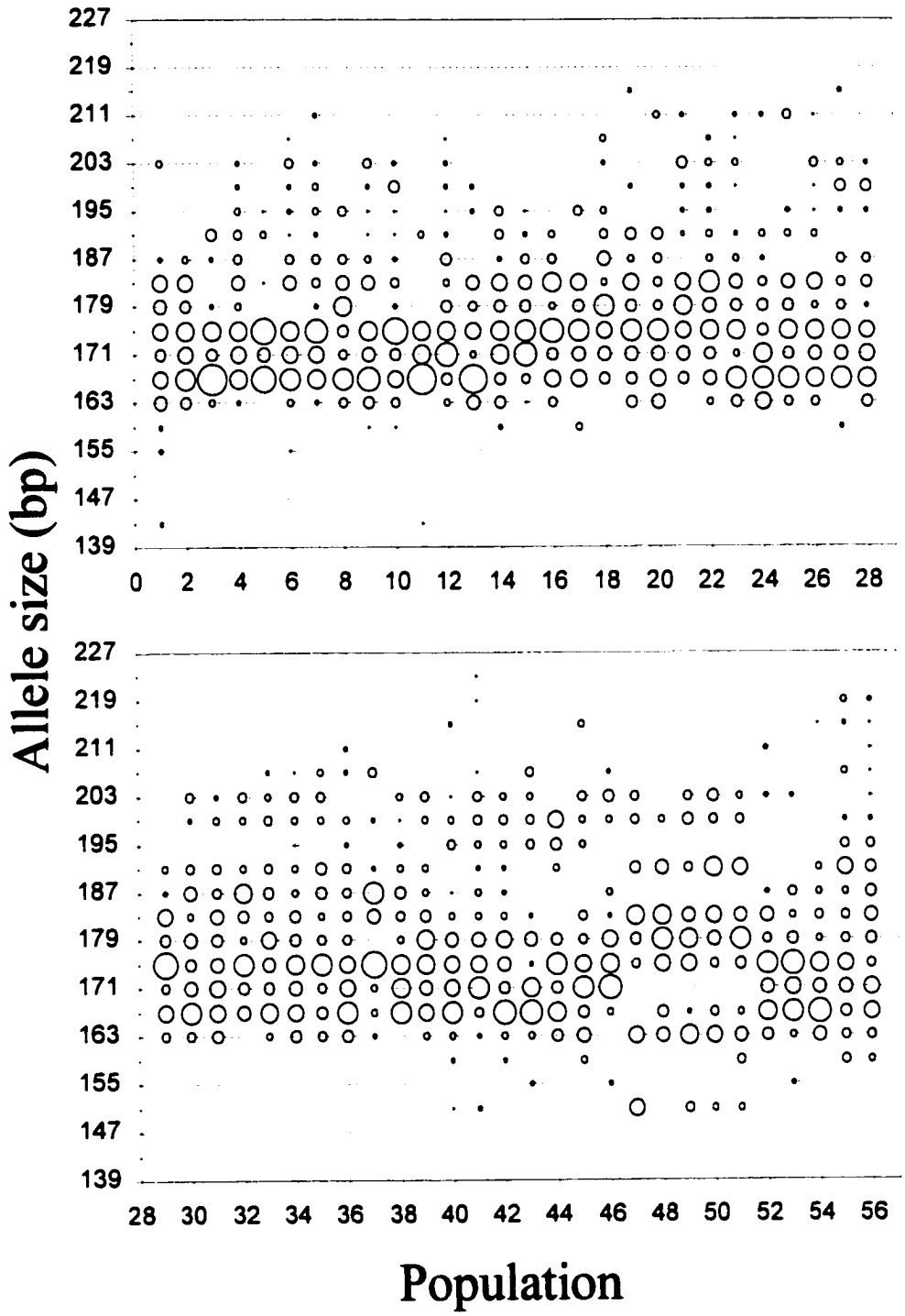

Figure 5-5. Allele frequencies at *Ssa197* for each collection. Relative allele frequency for each collection is indicated by the size of the circle; collection numbers are as defined in Table 5-2 (Pt. Wolfe: 1-13; Big Salmon: 14-26; Petitcodiac: 27-28; Upper Salmon: 29-39; Stewiacke: 40-46; Gaspereau: 47-51; Hammond: 52-54; Margaree: 55-56).

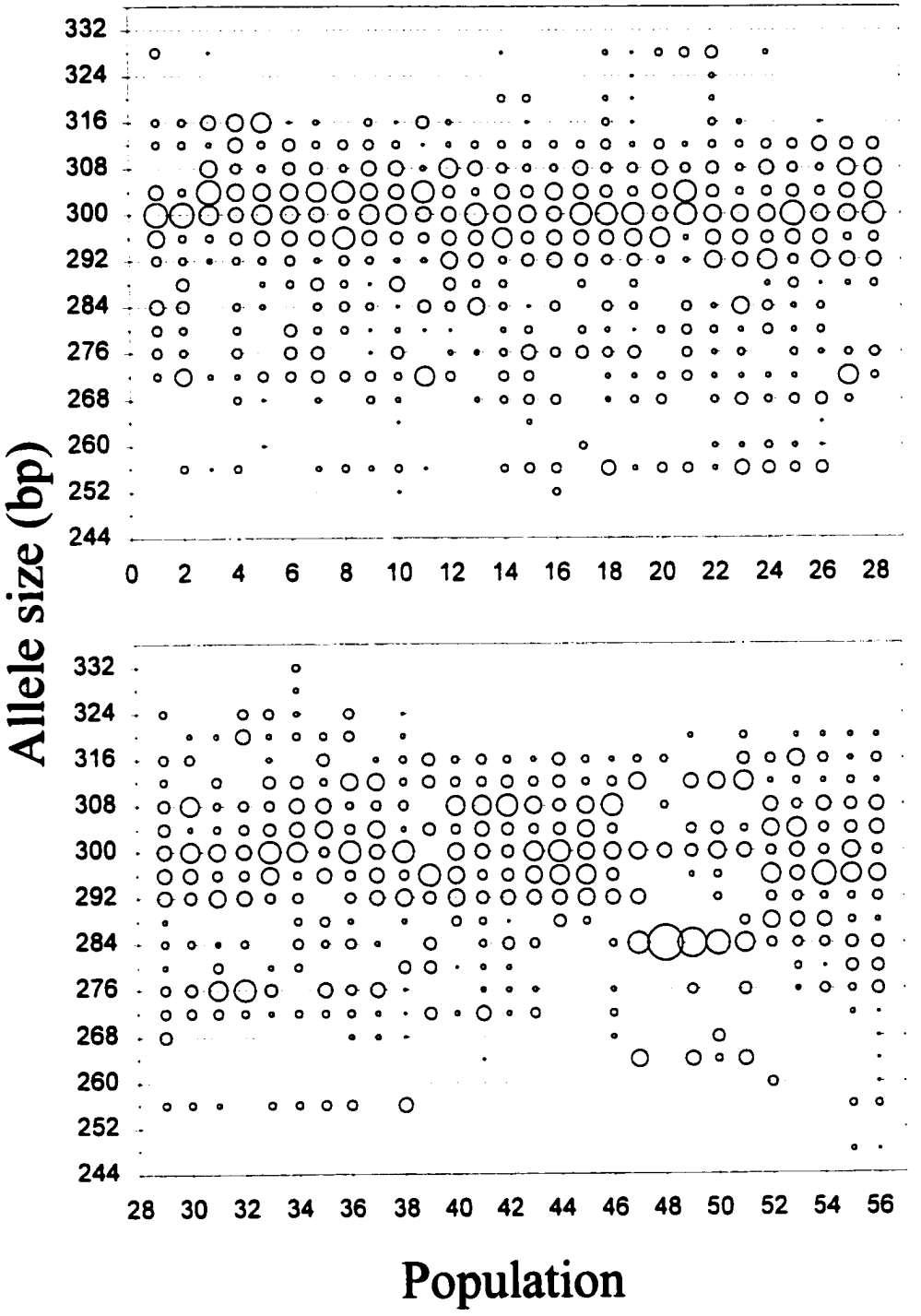

Figure 5-6. Allele frequencies at *Ssa202* for each collection. Relative allele frequency for each collection is indicated by the size of the circle; collection numbers are as defined in Table 5-2 (Pt. Wolfe: 1-13; Big Salmon: 14-26; Petitcodiac: 27-28; Upper Salmon: 29-39; Stewiacke: 40-46; Gaspereau: 47-51; Hammond: 52-54; Margaree: 55-56).

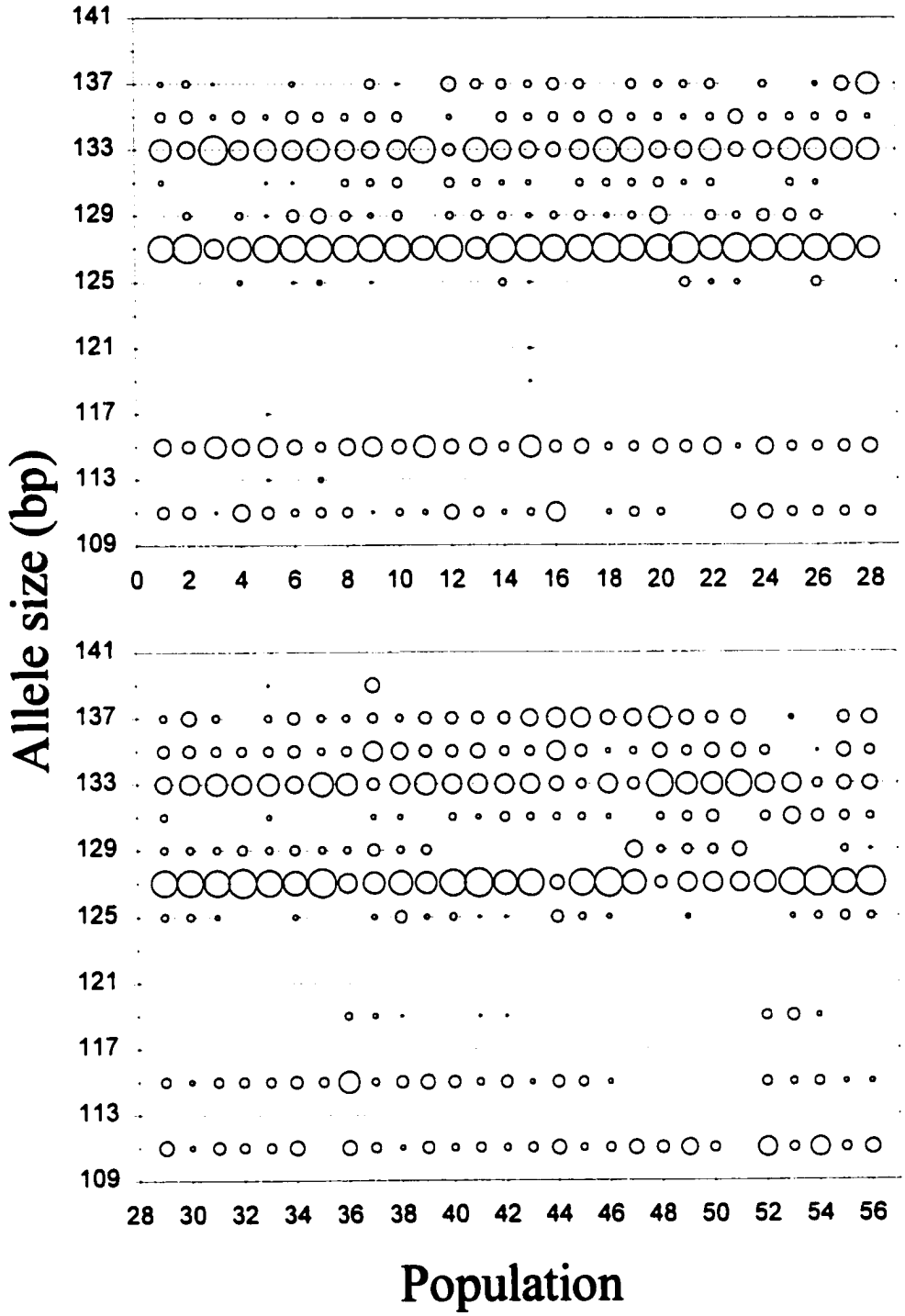

Figure 5-7. Allele frequencies at *Ssa85* for each collection. Relative allele frequency for each collection is indicated by the size of the circle; collection numbers are as defined in Table 5-2 (Pt. Wolfe: 1-13; Big Salmon: 14-26; Petitcodiac: 27-28; Upper Salmon: 29-39; Stewiacke: 40-46; Gaspereau: 47-51; Hammond: 52-54; Margaree: 55-56).

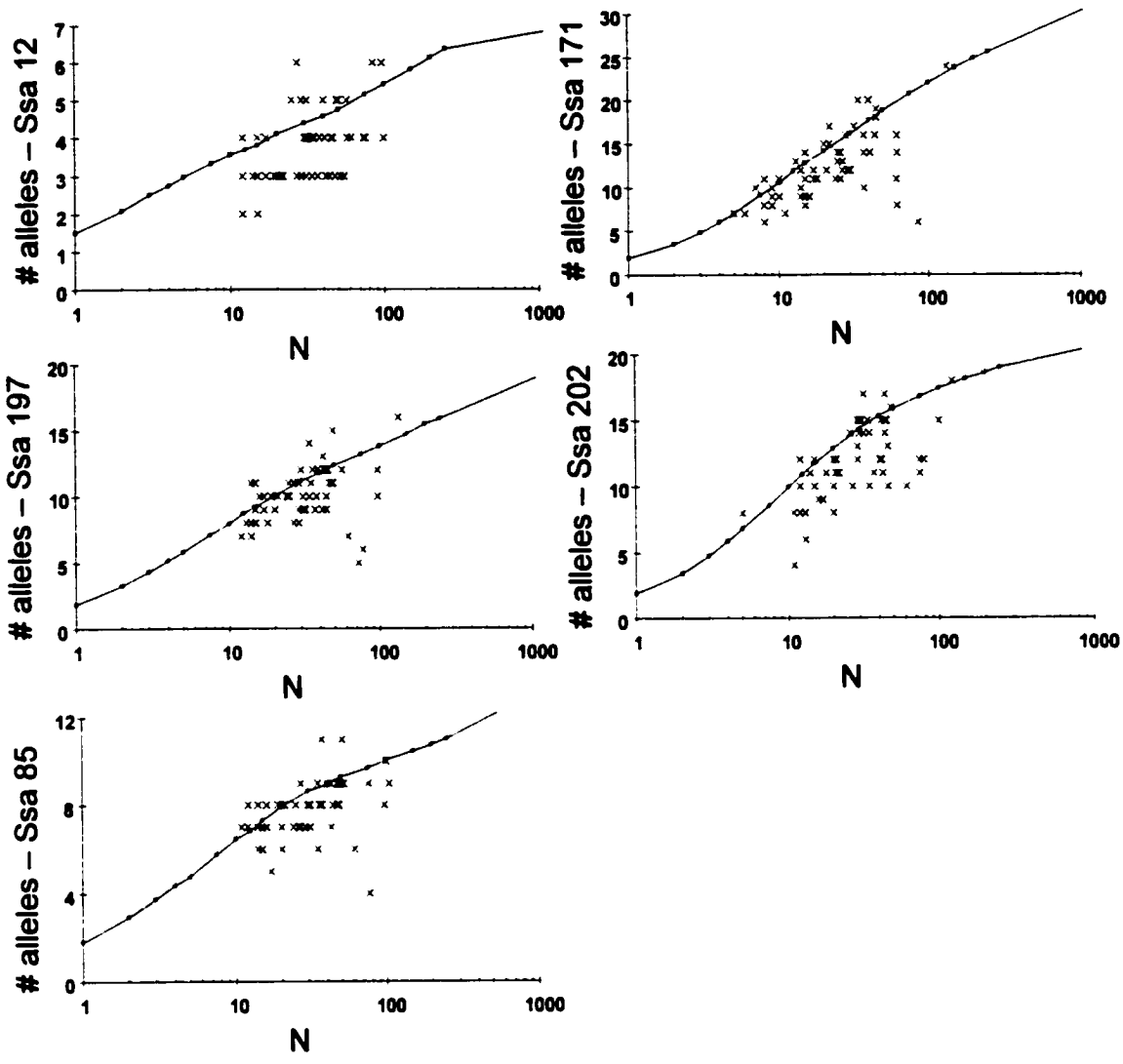

Figure 5-8. Number of alleles per locus as a function of sample size for all collections. The squares joined by the solid line represent bootstrapped values using all individuals from the Inner Bay of Fundy, with a final value representing the actual number of alleles observed at each locus occurring off the scale.

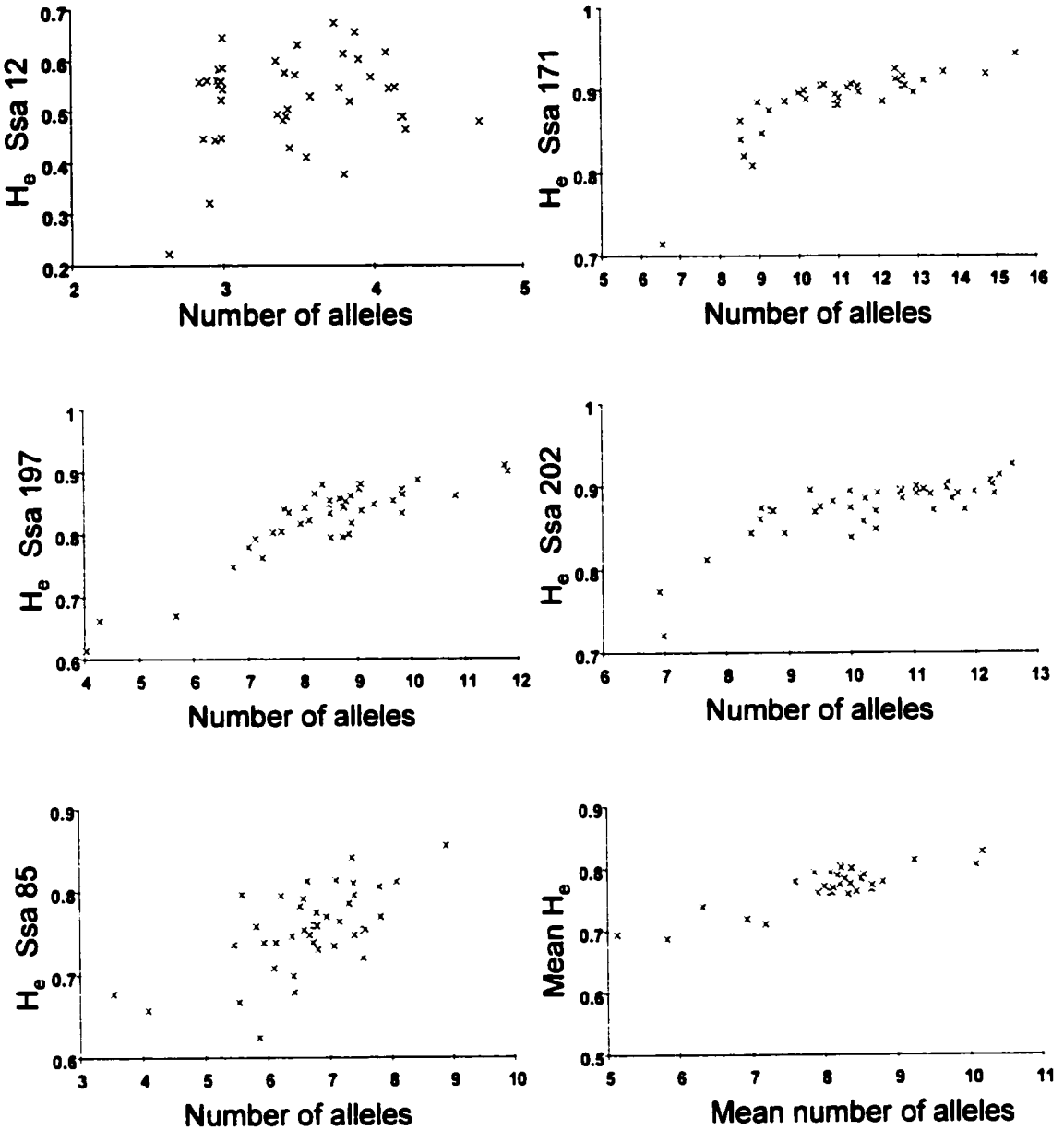

Figure 5-9. Gene diversity (H_e) as a function of bootstrapped number of alleles for all loci and mean of all loci for each collection with $n \geq 20$.

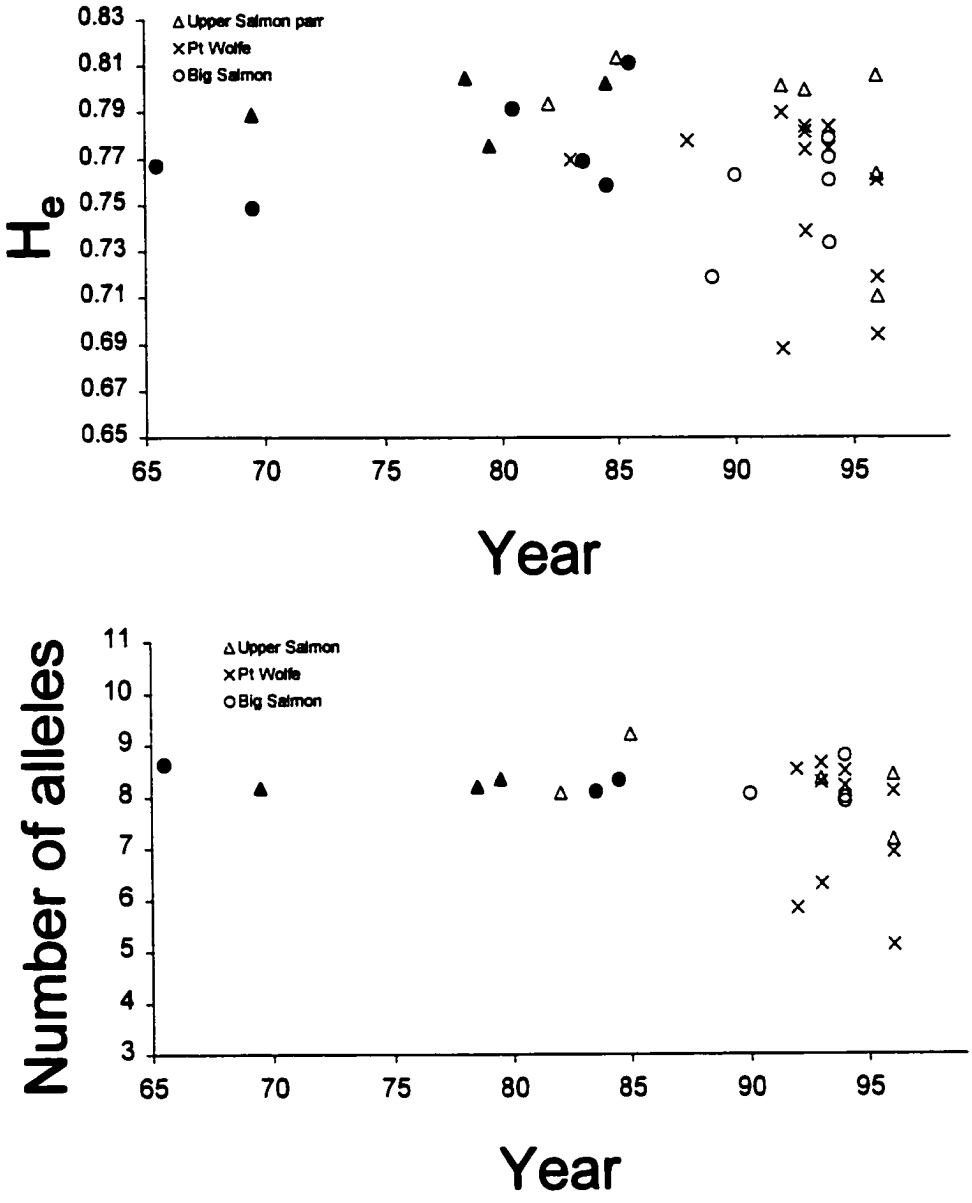

Figure 5-10. Gene diversity (H_e; upper graph) and bootstrapped number of alleles in the Upper Salmon, Point Wolfe and Big Salmon River collections for all year classes sampled (year-class for adult salmon (dark points) was approximated as (year collected - 4.5 years)).

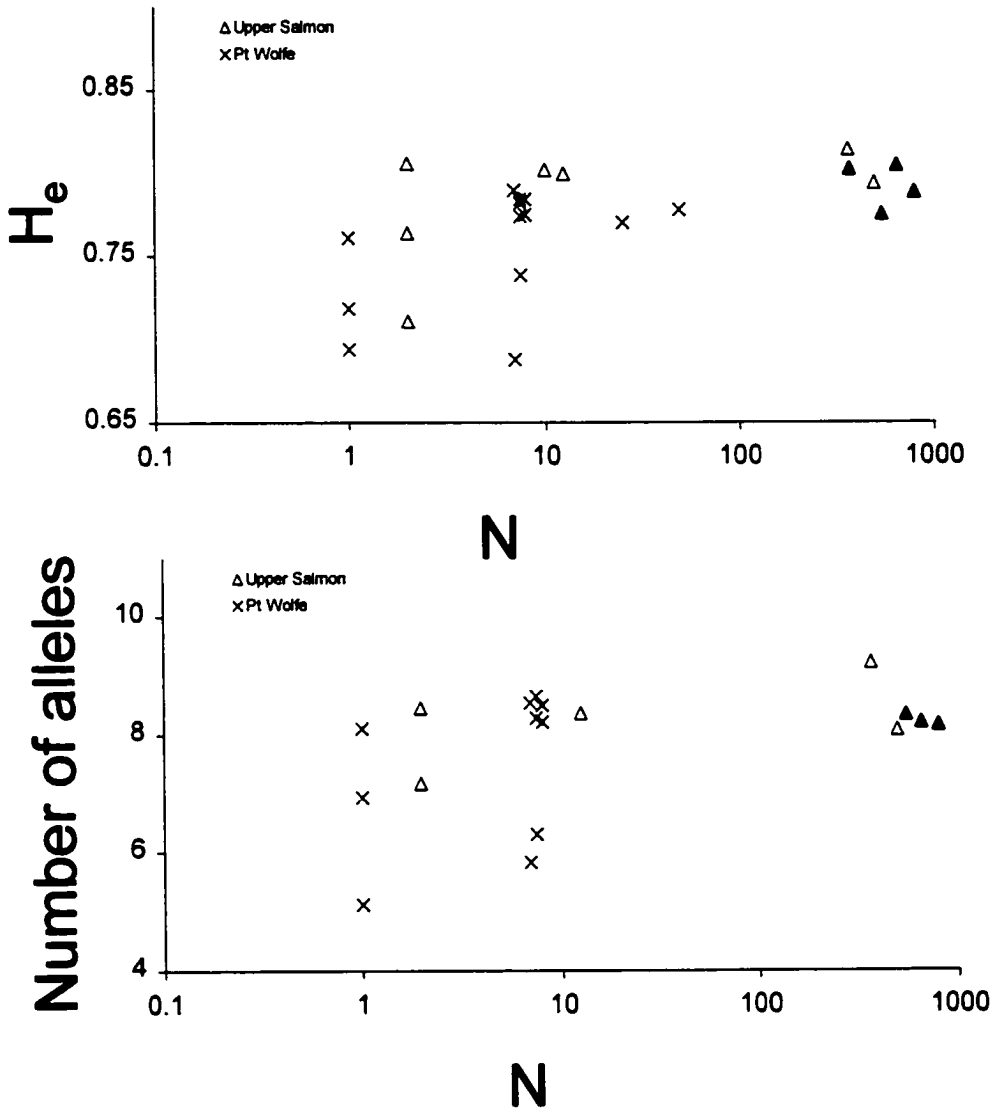

Figure 5-11. Gene diversity (H_e; upper graph) and bootstrapped number of alleles in the Upper Salmon and Point Wolfe collections as a function of the number of anadromous salmon observed in each river for the year classes sampled (year-class for adult salmon (dark points) was approximated as (year collected - 4.5 years); N was estimated for these years as a mean of the adjacent years. Similarly, the mean of the preceding and following N was used in two instances where no estimate of N existed).

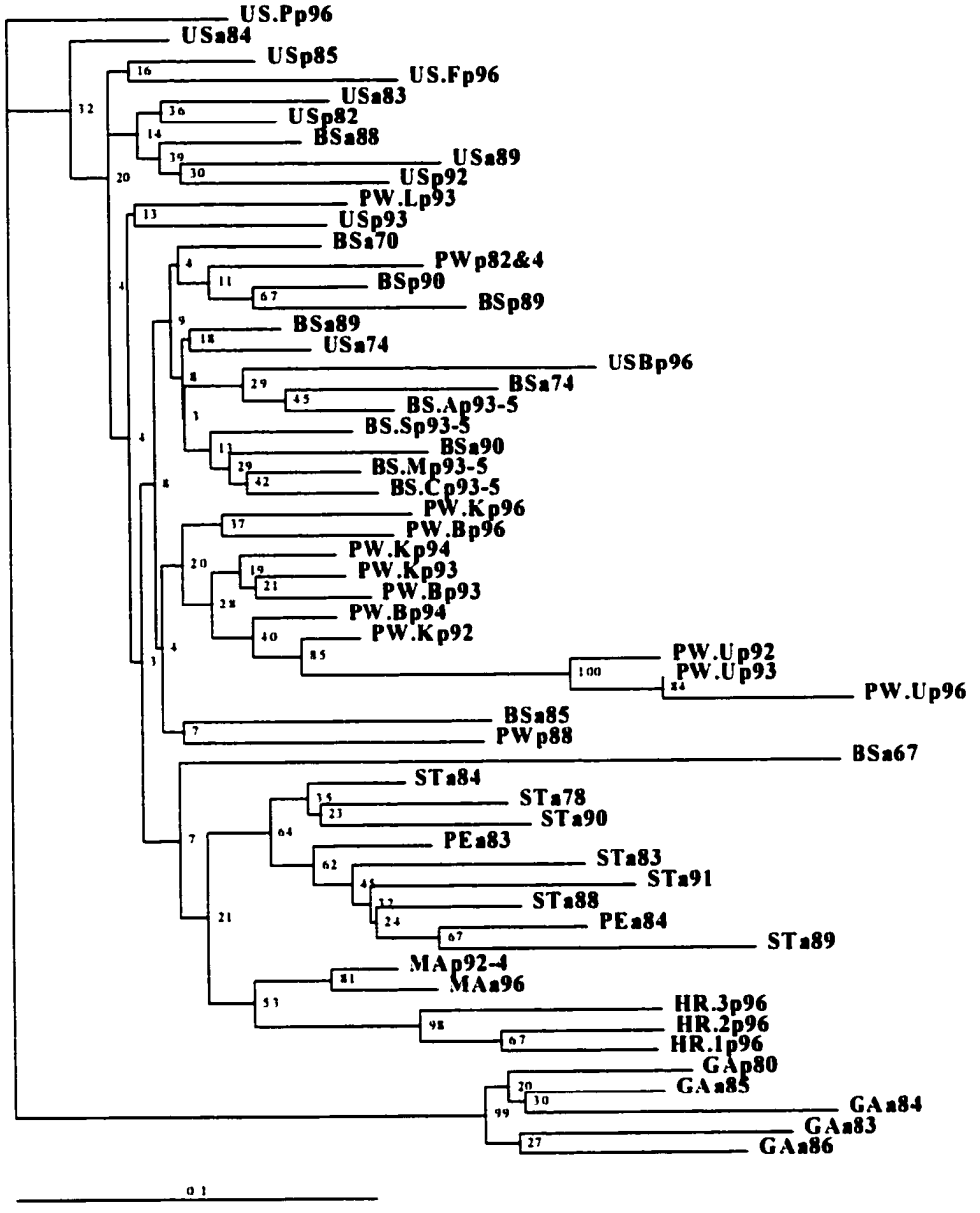

Figure 5-12. Neighbour-joining dendrogram of Atlantic salmon collections using D_A.

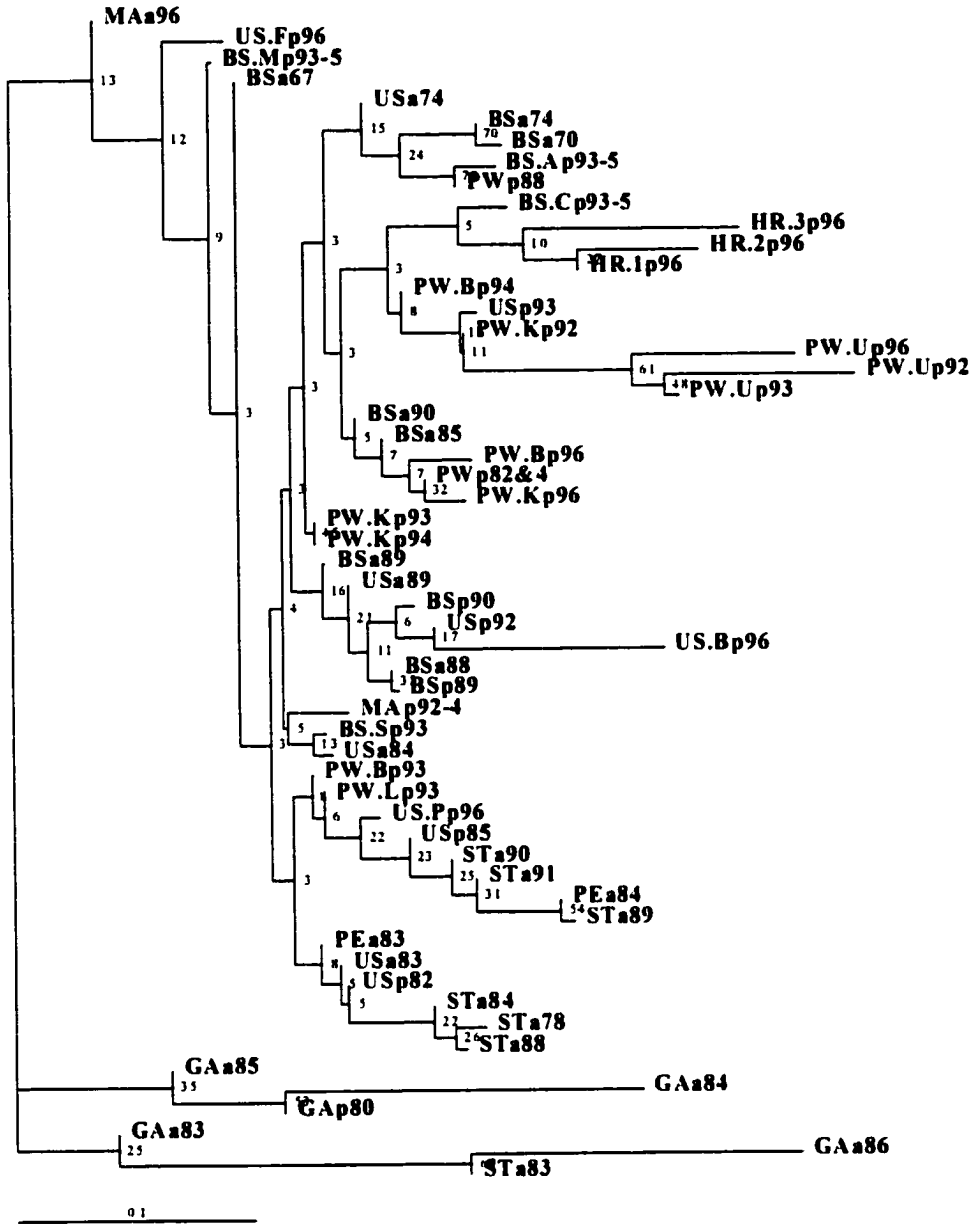

Figure 5-13. Neighbour-joining dendrogram of Atlantic salmon collections using D_{SW}.

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