Ecological correlates and long-term consequences of hatchery-wild hybridization

A thesis
In the Department
of
Biology

Presented in Partial Fulfillment of the Requirements
For the Degree of Master of Science (Biology) at
Concordia University
Montreal, Quebec, Canada

August 2012

© Andrew B. Harbicht, 2012

CONCORDIA UNIVERSITY

School of Graduate Studies

This is to certify that the thesis prepared

By: Andrew B. Harbicht

Entitled: Ecological correlates and long-term consequences of hatchery-wild hybridization

and submitted in partial fulfillment of the requirements for the degree of

Master of Science (Biology)

complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the final Examining Committee:

Dr. Vladimir Titorenko_____ Chair

Dr. Robert Weladji_____ Examiner

Dr. Selvadurai Dayanandan_____ Examiner

Dr. Daniel McLaughlin_____ External Examiner

Dr. Dylan Fraser_____ Supervisor

Approved by Dr. Selvadurai Dayanandan_____
Chair of Department or Graduate Program Director

August 16th, 2012 Brian Lewis_____
Dean of Faculty of Arts and Science

Abstract

Ecological correlates and long-term consequences of

hatchery-wild hybridization

Andrew Harbicht

The intentional and unintentional removal of reproductive barriers between populations is resulting in ever increasing occurrences of intraspecific hybridization between genetically distinct populations, in many cases of domestic or hatchery origin. To appropriately manage the potential risks to nearby wild populations such intraspecific hybridization represents conservation and wildlife managers must understand what factors affect the extent of hybridization as well as what the long-term outcomes are for affected populations. This study presents the results of two separate but related investigations into this phenomenon. The first addressed whether environmental or anthropogenic variables can be used to predict the extent of admixture following hybridization between wild and hatchery brook trout populations. The results suggest that populations inhabiting less productive habitats (lower pH, higher elevation, reduced littoral zones) experience greater admixture following hybridization with hatchery fish, as do populations exposed to increased human activity (elevated fishing pressure and stocking frequencies). The second portion of this study addressed the long-term consequences of hybridization on fitness and adaptability through experimental matched plantings of wild, admixed and hatchery strains into three new environments roughly 800 km away. Results indicated that among the populations studied, there was no evidence for long-term deleterious effects of hybridization after as few as 7 generations following the cessation of stocking. The results of these two studies have implications for the management of hybrid

populations and hybridization events following accidental introductions by proposing measures that may limit the resulting amount of admixture as well as for the conservation and restoration of native populations in areas exposed to hybridization with conspecifics as the results suggest that any negative effects of hybridization may be transient, with populations displaying levels of survival and phenotypic plasticity comparable to wild populations in a time span of fewer than ten generations.

Acknowledgments

I would like to formally thank my supervisor Dylan Fraser for all the assistance and guidance he has offered over the span of this project and Mohammed Alshamlih who provided the raw genotype data for chapter one. I would also like to thank my pseudo co-supervisor Chris Wilson for the help provided with the writing, genotyping and the field components of this project. Without his help and the help of other Ontario Ministry of Natural Resources staff; Bill Sloan, Ola McNeil, Jim Trottier, Anne Kidd, and Jennifer Bronnenhuber, this project would not have been possible. Thanks must also be given to both of my excellent field assistants; Steve Harbicht and Emily Fobert for their hard work and dedication in the field as well as Dr. Paul Morgan who offered his fishing lodge (Blue Fox Lodge) as a base camp for field operations. I would also like to thank Anna Calvert for her excellent advice regarding editing of this manuscript and Morgane Bonamy who provided the constant motivating force that resulted in this project being finished on time.

Dedication

The following thesis is dedicated to my mother, Jill Ann Harbicht, who passed away last year. She is greatly missed

Contribution of Authors

The research, statistical analyses, and writing for this project was performed by the principle author of this study, , with extensive amounts of advice in regards to the execution of field work, the statistical tests employed and writing provided by Dylan Fraser and Chris Wilson. The raw genetic information employed in chapter 1 of this project was provided by Mohammed Alshamlih as per an agreement made with Mohammed and his supervisor, Chris Wilson. The collection of gametes for chapter 2, their fertilization as well as care provided pre-stocking was provided by Bill Sloan at the Codrington Research Hatchery.

Table of Contents

List of Figures

dorsal limit of operculum; 4, the anterior insertion point for dorsal fin; 5, the anterior limit of adipose fin; 6, the dorsal terminus of the caudal flexure; 7, the ventral terminus of the caudal flexure; 8, the anterior insertion point of the anal fin; 9, the anterior insertion point for the left pelvic fin; 10, the anterior insertion point for the left pectoral fin; 11, the meeting point of the gill plate and the ventral midline; 12, the most posterior point on upper mandible; 13, the most anterior point on the eye; 14, the most posterior point on the eye; 15, the most posterior point on the operculum; 16, the dorsal position above the thinnest part of the caudal peduncle; 17, the ventral position below the thinnest part of the caudal peduncle.

Figure 2.2: Estimated population abundances and 95% confidence limits in three study lakes north of Lake Huron, Ontario, Canada, five month post stocking. Estimates were made using the Schnabel method with the Chapman modification and confidence intervals were based on the Poisson distribution.

Figure 2.3: Mean (± SE) number of fish from each of five populations caught per net set (ranging in soak time from 15 min to 7 hours) in three study lakes north of Lake Huron, Ontario, Canada.

Figure 2.4: Mean (± SE) centroid size for five populations of brook trout in three study lakes north of Lake Huron, Ontario, Canada. Centroid sizes were calculated using tpsRelW by Rolph (2006) and photographs of the left side of fish captured using short-set gill net sampling.

Figure 2.5: Mean (± SE) relative warp values for five populations of brook trout in three study lakes north of Lake Huron, Ontario, Canada. Relative warp values were produced using the program tpsRelW by Rolph (2006) and photographs of the left side of fish captured using short-set gill net sampling. Relative warp 1 (A) corresponds to deeper bodies at higher values, relative warp 3 (B) corresponds to shorter stockier fish higher values, and relative warp 4 (C) corresponds a lengthening of the caudal.

List of Tables

and the standard error for each parameter against hatchery admixture. Models were created using both beta regressions on lake mean admixture values and generalized linear models on individual admixture values. The method of estimation used for the relative variable importance was that of Burnham and Anderson (2002) with a maximum model size of three parameters.

Table 1.4: The three best-fit models from an exhaustive set of all possible additive and interactive models containing various combinations of five explanatory variables found to be important in modeling the amount of hatchery-wild admixture in populations of brook trout. Models were constructed using two parallel methods; beta regression and generalized linear mixed models with beta error distributions.

Table 2.1: Environmental characteristics of source population habitat and experimental lakes used in a matched planting experiment as well as information on the hatchery admixture proportion of each source population based on estimates from STRUCTURE (Pritchard et al. 2000), the number of full and half sibling families used for matched planting and the number of families represented among the fish present in the study lake at the end of the experimental period (five months). NA represents non-applicable, as is the case with environmental characteristics for the hatchery and with the number of families formed or recovered from study lakes.

Table 2.2: Results of model selection using AIC values on the number of each of five populations of brook trout caught per net set in three study lakes north of Lake Huron, Ontario, Canada. Net sets varied in soak time from 15 minutes to 7 hours.

Table 2.3: Results of model selection using AIC values on the phenotypic parameters measured for five populations of brook trout matched planted into three study lakes north of Lake Huron, Ontario Canada. The morphological response variables; Centroid size and Relative Warp values RW1, RW3, and RW4, were produced in tpsRelW (Rohlf 2006).

LIST OF ABBREVIATIONS

Lakes sampled for Chapter 1

ANI…..Animoosh Lake

CHR…..Charles Lake

CHIP…..Chipmunk Lake

CDS…..Coldspring Lake

DIK…..Dickson Lake

FARN…..Farncomb Lake

FRK…..Frank Lake

GUSK…..Guskewau Lake

HRY…..Harry Lake

HOG…..Hogan Lake

HND…..Hound Lake

LAV…..Lavieille Lake

LCRO…..Little Crooked Lake

LDIK…..Little Dickson Lake

LMYK…..Little Mykiss Lake

MJR…..Major Lake

MYK…..Mykiss Lake

OWE…..Owenee Lake

PHP…..Philip Lake

RED…..Redrock Lake

REN…..Rence Lake

SAL…..Salvelinus Lake

SCOT…..Scott Lake

SHAL…..Shallnot Lake

STRK…..Stringer Lake

WELC…..Welcome Lake

WEST….. Westward Lake

General Introduction

A problem often encountered by conservation organizations such as the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) or those tasked with enforcing the Endangered Species Act in the U.S.A. is what to do with hybridized populations (Allendorf et al. 2004). Human activities have resulted in the breakdown of many isolating barriers among populations, resulting in an ever-increasing number of hybridized populations (Allendorf et al. 2001). The immediate consequences of such hybridization may range from the genetic rescue of a small population (Hogg et al. 2006), to considerable deleterious effects when genetic differentiation between hybridizing populations produces genetic incompatibilities or the loss of local adaptations (Edmands 1999).

In many cases the effect of hybridization is in part dependent on the extent of hybridization, which can vary from being limited to the F1 generation when sterile offspring are produced, to the introgression of genetic material from one population to another when offspring are fertile and backcrossing occurs. Given enough time such backcrossing often results in complete admixture whereupon no individual in a hybridized population can be classified as a non-hybrid as each member of the population possesses some amount of transgenetic material (Allendorf et al. 2001). Large variation in the extent of admixture can exist among groups of populations exposed to similar conditions (Halbisen and Wilson 2009) and the reasons for this variation are largely unknown. Knowledge of environmental or anthropogenic variables which may be

correlated to admixture levels would assist conservation organizations in locating populations that have resisted admixture following hybridization events, or populations that might be at risk of undesirable hybridization based on their biotic, abiotic and anthropogenic environments.

Unfortunately the problem with hybridizing populations does not end with their identification. There is also the question of whether or not they still possess any conservative value biologically. In situations where hybridization is entirely the result of human activity, hybrid populations are generally seen as being a separate entity from the original species or population and as such are omitted from any protection that may have been afforded to their progenitors while even being seen as a threat in some situations (COSEWIC 2011). The reason for this treatment is generally attributed the decreased fitness exhibited by hybrids compared to wild individuals as a result of outbreeding depression (Edmands 1999, Allendorf et al. 2004). But while this classification may last indefinitely, theory predicts that the negative fitness effects of outbreeding depression will not (Tallmon et al. 2004).

In the following chapters I will address these two important issues by making use of the hybridized populations of brook trout in Algonquin Provincial Park, Ontario, Canada as a model system. Within the park there are multiple populations that have experienced stocking of hatchery fish in the past. The result of this past stocking has been that populations across the park now display varying amounts of hatchery admixture despite having experienced similar conditions among them. This provides not only an

opportunity to test for environmental and anthropogenic correlates of hatchery admixture and potentially identify ecological drivers of admixture, it also provides a source of multiple hybridized and wild populations. As stocking of naturally self-sustaining brook trout populations nearly completely ceased within the park over 7 generations ago, it is possible to address the issue of what the long-term consequences of hybridization are for fitness by experimental means. By addressing these issues I intend to provide management and conservation organizations with valuable information that will assist them in situations where hybridization is a potential or principle issue such as in situations of biological invasions, natural restoration or conservation management.

CHAPTER 1:

ENVIRONMENTAL AND ANTHROPOGENIC CORRELATES OF HYBRIDIZATION BETWEEN HATCHERY AND NATIVE TROUT IN WILD POPULATIONS

Andrew B. Harbicht[1], Mohammed Alshamlih[2], Chris C. Wilson[3], Dylan J. Fraser[4]

[1] Concordia University, Montreal, QC Canada, aharbicht@yahoo.ca

[2] Trent University, Peterborough, ON Canada, alshamlih@gmail.com

[3] Ontario Ministry of Natural Resources, Peterborough, ON Canada, chris.wilson@ontario.ca

[4] Concordia University, Montreal, QC Canada, dylan.fraser@concordia.ca

ABSTRACT

Interbreeding between stocked and wild fish is increasingly common due to the routine stocking of hatchery-reared fish into lakes and streams containing wild populations. The present study assessed whether specific human actions and/or environmental characteristics were associated with the extent of hybridization between hatchery and wild brook trout occupying seventeen lakes in Algonquin Park, Ontario, Canada. We found that hatchery and wild trout were most likely to hybridize in lakes with less productive environments for wild trout (lakes with lower pH, smaller littoral zones, and situated at higher elevations), with repeated stocking events of hatchery trout, and with greater exposure to other human activities (higher accessibility, angling pressure). Conversely, other stocking factors such as the stocking density, total number stocked and life stage of hatchery fish stocked did not show significant influence on the extent of hybridization. Our results highlight how particular human activities may interact with specific environmental conditions to elicit hybridization in wild populations. Hence, the risk of hybridization might be anticipated based on readily available environmental and land use data. The results further provide a potentially economical means of identifying indigenous populations with minimal introgression.

KEY WORDS:

intraspecific hybridization, introgression, salmonid, brook trout, hatchery, stocking, *Salvelinus fontinalis*, conservation

INTRODUCTION

Anthropogenic hybridization within species is a common occurrence and has been observed in many different species from wild and domestic cats in Europe (Pierpaoli et al. 2003*)* to reindeer and caribou in Alaska (Cronin et al. 1995) and can have both positive and negative ecological and genetic effects. On one hand, it may facilitate the genetic rescue of a population by adding new alleles necessary to stave off inbreeding depression and extinction (Hogg et al. 2006), or enhance a population's adaptive potential in new or changing environments (Arnold 1992). On the other hand, hybridization can reduce a population's fitness through outbreeding depression, potentially resulting in the loss of local adaptations and ultimately reduced population viability (Rhymer and Simberloff 1996, Tallmon et al. 2004).

Introgressive transfer of genetic material between two groups can result in novel, untested genes being introduced into a gene pool that subsequently lower the fitness of all individuals possessing such transgenes (Rhymer and Simberloff 1996, Burke and Arnold 2001). Following continued backcrossing, transgenes may spread throughout a population, eventually resulting in most individuals containing some portion of hybrid genotype. Such populations are referred to as being admixed (Allendorf et al. 2001). The occurrence and extent of admixture following hybridization between different populations are highly variable (virtually ranging from 0 to 100%: e.g. Hindar et al. 1991; Hansen 2002; Hansen et al. 2009; Marie et al. 2010). The causes of such variable admixture have not been studied thoroughly in most instances, but they are likely influenced by a number of ecological factors that affect both the frequency of

hybridization and the fitness of hybrid individuals relative to the parents (Utter 2000; Hails and Morley 2005).

Human-induced hybridization is an increasing occurrence in many taxa (Cronin et al. 1995; Pierpaoli et al. 2003; Hails and Morley 2005). In fishes, escapes from aquaculture facilities and the intentional release of domestic or hatchery fish to supplement exploited wild populations have resulted in a growing number of cases of intraspecific hybridization (Rhymer and Simberloff 1996; Hutchings and Fraser 2008). Such human-induced hybridization often has had negative effects on wild populations, most commonly in the form of reduced fitness in hatchery-wild hybrids relative to wild conspecifics (Fraser 1981; Webster and Flick 1981; Einum and Fleming 1997; McGinnity et al. 2003; Araki et al. 2007; Fraser et al. 2010b; but see Fraser et al. 2008), and homogenization of genetic differentiation among wild populations (Ryman 1991; Hutchings and Fraser 2008; Matala et al. 2008; Marie et al. 2010).

Recent studies have found that the extent of admixture from fish stocking can be correlated with a species' life history (Utter 2000), stocking intensity (Almodovar et al. 2006), fishing pressure (Garcia-Marin et al. 1998), population size (Hansen et al. 2009), abiotic factors (Marie et al. 2012), and human activities such as logging (Heath et al. 2010). Few studies have rigorously examined the relative influence of multiple environmental and stocking factors in driving variation in hatchery-wild admixture among populations (but see Marie et al. 2012), but such investigations may bear considerable fruits. First, they may shed light on which indigenous populations/species

are more at risk of hybridizing. Second, they may provide an efficient and a cost effective means of identifying indigenous populations that have resisted admixture with previously stocked hatchery fish using readily available data, an increasingly common situation for restoration and biodiversity conservation (see Meraner et al. 2008; Baric et al. 2010). The brook trout (*Salvelinus fontinalis*) of Algonquin Park (Ontario, Canada) provide an opportunity to address the issue of which environmental variables and human factors may affect hatchery-wild admixture. The long stocking history of lakes in the park since the 1930s (Danzmann and Ihssen 1995) is well documented and considerable environmental and ecological information is available on stocked lakes.

The purpose of this study was therefore to identify possible ecological correlates of hatchery-wild admixture from lakes within Algonquin Provincial Park, and thus shed light on possible mechanisms that determine the extent of admixture following stocking events. As stocking has not taken place in the park's lakes since at least 1989, a *post hoc* data analysis approach was implemented whereby the relative importance of variables was analyzed and explanatory models were constructed. This method of data analysis can potentially provide ecologists, conservationists and managers with useful tools for identifying populations that have resisted hybridization and for further understanding the drivers of introgressive hybridization at the population level.

METHODS

STUDY POPULATIONS AND THEIR HISTORY OF HATCHERY TROUT STOCKING

Algonquin Provincial Park covers an area of 7 650 km^2 and contains approximately 250

naturally self-sustaining populations of brook trout (Quinn 1995) spanning 6 watersheds (Danzmann and Ihssen 1995). Between 2002 and 2009, tissue samples were collected from a total of 1071 adult brook trout from a total of 27 brook trout populations; 23 of these were within Algonquin Provincial Park lakes, another two lakes bordering the park; those remainding were the two hatchery brood stocks that have historically been stocked in Algonquin Park (Figure 1.1, Table 1.1). Note that previous work and genetic analysis done for this project suggest that each lake sampled represented an isolated population with the exception of three lakes (Harry, Rence, and Welcome lakes) which are connected and exchange high levels of gene flow (Addison and Wilson 2010). As such, for the purposes of this study, these lakes were treated as one large lake. Of the 25 sampled lake populations, 17 had been stocked in the past with hatchery trout; the remaining eight lake populations (and two hatchery brood stocks) were used as 'controls' for determining the degree of hatchery-wild admixture in stocked lakes in analyses below (Table 1.1).

Stocking in Algonquin Park began in the 1930s and was variable in its frequency and intensity. Some lakes received regular stocking, while others were stocked only once or twice for research or remediation purposes (Ontario Ministry of Natural Resources fish stocking database). All stocking of Algonquin Park lakes (and the lakes studied outside the park) occurred before 1989, with the exception of Charles Lake which received 1500 hatchery fish in 1994, and Major Lake outside the park which received 334 wild fish in 2006. Three hatchery strains of brook trout were stocked within the park. The principal strain used, Hills Lake, originated in Pennsylvania, approximately 500 km south of

Algonquin Park, and has been maintained as a brood stock in the Ontario hatchery system over the entire period that stocking in Algonquin Park took place (Kerr 2000). The second strain originated from Lake Nipigon (approximately 850 km west of the park) and was stocked into several lakes within the park, though to a much lesser extent than the Hills Lake strain. The third strain previously stocked into the park was a first generation cross between the Hills Lake and Nipigon Lake strains and was only stocked in a few instances as part of a study on the fitness of F_1 hatchery-wild hybrids (Fraser 1981).

ENVIRONMENTAL AND STOCKING DATA

We collated environmental data and stocking records for each study lake stocked with hatchery trout from government records obtained from the Ontario Ministry of Natural Resources (OMNR), local records kept by the provincial park authorities (Friends of Algonquin Park), the Algonquin Forestry Authority (AFA), and to a lesser extent published primary literature sources. Variables analyzed are outlined in Table 1.2 and include chemical, physical and biological variables known to be important for the success of stocked brook trout (Kerr 2000). In cases where years in which the data was collected differed from one lake to another, only variables recorded around the same time of year (mid-summer) were used, to minimize differences between lakes resulting from seasonal changes. Of the data available, only variables that offered complete data sets for each lake were used, with the exception of age-at-stocking for which no information was available for two stocking events in Hound Lake.

DNA ANALYSES

Over seven non-consecutive sampling seasons, fin clips were non-lethally collected from adult fish in the 27 populations/hatchery strains sampled and stored in ethanol until the DNA was extracted using a salt extraction method. Individuals were then genotyped using 14 microsatellite loci (*Sfo18, Sfo23, Sfo12*: Angers et al. 1995; *SfoC24, SfoD28, SfoC38, SfoD75, SfoC88, SfoC100, SfoC113, SfoC86, SfoC115, SfoC129*, and *SfoB52*: T. King and M. Burnham- Curtis, U.S. Geological Survey, unpublished data). Each 10 µL multiplex PCR reaction consisted of 6.0 ng of DNA, 2x polymerase chain reaction (PCR) buffer (Promega Flexi Go Taq), 1X BSA (Bioshop), 1.5 mM MgCl (Promega Flexi Go Taq), 0.2 mM of dNTPs (Bioshop), 0.025 units of Taq polymerase (Promega Flexi Go Taq), between 0.03 to 0.3 µM of each primer pair (Operon and ABI) and double distilled H_2O. PCR amplifications were completed under the following conditions: 94.0 ºC for 5 min, 36 cycles of 94.0 ºC for 1 min, 58.0 ºC or 60.0 ºC (multiplex specific) for 1 min, 72.0 ºC for 1 min, and a final extension at 60.0 ºC for 45 min. Amplified PCR product was visualized using capillary electrophoresis on a ABI 3730 automated sequencer (Life Technologies Inc., Carlsbad, California, USA). Genotypes were scored using ABI GeneMapper v 4.0 and visual proofreading.

WITHIN-LAKE MEASURES OF GENETIC DIVERSITY

To determine the effect of hatchery stocking on microsatellite allele frequencies, admixture was compared to measures of allelic diversity calculated for all sampled populations/hatchery strains using Genetic Analysis in Excel (Peakall and Smouse 2006). These measures included the observed (H_o) and expected heterozygosity (H_e), and the

mean number of alleles per locus (N_a). GENEPOP 4.0.10 (Raymond and Rousset 1995) was also used to test for genetic linkage disequilibrium among loci within populations as well as deviations from Hardy-Weinberg equilibrium HWE within populations, as the program used to determine the extent of hatchery-wild admixture below (STRUCTURE; Pritchard et al. 2000) assumed linkage equilibrium and HWE within populations.

ESTIMATION OF THE DEGREE OF HATCHERY-WILD ADMIXTURE

The extent of admixture between hatchery and wild trout in different lakes was assessed using the Bayesian clustering methods implemented in the program STRUCTURE. First, the total number of clusters present within reference samples was estimated separately for hatchery reference samples (Hills Lake and Lake Nipigon) and non-stocked reference samples (non-stocked lakes within the park). This involved employing the methods of Evanno et al. (2005) with 10 replicate trials over a range of K values (from $K = 1$ to $K = n+3$ where n is the actual number of populations sampled) and a burn-in period of 50 000 replicates followed by 50 000 Markov chain Monte Carlo (MCMC) replicates. As the two hatchery reference samples clustered separately and only two clusters were present within the non-stocked reference samples (referred hereafter as 'Algonquin East' and 'Algonquin West'), two separate analyses were undertaken. The first involved pooling all populations that had been stocked with only the Hills Lake strain into a single dataset along with the Hills Lake and Algonquin (East and West) reference samples. STRUCTURE was then told which samples were reference samples and asked to assign the remaining samples to the three clusters (Hills lake, Algonquin East, Algonquin West) allowing for admixture and correlated allele frequencies and using a burn-in period of

$1x10^5$ replicates followed by $5x10^5$ MCMC steps. The second analysis was conducted in the same manner but only populations receiving hatchery fish from both the Hills Lake and Lake Nipigon strains (or their hybrid) were included along with all four reference sample clusters (Hills Lake, Lake Nipigon, Algonquin East, Algonquin West). More details about the procedures used are found in Appendix 1.

ECOLOGICAL CORRELATES OF HATCHERY-WILD ADMIXTURE

To determine which environmental and stocking variables could be potentially important for predicting hatchery-wild admixture, we used a model selection method slightly modified from that described by Burnham and Anderson (2002) whereby relative variable importance was first estimated using an exhaustive set of simple additive 1, 2, and 3 parameter models using variables listed in Table 1.2. Cumulative Akaike weights ($\Sigma\omega$) were then calculated for each variable and any variables deemed to be unimportant were excluded from further modeling. This overall procedure was conducted with two separate modeling methods: (i) beta regressions, using mean admixture values for each lake (hereafter referred to as the BETA method), and (ii) generalized linear mixed-effects models (hereafter referred to as the GLMM method), using a beta error distribution and individual admixture values within lakes as the response variable. Lakes were used as random effects. Both the BETA and GLMM methods were used to ensure that our analysis of ecological variables and hatchery-wild admixture captured the relationship at the population and individual levels, respectively. This also allowed us to account for any effect of variation in individual admixture levels. To determine the relationship between the top five variables resulting from each method, which were concordant, and the

resulting hatchery-wild admixture levels a second exhaustive set of models was tested. This set included all possible additive and interactive models containing the five important variables and interactions were restricted to a maximum size of a two-way interaction because more complex models were difficult to interpret biologically. Models were then compared using Akaike's Information Criterion (AIC) values for the GLMM method, and second order (AICc) values for the BETA method due to smaller sample sizes (Burnham and Anderson 2002).

RESULTS

WITHIN-LAKE MEASURES OF GENETIC DIVERSITY

Of the 27 populations/hatchery strains sampled, 12 exhibited significant deviation from Hardy-Weinberg expectations (HWE) after Bonferroni corrections ($\alpha = 0.0036$, $k = 14$) (Table 1.1). Among these, ten displayed heterozygote deficiencies, but these were spread out over the fourteen different loci and depended on the particular population (In total 76 loci deviated significantly from HWE out of 378 tests, the maximum and minimum number of loci that deviated significantly within a population was 6 and 1 respectively); this suggested biological explanations for them. Two of the deficiencies were found in the hatchery strains; the Hills Lake strain has been maintained within the Ontario hatchery system for over 40 years with few genetic introductions (Fraser 1981); and the Lake Nipigon strain, which was established from wild spawn collections in the late 1970s (Kerr 2006). Of the remaining eight lakes displaying heterozygote deficiencies, three had large surface areas (>181 ha) and a fourth (HRW) was comprised of three connected lakes where possible population substructure was present and hence a Wahlund effect

was not unexpected (Wahlund 1928, but see Addison and Wilson 2010). Heterozygote deficiencies in the smaller lakes have been detected in other brook trout populations and may be due to limited suitable breeding sites being available, which can reduce dispersal from spawning sites and increase occurrences of inbreeding compared to larger lakes (Castric et al. 2002). Of the 2457 tests of linkage disequilibrium across populations (ninety-one tests per population), only 35 were significant after Bonferroni corrections ($\alpha = 0.000549$, $k = 91$) and these were spread out over all loci and in 15 different populations. All three measures of genetic diversity displayed curved, polynomial relationships best described by quadratic relationships between each variable and the extent of hatchery admixture, wherein intermediate admixture values around or slightly above 0.5 displayed the highest values (all p-values < 0.01) (Figure1.2).

DEGREE OF VARIATION IN ADMIXTURE AMONGST STUDY LAKES

The mean (± S.D.) admixture across all previously stocked lakes was 0.34 ± 0.34, yet few lakes displayed intermediate admixture: roughly half of the lakes had mean admixture ranging from 0.18 to 0.90 while the other half demonstrated little admixture (<0.05)(Table 1.1). Within-lake variance in admixture was generally low (0.00002 to 0.04) which is to be expected since in most cases at least 7 generations have passed (Liskauskas and Ferguson 1991) since the park ceased stocking naturally self-sustaining populations.

ECOLOGICAL CORRELATES OF ADMIXTURE

Among the five most important predictive variables (Table 1.3) were three environmental factors (pH, elevation, depth), one related to stocking (number of stocking events) and one related to other human influences (accessibility). pH was the explanatory variable with the most support, with a cumulative AIC weight ($\Sigma\omega$) double or nearly double that of the explanatory variable with the second-most support (elevation) for the GLMM and BETA methods respectively. The remaining environmental variable was mean depth. In the BETA model, the number of stocking events ranked slightly higher than the accessibility of a lake (trail length) while in the GLMM the relative importance of the number of stocking events was less than both accessibility and mean depth.

Notable omissions from the best five variables included admixture period, the amount of time between the first stocking event and the collection of genetic samples for admixture analysis, which ranked second to last in both the BETA and GLMM methods. The total number of hatchery trout stocked and the mean stocking density also ranked low with both the BETA method (11^{th} and 12^{th} out of 14 variables tested) and the GLMM method (10^{th} and 12^{th} respectively). Interestingly, both modeling methods assigned the lowest relative variable importance to the lake surface area although this variable was historically used to calculate stocking numbers (Kerr 2006).

MODEL SELECTION

Both modeling methods (BETA and GLMM) resulted in the same best-fit model, an additive relationship between pH and stocking events with an interactive relationship

between elevation and mean depth. The 2nd and 3rd place models were the same in both methods, however the orders were reversed (Table 1.4). Beyond these three (not shown) the BETA method tended to favor simpler models while the GLMM method favored more complex models with more interactive effects. In all of the top three models each parameter and interaction was significant with the exception of accessibility (trail length), which was non-significant in both of the models in which it appears for each modeling method (BETA and GLMM). The interaction between pH and stocking events in the 2nd and 3rd models of the BETA and GLMM methods respectively was also non-significant, suggesting that this model possessed no more explanatory power than the best fit model.

In general, the relationships between each explanatory variable and the degree of hatchery-wild admixture were fairly weak with the exception of pH, which displayed a significant negative correlation with admixture (p-value > 0.01 for both BETA and GLMM methods). Elevation had a positive correlation with admixture as did accessibility, i.e. lakes with shorter trail distances had higher levels of admixture. Mean depth was negatively correlated with admixture while the number of stocking events was positively correlated (Table 1.3). Upon inspection of the graphs (Fig. 1.3) it became apparent that one data point in the relationship between admixture and mean lake depth, corresponding to Westward Lake, was highly influential in determining the slope of the relationship. A supplemental analysis with this point removed resulted in the reversal of the slope from negative to positive. The effect of the interaction between mean depth and elevation was to reduce the increase in admixture experienced by high altitude lakes when mean depth was shallow (increased littoral zone).

DISCUSSION

Our results suggest that, among Algonquin Park lakes previously stocked with hatchery brook trout, few environmental and stocking variable were effective at predicting the genetic outcome of stocking hatchery conspecifics into wild populations of brook trout. Variables correlated to hatchery-wild hybridization tended to be only weakly so and possessed little or no statistical significance on their own, with the exception of pH. The general ineffectiveness of individual variables to predict hatchery wild admixture suggests that ecological determinants of hatchery-wild admixture are likely combinations of several factors. For the most part, these factors appear to be associated with lake productivity and brook trout density, specifically pH, mean depth and elevation (see below). Certain anthropogenic variables also proved to be important, such as the number of stocking events and lake accessibility. Other anthropogenically-driven variables, including the total number of hatchery fish stocked, did not explain the extent of admixture.

The low relative importance of the admixture period indicates that populations that were stocked earlier and therefore have had a greater number of generations exposed to hybridization and introgression do not necessarily possess higher levels of admixture than populations which weren't stocked until later. This suggests that the current admixture levels are most likely the result of initial determinants of the extent of hybridization rather than the result of transgenes increasing or decreasing in frequency as a function of time.

ENVIRONMENTAL CORRELATES OF HATCHERY ADMIXTURE

After examining the relationship between morphological lake parameters (mean depth and surface area) as well physicochemical parameters (dissolved oxygen, pH, and temperature) with hatchery-wild admixture in brook trout populations in Quebec, Marie et al. (2012) proposed a link between the environment and admixture based on environmental constraints and their effect on the amount of contact between hatchery and wild fish within a lake. They proposed that in more constrained environments there would be increased contact between hatchery and wild fish, and therefore increased mating opportunities. Conversely, our study suggests that the relationship between the environment and hatchery-wild admixture may be density driven. While information on the densities of wild and naturalized brook trout within each study lake is not available, we can hypothesize the effect density has on admixture by looking at the relationship between particular environmental variables and brook trout density.

First, pH has been shown to be positively correlated to brook trout density in several instances (Codbout and Peters 1988, Kwak and Waters 1997, Nislow and Lowe 2003), a relationship which likely results from the effect that pH has on macroinvertebrate densities (Kreuger and Waters 1983) or on reproductive success (Warren et al. 2005). Second, elevation has also been linked to lake productivity in ways that can extend up the food web (Karlsson et al. 2005). For instance, in a neural net model of the relationship between brown trout densities and environmental parameters Lek et al. (1996) found that trout densities initially increased with increasing elevation, but beyond an elevation of

900 m a.s.l. this relationship became strongly negative. It is possible then that for brook trout a similar relationship exists but that this critical elevation is lower than for brown trout. The range in elevation of our study lakes (352 – 473 m a.s.l.) may include or exceed the critical elevation, thereby resulting in a negative correlation between elevation and brook trout densities. Third, a positive relationship between mean depth and brook trout densities could result from the correlation between mean depth and the amount of littoral area in a lake. Brook trout flourish in lakes with larger littoral zones, covering 30-80% of their total area (Kerr 2000). As mean depth increases, the area of littoral zone decreases and the environment becomes less suitable for brook trout (Cote et al. 2011). The positive correlation of pH and the negative correlations of mean depth and elevation to brook trout densities found in the literature mentioned, as well as their similar correlations to hatchery wild admixture suggest that the density of brook trout has a negative correlation with the extent of hatchery hybridization in the wild. A similar correlation has been observed and commented on regarding hybridized populations of brown trout in Europe (Hansen et al. 2009)

ANTHROPOGENIC CORRELATES OF HATCHERY ADMIXTURE

One of the two anthropogenic variables that proved to be important was lake accessibility measured as the trail length to reach the lake, which is negatively correlated to angling intensity (Kaufman et al. 2009) and which was found to be negatively correlated to admixture in this study, suggesting a positive correlation between angling intensity and admixture. Although this contradicts some previous findings in which fishing was negatively correlated with hatchery admixture (Garcia-Marin et al. 1998), potentially due

to fishers being more efficient at catching hatchery fish than wild fish (Mezzera and Largiader 2001, Champigneulle and Cachera 2003), it does supports the hypothesis of negative, density dependent admixture. Such a hypothesis has already been suggested by Evans and Willox (1991) who, through modeling, has shown that fishing preferentially removes large, highly fecund wild and hatchery fish, however, while wild fish have only one source of replacement, natural recruitment, hatchery fish are potentially replaced by both natural recruitment and further stocking events.

The importance of the positive correlation between the number of stocking events and hatchery-wild admixture supports the idea of a migration-selection balance (Haldane 1930) whereby the balance between migration of hatchery genes into a populations and the selection against those genes can be overcome by increasing the number of hatchery introductions over multiple generations. This has been suggested as an explanation for differential admixture levels among two hybridized populations of brown trout in Denmark (Hansen 2002). Given enough time, unless selection against hatchery genes is extremely strong, continual pulses of immigrants will increase the proportion of relatively benign hatchery genes in a wild population and complete admixture may occur. Our data suggest that this potentially continuous, 'low-level pulsing' of genes into a population has a greater effect on hatchery-wild admixture than the total number of fish stocked or the density at which fish are stocked.

An alternative, but not mutually exclusive explanation for the relationship between stocking frequency and admixture is the positive correlation between fishing pressure and

stocking effort often observed among many sport fish species (Loomis and Fix 1998, Post et al. 2008) as well as in this study (data not shown, $R^2 = 0.25$, $p < 0.05$). Lakes that experience more frequent stocking events are more often frequented by anglers (Post et al. 2008), and conversely lakes that are more accessible to anglers, and therefore more frequented by anglers (Hunt and Lester 2009), receive more attention from hatchery authorities. This makes it difficult to differentiate between the effects of angling and stocking frequency on hatchery admixture, however it is highly likely as suggested by Evans and Willox (1991) that these two elements likely work synergistically to increase hatchery-wild admixture.

DENSITY DEPENDENCE

The postulated relationship between hatchery admixture and brook trout density might be the result of several negative density dependent factors affecting the fitness of hatchery and domesticated strains. A limited number of spawning sites is one possible explanation. Density-dependent reproductive success, whereby hatchery fish experience greater reproductive success at lower densities, has been observed in Pacific salmon, where hatchery fish are regularly outcompeted by wild fish at higher densities (Fleming and Gross 1993). If a similar situation exists among the brook trout populations of Algonquin Park, as suggested by Blanchfield and Ridgway (2005), then we would expect hatchery fish to experience reduced reproductive success at higher densities and therefore reduced hatchery-wild admixture in environments with greater intraspecific competition. A second possible explanation is density-dependent mortality. Hatchery rainbow trout have been shown to take greater risks in natural settings than wild fish in order to maintain

their higher growth rates (Biro et al. 2004). They have also been found to venture further into riskier, but more productive, habitat at higher densities (Biro et al. 2003). Together, these two phenomena suggest that in natural settings domesticated hatchery fish with high growth rates and shorter lifespans, such as the Hills Lake strain (OMNR 1999), likely experience greater mortality rates than wild trout at high densities. This would result in reduced survival to maturity and therefore reduce hatchery-wild admixture.

SUMMARY AND IMPLICATIONS

More generally, our results suggest that populations found in habitats that typically support lower densities of brook trout are more at risk of introgressive hybridization and admixture from conspecifics than those at high densities, and that the frequency of gene flow and the intensity of angling pressure have a positive effect on admixture. This has implications for conservation efforts seeking to identify non-introgressed individuals/populations or to control undesirable anthropogenic hybridization, (e.g. see refs) as well as for risk assessment analyses of projects that may potentially result in unintentional hybridization (e.g. Hutchings and Fraser 2008). First, researchers and conservationists searching for non-introgressed populations or individuals should focus their efforts on areas where few introductions are likely to have occurred, regardless of the size of those introductions as the introductions of hatchery genes in such cases may not have be sufficiently high to overcome selection against those genes (Hansen 2002). Second, more effort could be given to areas that experienced conspecific hybridization, but where environmental parameters are likely to have supported a high density of native individuals at the time of hybridization as larger densities of wild populations reduces the

relative fitness of domestic conspecifics (Naylor et al. 2005). Third, when attempting to remove hybrids from a system (if this is deemed to be desirable), one solution may be to alter the environment or change management strategies so as to increase the density of both wild and hybridized individuals to the point where density dependent natural selection can exhibit stronger selection against those individuals lacking local adaptations. Fourth, the decision to non-discriminately remove individuals from a population undergoing admixture may need to be considered judiciously, as such removal may lower overall density and potentially increase the reproductive success of foreign conspecifics (e.g. see ref). And finally, in situations where unintentional hybridization is a possibility, the number of accidental introductions should be minimized wherever possible, and the choice of locations for such activities as aquaculture and farming should focus on areas where environmental conditions are likely to greatly reduce the reproductive success of any potential escapees (e.g. Naylor et al. 2005, Hutchings and Fraser 2008; Thorstad et al. 2008).

Table 1.1: Information on the 27 lakes (H-R-W represents the combination of Harry, Rence and Welcome Lakes) and the two hatchery strains sampled genetically between 2002 and 2009. Sample sizes are the number of adult brook trout genotyped for admixture analyses. The hatchery strain, Hills Lake (HL) or Lake Nipigon (LN), represents the strain stocked into each study lake based stocking records. Measures of genetic diversity within sampled lakes and hatchery sources are represented by observed (H_o) and expected (H_e) heterozygosities and the mean number of alleles per locus (N_a). Populations deviating significantly from Hardy-Weinberg equilibrium after Bonferroni corrections are marked with asterisks. Admixture levels reported are mean proportions of individuals' genotypes attributed to hatchery origins (either HL, LN, or both) as estimated using the program STRUCTURE (Pritchard et al. 2000). Populations with NA's represent either non-stocked populations or hatchery sources.

Lake	Initial Stocking	Final Stocking	Genetic Sampling	Sample Size (n)	Hatchery Strain stocked	N_a	H_o	H_e	Hatchery Admixture (S.D.)
Animoosh	1966	1970	2008	14	HL	3.5	0.465	0.435	0.007 (0.009)
Charles	1954	1994	2007	31	HL	2.714	0.349	0.341	0.009 (0.007)
Chipmunk	1973	1989	2007	47	HL, LN	4.786	0.531	0.556	0.052 (0.056)
Coldspring	1961	1961	2007	32	HL	3.357	0.362	0.376	0.008 (0.013)
Farncomb	1950	1950	2009	20	HL	3.643	0.482	0.467	0.006 (0.009)
Frank	1958	1981	2002	28	HL	6.714	0.677	0.707	0.660 (0.111)
Hound	1987	1989	2009	14	HL, LN	3.357	0.695	0.581	0.837 (0.121)

H-R-W*	1940	1978	2002	93	HL	8.357	0.678	0.739	0.677 (0.142)
L.Mykiss	1962	1973	2009	33	HL	6.071	0.698	0.705	0.568 (0.117)
Major*	1951	2006	2009	21	HL	4.429	0.542	0.547	0.182 (0.208)
Mykiss	1975	1985	2008	39	HL, LN	6.714	0.598	0.612	0.29 (0.164)
Philip*	1961	1963	2006	18	HL	3.286	0.36	0.456	0.005 (0.005)
Redrock*	1954	1970	2009	49	HL	5.857	0.515	0.543	0.035 (0.057)
Scott	1954	1977	2006	32	HL, LN	5.857	0.635	0.606	0.904 (0.065)
Shallnot*	1962	1989	2009	70	HL, LN	5.643	0.584	0.63	0.330 (0.17)
Stringer*	1954	1977	2007	40	HL	5.286	0.671	0.629	0.818 (0.108)
Westward*	1951	1968	2005	47	HL	3.786	0.389	0.442	0.009 (0.016)
Dickson*	NA	NA	2006	61	NA	5.071	0.48	0.515	NA
Guskewau	NA	NA	2008	35	NA	4.214	0.404	0.422	NA
Hogan	NA	NA	2009	29	NA	3.786	0.394	0.444	NA
L.Crooked*	NA	NA	2009	41	NA	3	0.382	0.413	NA
L.Dickson	NA	NA	2009	27	NA	4.214	0.5	0.51	NA
Lavielle	NA	NA	2009	29	NA	4.143	0.455	0.455	NA
Owenee	NA	NA	2008	31	NA	4.643	0.525	0.512	NA
Salvelinus*	NA	NA	2007	29	NA	3.5	0.479	0.468	NA
Hills*	NA	NA	2009	80	HL	8.286	0.715	0.737	NA
Nipigon*	NA	NA	2006	81	LN	4.143	0.392	0.403	NA

Table 1.2: Environmental and anthropogenic variables considered for each of the previously stocked populations of brook trout from Algonquin Provincial Park, Ontario, used in this study. Minimums, maximums and means are lake values, taken in early summer.

Parameter	Description	Minimum	Mean	Maximum
Surface Area (ha)	Lake surface area in hectares	9.3	81.27	469.7
Shoreline Development Index	A measure of the amount of shoreline compared to lake volume	1.14	1.73	2.89
Elevation (m)	Meters above sea level	352	424	473
pH	Mid-summer pH value	6	6.7	7.5
Morphoedaphic Index	Total dissolved solids (ppm) / Mean depth (m)	0.84	7.25	16.79
Mean Depth (m)	Mean lake depth	2	6.2	20.9
Species Present (n)	Total number of fish species present in lake	2	8.7	19
Trail Length (km)	Shortest trail distance from nearest road	0.06	19.77	49
Mean Stocking Month	Mean month of year brook trout were stocked into lake	3.88	6.28	10
Total Stocking Events (n)	Total number of years in which stocking occurred	1	8.8	36
Mean Stocking Age (months)	Mean age of fish at time of stocking	6	11.7	15.6
Mean Stocking Density (n/ha)	Mean number of fish stocked per hectare	2.75	47.08	148.55
Total Fish Stocked (n)	Total number of fish stocked into lake	1000	20916	137052
Admixture Period (years)	Time between initial stocking event and genetic sampling	22	47.4	62

Table 1.3: The explanatory variables examined ranked according to their relative variable importance based on cumulative Akaike weights (Σω) from all possible one, two and three parameter additive models as well as the estimate of the slope of the correlation and the standard error for each parameter against hatchery admixture. Models were created using both beta regressions on lake mean admixture values and generalized linear models on individual admixture values. The method of estimation used for the relative variable importance was that of Burnham and Anderson (2002) with a maximum model size of three parameters.

	BETA				GLMM			
Rank	**Parameter**	**Σω**	**Slope**	**SE**	**Parameter**	**Σω**	**Slope**	**SE**
1	pH	0.516	-2.02E+00	6.40E-01	pH	0.729	-3.24E+00	8.25E-01
2	Elevation	0.343	2.03E-02	8.20E-03	Elevation	0.326	3.45E-02	1.21E-02
3	StEvents	0.293	4.11E-02	3.51E-01	Access	0.308	-7.32E-02	2.53E-02
4	Access	0.292	-3.92E-02	1.72E-02	MDepth	0.304	-8.86E-02	1.20E-01
5	MDepth	0.282	-3.06E-02	6.89E-01	StEvents	0.262	5.22E-02	5.89E-02
6	StAge	0.205	1.16E-01	1.22E-01	StMonth	0.144	-4.46E-01	2.88E-01
7	StMonth	0.113	-2.14E-01	1.74E-01	SDI	0.122	6.27E-02	1.14E+00
8	SDI	0.097	-2.35E-02	6.53E-01	SPresent	0.101	-1.67E-01	9.92E-02

9	SPresent	0.091	-7.37E-02	6.05E-02	StAge	0.090	-6.19E-02	1.40E-01
10	MEI	0.089	3.69E-02	7.07E-02	StDensity	0.089	1.40E-02	1.39E-02
11	StDensity	0.089	9.19E-03	8.26E+03	MEI	0.084	6.31E-02	1.21E-01
12	StTotal	0.080	8.18E-07	8.77E-06	StTotal	0.082	-4.71E-06	1.52E-05
13	AdmixPeriod	0.079	-1.91E-02	2.90E-02	AdmixPeriod	0.078	-3.49E-02	4.94E-02
14	SArea	0.073	6.51E-04	2.39E-03	SArea	0.070	-9.53E-04	4.19E-03

Table 1.4: The three best-fit models from an exhaustive set of all possible additive and interactive models containing various combinations of five explanatory variables found to be important in modeling the amount of hatchery-wild admixture in populations of brook trout. Models were constructed using two parallel methods; beta regression and generalized linear mixed models with beta error distributions.

Model	Log Likelihood	K	AIC	ΔAIC
BETA method				
pH+Elevation*Mdepth+StEvents	22.55	5	-25.65	0
pH*StEvents+Elevation*Mdepth	23.84	6	-23.29	2.35
pH+Access+Elevation*Mdepth+StEvents	23.29	6	-22.18	3.47
GLMM method				
pH+Elevation*Mdepth+StEvents	934.99	5	-1854	0
pH+Access+Elevation*Mdepth+StEvents	935.39	6	-1852.8	1.20
pH*StEvents+Elevation*Mdepth	935.14	6	-1852.3	1.20

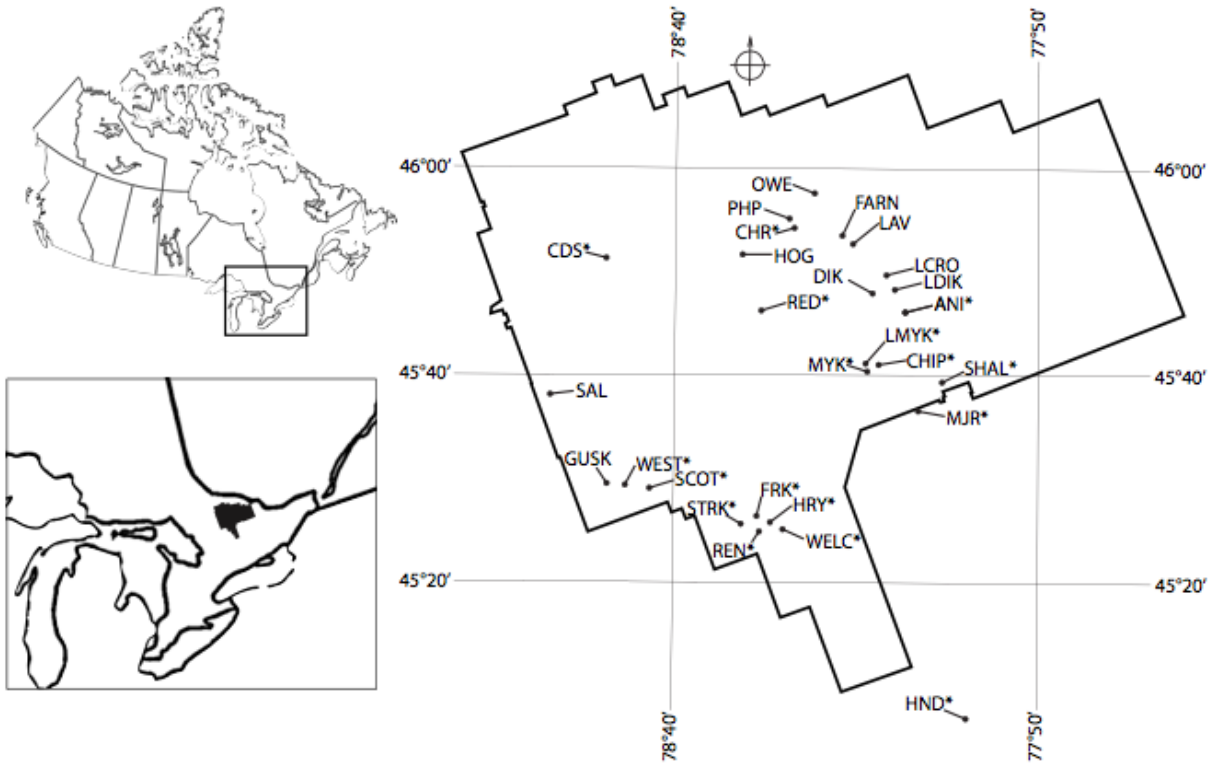

Figure 1.1: Algonquin Provincial Park in Ontario, Canada, with the locations of the 27 study lakes. Asterisks indicate previously stocked populations. The following are abbreviations of the lakes sampled: ANI Animoosh, CHR Charles, CHIP Chipmunk, CDS Coldspring, DIK Dickson, FARN Farncomb, FRK Frank, GUSK Guskewau, HRY Harry, HOG Hogan, HND Hound, LAV Lavieille, LCRO Little Crooked, LDIK Little Dickson, LMYK Little Mykiss, MJR Major, MYK Mykiss, OWE Owenee, PHP Philip, RED Redrock, REN Rence, SAL Salvelinus, SCOT Scott, SHAL Shallnot, STRK Stringer, WELC Welcome, WEST Westward.

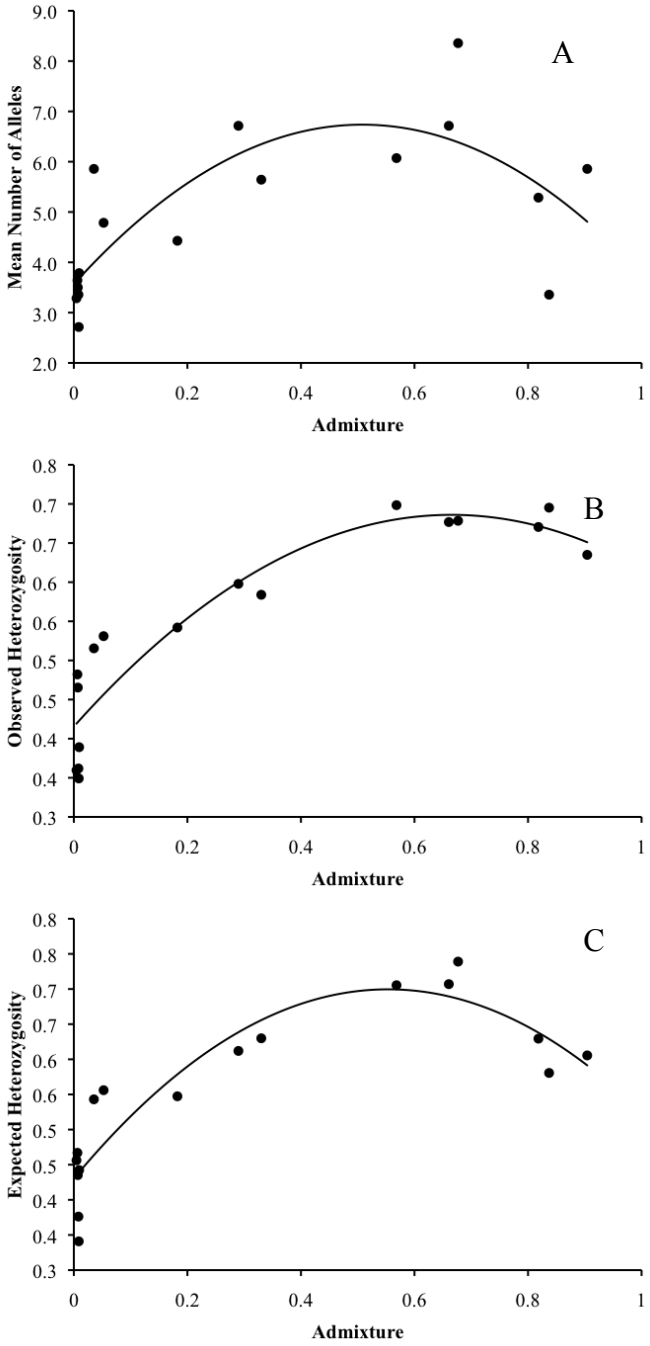

Figure 1.2: Relationships between admixture values for 17 lakes in Algonquin Provincial Park, Ontario, Canada previously stocked with the Hills Lake or Lake Nipigon strains of hatchery brook trout and measures of genetic diversity including; A) the mean number of alleles per locus measured over 14 microsatellite loci, B) the observed heterozygosities, and C) the expected heterozygosities.

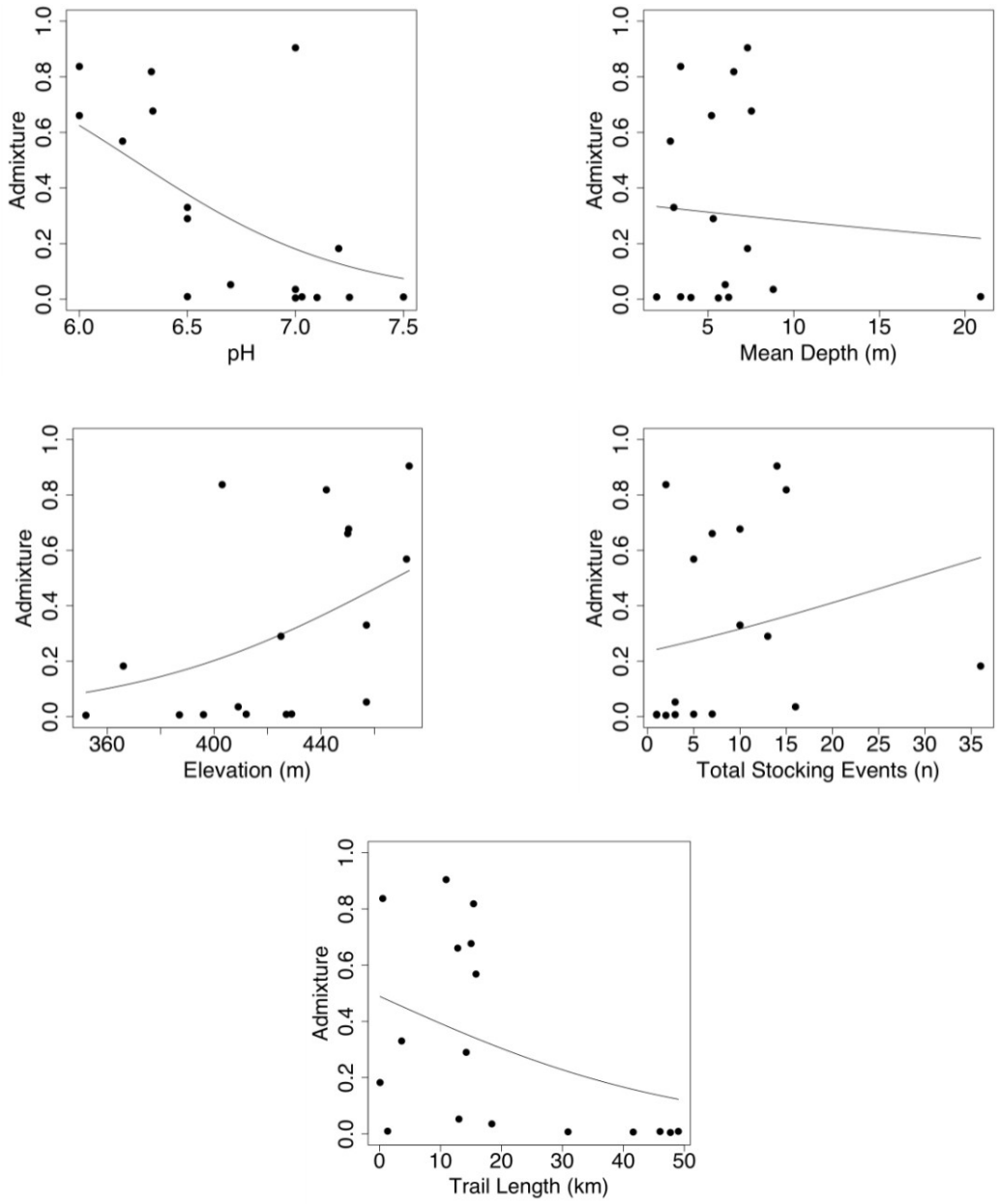

Figure 1.3: Relationships according to beta regression models between mean hatchery admixture levels and the best five ecological variables according to their relative importance based on cumulative AICc values.

CHAPTER 2:

LONG-TERM EFFECTS OF VARIABLE CONSPECIFIC ADMIXTURE ON FITNESS AND PHENOTYPIC PLASTICITY

Andrew Harbicht[1], Dylan Fraser[1], Chris Wilson[2], Bill Sloan[3]

[1] Concordia University, Biology Department, Montreal, Quebec.

[2] Ontario Ministry of Natural Resources, Peterborough, Ontario

[3] Ontario Ministry of Natural Resources, Codrington Research Hatchery, Codrington, Ontairo

Abstract:

The negative fitness associated with outbreeding depression that occurs when hatchery or domestic fish from aquaculture facilities hybridize with wild fish may have serious consequences on the wild population's biological and conservational outlook. Current conservation practices typically exclude anthropogenically-hybridized populations from protection on the basis that the genetic effects of hybridization on their fitness and long-term viability are irreversible; theory predicts otherwise. Through the use of matched plantings of wild, admixed and hatchery fish into new environments this study addressed the issue of whether the reduction in fitness typically exhibited by hybridized populations persists in the long-term or if natural selection returns affected populations to a state where fitness and adaptability are equal to pre-hybridization levels. Results indicate that survival and adaptability of hybridized populations are not significantly reduced. In fact, the highest survival among the five populations tested belonged to a previously stocked population that had resisted admixture. By far the lowest survival was that of the hatchery strain. Significant differences in phenotypic characters did exist among populations, however there was no evidence that admixed strains differed from wild populations in their amount of phenotypic plasticity. The results of this study have implications for the use of remnant wild populations that have resisted admixture as potential sources for brood stocks in genetic remediation programs as well as highlighting the potential value of admixed populations.

KEY WORDS:

hybridization, phenotypic plasticity, adaptability, survival, variable admixture

Introduction:

Human activities are increasing the incidence of intraspecific hybridization around the globe (Allendorf et al. 2001). Although the outcome of such hybridization events are somewhat unpredictable and can range from decreasing a population's fitness due to outbreeding depression (Edmands 1999) to increasing genetic diversity in small inbred populations, thereby increasing the population's fitness (Westemeier et al. 1998), what is certain is that when such events are unintentional, they are generally seen as a problem (Rhymer and Simberloff 1996). Much of the attention given to this issue has focused on the immediate effects, or concerns about possible long-term effects based on what population genetic changes could signify. The long-term fitness effects remain largely untested (but see Johnson et al. 2010) and not in natural environments. As a result, regulatory decisions are currently being made regarding hybridized populations based on the assumption that the initial effects of hybridization on fitness persists into the future, as the long-term genetic effects of hybridization do (COSEWIC 2010). Theory, however, predicts this may not be the case (Tallmon et al. 2004). With growing rates of conspecific hybridization worldwide, more scientific knowledge is needed to assess the conservation value of already hybridized populations, and decide how to best manage them in the context of biodiversity conservation (Allendorf et al. 2001), native species restoration (Hansen and Mensberg 2009), invasive species biology (Fraser et al. 2010a), and fisheries/wildlife management (Araki et al. 2007).

Hybridization has been a common problem within many species of exploited fish of high economic, recreational and cultural value (Allendorf et al. 2001, Hansen et al. 2009).

Salmonid fishes are a widespread family of fishes whose ranges encompass much of the northern hemisphere. They are further subdivided into many discrete species of high economic and social importance consisting of multiple populations, many of which display adaptations to their local environments, making them vulnerable to habitat alterations (Taylor 1991). The high socio-economic importance of salmonid fishes has led to the widespread stocking of hatchery-reared conspecifics into wild populations to compensate for population declines resulting from habitat alteration or fishing pressure (Aprahamian 2003, Hansen et al. 2009, Fraser et al. 2008). This stocking has resulted in the widespread hybridization of wild populations with hatchery fish.

In situations where populations possess local adaptations stocking of local (created from local populations) and even more so with non-local (originating from an external population source) hatchery strains that have experienced intentional or unintentional selection in the hatchery environment, generally results in the hatchery and hybrid individuals displaying reduced fitness (Araki et al. 2007, Hansen et al. 2009, Fraser et al. 2008). This may result from a lack of local adaptations to the receiving environment (Allendorf et al. 2001, Hansen et al. 2009) or the deleterious effects of partial genetic incompatibilities between the genetically distinct parental sources (Edmands 2007). In either case the effects are usually immediate and the duration variable (Edmands 1999).

How long these maladaptations persist is often unknown owing to a number of non-mutually exclusive outcomes. If hybrid fitness depends on the environment (the disruption of local adaptations), then natural selection should revert any maladaptive

phenotypes back to the environmental phenotypic optimum (Edmands 2007) given enough time and provided the immigration rate of hatchery fish ceases or does not become too high (Hansen et al. 2009). However, if hybrid fitness depends on interactions between parental gene combinations rather than on the environment (intrinsically coadapted gene complexes), such a reversion back to the phenotypic optimum might be more difficult over the long-term (Johnson et al 2010, Allendorf et al. 2004). In fact, it might not happen at all if wild populations are quite small and have been for some time (limited genetic diversity) as new gene combinations resulting from hybrid genotypes may have fitness equal or superior to wild populations resulting in a new phenotypic optimum (Rieseberg et al. 1999, Ellstrand and Schierenbeck 2006, Araki et al. 2007).

Traditionally, the fitness reductions observed in the first few generations of hatchery-wild hybridization have been thought to be long-term, resulting from the loss of local adaptations and a decreased probability of population persistence due to the introgression of maladapted hatchery genes (Allendorf et al. 2001, Allendorf et al. 2004, Araki et al. 2007, Hansen et al. 2009, Hansen and Mensberg 2009). The loss of such long-term local adaptations in wild populations might be difficult to detect if they only affect fitness during periodic episodes of extreme environmental conditions (e.g. every few decades), such as intense winters, floods, or drought (Allendorf et al. 2004). As a result, numerous resources are routinely invested into genetic restoration projects to restore wild populations where hatchery-wild mixing has occurred should such extreme conditions occur (Allendorf et al. 2004, Hansen et al. 2009, Hansen and Mensberg 2009). Nevertheless, in some cases, stocking has ceased for multiple generations and despite

considerable levels of hatchery-wild admixture, the hybridized populations persist at what are considered normal densities (Hansen and Mensberg 2009).

That densities of previously stocked populations do not decrease gradually, eventually resulting in the loss of the population may be seen as evidence that selection against maladapted gene combinations following hybridization is capable of returning a population's fitness to levels capable of supporting 'normal' densities. This is rarely tested however as to do so requires making direct comparisons of the fitness of wild populations to that of hybridized populations once natural selection has acted for multiple generations. The inherent difficulties in doing this type of experiment include avoiding further hybridization of wild populations, the need for multiple hybridized populations that have experienced selection over sufficient generations and multiple closely related wild populations. To compare fitness directly both hybridized and wild populations must be placed into common environments to which neither has an inherent advantage (no home team). A new environment is therefore needed and as a result testing for local adaptation is difficult. More often what is tested is each population's ability to adapt to an environmental change. For these reasons, among others, such experiments are not often performed, despite the considerable benefit such information might offer with respect to improving frameworks for setting conservation priorities and sound management practices dealing with the growing numbers of hybridized populations.

I will attempt to address this question using the brook trout of Algonquin Provincial Park as a model species. Populations within the park have a long and well-documented

stocking history, which has resulted in many hybridized populations with varying levels of admixture (Harbicht unpublished, AlShamlih personal communication). Stocking ceased within the park for all naturally self-sustaining brook trout populations in 1989 with very few exceptions (but see Charles Lake below), so populations within the park now represent admixed populations exposed to natural and artificial (angling) selection for at least 7 generations (Liskauskas and Ferguson 1991). Many populations within the park were also omitted from stocking and represent closely related and genetically 'wild' populations against which the fitness of hybridized populations may be compared.

By performing matched plantings of hybridized populations with varying levels of hatchery admixture, as well as hatchery and wild populations into three new environments we will attempt to answer the following questions: does natural selection restore a hybridized population's capacity to adapt to environmental change to levels comparable to that of a wild population, and does the introduction of new genes to a population through introgression increase a hybrid population's adaptive potential, providing it with greater phenotypic plasticity? Based on our knowledge that hatchery fish generally do more poorly in nature than wild or hybridized fish (Araki et al. 2007) and that natural selection filters out maladaptive introduced loci over time (Tallmon et al. 2004) in some cases quite rapidly (Nagy 1997) we can make the following hypothesis. As admixed populations within Algonquin Park have persisted for 7+ generations following the cessation of stocking (Liskauskas and Ferguson 1991), natural selection has removed maladaptive genes from the gene pool, returning hybridized populations to their original, or a novel phenotypic optimum. We therefore predict that admixed populations will

exhibit similar abilities to adapt to environmental change compared to wild fish resulting in both wild and hybrid populations exhibiting greater survival than hatchery fish in all three new environments. An alternative hypothesis is that despite both admixed and wild populations being near or at their phenotypic optimum and therefore possessing similar fitness levels in their respective environments, hybridized populations will exhibit greater fitness in a new environment as a result of greater genetic diversity and adaptive potential (Burke and Arnold 2001). In this case we predict that admixed populations will exhibit greater survival in the new environments as well as greater variation among phenotypic traits within each environment as well differing reaction norms across environments compared to a wild population. A final hypothesis is that while hybrid populations may have persisted within the park, selection upon maladaptive gene complexes is weak and fitness has not returned to pre-stocking levels despite selection over 7+ generations. In which case we predict admixed populations will possess intermediate survival compared to wild and hatchery populations as well as increased phenotypic variation.

Methods:

SOURCE POPULATIONS

In total, five populations were used in this study (Table 2.1). Two were admixed populations, Shallnot Lake and Welcome Lake (hereafter referred to as Shallnot and Welcome) known to have moderate (~30%) and high (~70%) admixture levels respectively (Harbicht et al. unpublished data), as well as one previously stocked lake, Charles Lake (hereafter referred to as Charles), shown to have completely resisted hatchery admixture despite being one of several populations within the park to have

received hatchery fish post 1989 (1500 hatchery fish in 1994). This population will be considered wild for the purposes of this study. The two other populations included in this study comprise both ends of the admixture spectrum, the fourth population, Dickson Lake (hereafter referred to as Dickson) has never been stocked with hatchery or any other strain of fish while the fifth population, the Hills Lake hatchery strain (hereafter referred to as Hatchery) from the Codrington research hatchery in Ontario, Canada, is the hatchery strain predominantly used within Algonquin Park and much of southern Ontario.

Gamete Collection

Male and female gametes were collected and combined in the field during October 2010. A minimum of 12 full-sibling families were created from each population (Table 2.1). Exceptions were the result of insufficient females being available at the time gametes were collected. In such cases two half-sibling families were created from a single female. Fertilized eggs were then incubated at the Codrington research hatchery 500 km south of Algonquin Park. All families were kept separate from one another while experiencing identical conditions (water source, feeding schedule, temperatures). Following the absorption of the yolk sacs families were combined according to the population of origin and densities among populations were equalized.

Stocking

Two weeks prior to stocking, all five populations were combined in equal proportions into three different holding tanks corresponding to the three study lakes north of Lake Huron: Penikett Lake, Woodside Lake, and Roy Berry Lake (hereafter referred to as

Penikett, Woodside and Roy Berry respectively). Stocking densities were high and constant among lakes based on a stocking ratio of 1000 fry/ha. Both Penikett and Woodside contained minnow species (Notropis sp.), and all three lakes were otherwise fishless. Stocking was done by helicopter in mid May 2011 and the brook trout were then left to acclimate and grow throughout the spring, summer and fall seasons (5 months).

SAMPLING

The three study lakes were revisited in mid October 2011 and fish were sampled using short gill net sets (15 min to 7 hrs, soak time increased towards the end of the study to increase catches) with two 182.8 cm x 27.4 m gill nets consisting of three equally sized panels of 1.27 cm, 1.9 cm, and 2.54 cm stretched monofilament mesh. Captured fish were placed into a recovery pail and left for 15 minutes prior to being anesthetized with MS222. Anesthetized fish were then weighed, measured and photographed using a mounted overhead Nikon D40 DSLR camera. The adipose fin of each fish was then clipped and stored in separately in 95% ethanol. Adipose fin clips were used to identify previously captured fish in a mark-recapture experiment.

GENOTYPING

DNA was extracted from the adipose fin clips using a modified phenol–chloroform protocol. Extracted DNA was then washed using 95% ethanol and resuspended in 30 µ L of TE buffer. Fourteen polymorphic microsatellite loci in total were amplified (Sfo18, Sfo23, Sfo12: Angers et al. 1995; SfoC24, SfoD28, SfoC38, SfoD75, SfoC88, SfoC100, SfoC113, SfoC86, SfoC115, SfoC129, and SfoB52: T. King and M. Burnham- Curtis,

U.S. Geological Survey, unpublished data) using four separate multiplex PCR reactions. Each 10 µL PCR reaction contained 6 ng of genomic DNA, 2x PCR buffer (Promega Flexi Go Taq), 0.2 mM dNTP (Bioshop), 1X BSA (Bioshop), 1.5 mM MgCl (Promega Flexi Go Taq), between 0.03 and 0.3 µM of both forward and reverse primers depending on the locus (Operon and ABI), 0.025 units of Taq polymerase (Promega Flexi Go Taq) and double distilled H2O. All multiplex reactions used thermal cycling conditions of an initial denaturation at 94 °C for 5 min, followed by 36 cycles of 94 °C for 1 min, 58 °C or 60.0 ºC (multiplex specific) for 1 min and 72 °C for 1.5 min, followed by a final extension step at 60 °C for 45 min. Amplified PCR product was visualized using capillary electrophoresis on an ABI 3730 automated sequencer (Life Technologies Inc., Carlsbad, California, USA) and genotypes were then scored using Genemapper v4.0 and visually proofread.

MORPHOMETRIC ANALYSIS

To measure the extent of phenotypic variation, photos of each fish were uploaded into the program TPSDIG2 (Rohlf 2006). Seventeen landmarks were then collected (Figure 2.1) from each photo to be analysed in TPSRELW (Rohlf 2006). A consensus body shape (generalized orthogonal least-squares Procrustes mean) was first constructed using the mean landmark positions corrected for angle, scale and centroid size. The program then aligned each sample to the consensus using thin-plate spline analyses (Bookstein 1989). From this, a partial warp analysis (Bookstein 1991) was performed and two-dimensional relative warp values were returned, which represent the direction and magnitude of deviations from the consensus form. A single consensus shape and partial warp analysis

was performed using photos of fish from all 3 study lakes combined into a single dataset. Fish that deviated from the standard salmonid form due to damage suffered in the gill nets were omitted from this analysis.

Statistical Analysis

To estimate abundances within each study lake, the Schnabel method was employed using the Chapman modification (Chapman 1954) as implemented through the FSA package within R. A Poisson distribution was used to construct confidence limits as the total number of recaptured fish never exceeded 50 for any of the lakes throughout the sampling period (Krebs 1999).

To assess survival among cross types we first assigned each individual back to its respective parental pair, and therefore its population, by exclusion using the program PROBMAX (Danzmann 1997). Given the assumption that all populations had equal catchability in the size range of gill nets employed and as equal fishing pressure was applied to all three study lakes the relative number or fish from each population present in each study lake was estimated from the number of fish from each population captured in each netting event. A generalized linear model (GLM) with a negative binomial error distribution for overdispersed count data was then used to determine whether population or study lake affected the number of fish caught in each netting.

The extent of phenotypic plasticity was estimated in two ways. First, the morphometric traits: relative warp 1 (RW1), relative warp 3 (RW3), and relative warp 4 (RW4), which

accounted for 28.5, 9.7, and 7.7% of the total variation respectively, as well as the centroid size (the cumulative radial distances from a central position to each landmark) were modeled with the study lake and the source population as explanatory variables. Centroid size was also included when testing relative warp values to account for effects of allometric growth. By employing Akaike information criterion (AIC) values to select the best fit model it is possible to test for differences in reaction norms by testing for evidence of interactive effects between study lake and source population. The second measure of phenotypic plasticity was a comparison of variances for morphometric variables among populations within each study lake. A significant reduction in phenotypic variance by one population would suggest that said population lacked the plasticity to shift their phenotypic range towards the optimum of the new environment. In which case only individuals with genotypes (phenotypes) nearest the new optimum persist in the new environment.

Centroid size was used in place of length and mass as there was a highly significant linear relationship with length ($R^2 = 0.99$, $p << 0.01$) and consequently an exponential relationship with mass ($R^2 = 0.97$, $p << 0.01$). Higher values for RW1, RW3 and RW4 corresponded roughly to increased body depth, shortened stockier bodies, and lengthened caudal flexures respectively. The second relative warp (RW2), which accounted for 14.6% of the total variation, corresponded to the extent of bending of the spine and was only predominant among fish that were photographed post mortem and was likely the result of rigor mortis. It was therefore excluded from further analysis.

Results:

POPULATION DENSITIES AND SURVIVAL

The population estimates for Penikett, Woodside and Roy Berry (543, 505, 455 respectively) did not differ significantly from one another as the 95% confidence intervals were highly overlapping (Figure 2.2). All three lakes experienced similarly high mortalities during the study period ranging from 83% in Roy Berry to 94% in Woodside. The best-fit model for explaining variation in catches was an interaction between populations and study lake (Table 2.2). This interaction resulted in Charles (the previously stocked population that resisted hybridization with hatchery fish) being caught twice as often as the next most frequently caught population in two of the three lakes (54% of the catch in Penikett and 66% of the catch in Woodside) while in Roy Berry catches of Charles only differed significantly from Hatchery ($p < 0.01$), which made up the smallest portion of the catch in each of the three study lakes (Figure 2.3). The two admixed populations (Shallnot and Welcome) showed no significant difference from the non-stocked wild strain (Dickson) in any of the three study lakes although Shallnot consistently made up a greater proportion of the catches compared to Dickson and Welcome in all three study lakes while Welcome catches were roughly half those of Dickson and Shallnot.

PHENOTYPIC PLASTICITY

The variation in centroid sizes was best explained by an additive model including study lake and population (Table 2.3, Figure 2.4). Among the relative warps however, both RW1 and RW3 were best described by an interaction between study lake and population along with centroid size (Table 2.3, Figure 2.5) although in both cases, the simpler additive model of study lake, population, and centroid size was within two Δ AIC units an so there is little support for the interaction between study lake and population (Burnham and Anderson 2002). RW4 was best explained by an additive model of study lake, population, and centroid size.

Among the study lakes, all populations experienced an increase in growth (measured by the centroid size) of nearly 30% in Roy Berry compared to both Penikett and Woodside, which did not differ greatly. Among the populations, the admixed population Shallnot displayed significantly higher growth in the three study lakes (all $p < 0.05$), surpassed only by Hatchery, and only in two of the three lakes (Figure 2.4). The wild, non-stocked population Dickson displayed consistently reduced growth. Relative Warp 1, which corresponded to deeper body shapes at higher values, displayed a large range of values heavily influenced by the environment and ranging from generally shallow bodied fish in Penikett to deep bodied fish in Roy Berry (Figure 2.5). Woodside had intermediate values. The trend among populations was for admixed populations to possess deeper bodies than wild populations in all three environments. Hatchery displayed an interactive relationship with environment, whereby Hatchery were more deep bodied in Penikett relative to other populations and inversely more shallow bodied in Woodside compared

to the other populations. Relative Warp 3, which corresponded to shorter, stockier bodies at higher values showed little difference among populations or study lakes with the exception of Hatchery which displayed a greater range than any other population (from 0.007 to 0.02) and which at its lowest point was still three times greater than the next highest point (Shallnot in Penikett) corresponding to generally thicker fish from Hatchery. There were no other consistent trends among populations over the three study lakes. For Relative Warp 4 Dickson had the highest values in all three study lakes which corresponded to a longer caudal flexures while Hatchery had by far the lowest values in each environment. Charles and Shallnot displayed similar values of RW4 across environments while the heavily admixed Welcome had values more similar to the wild Dickson population in two of the three study lakes.

Plasticity in phenotypic expression within each study lake varied significantly among populations in only one of 12 tests, corresponding to centroid size in Roy Berry (Fligner-Killeen test, $X^2 = 10.9$, d.f. = 4, p = 0.02). Shallnot displayed the greatest variance for centroid size in Roy Berry, with more than twice as much variation in centroid size than both Charles and Welcome while Dickson and Hatchery both had intermediate levels of variance nearly double that of Charles and Welcome. Repeated F-tests where used to determine which variance differed significantly within Roy Berry, however after Bonferroni corrections to the alpha value ($k = 10$, $\alpha = 0.005$), non of the observed differences were significant.

Discussion

The principle goal of this study was to determine whether hybridization between hatchery and wild populations of brook trout has had the long-term effect of reducing admixed populations' fitness compared to wild populations in a natural environment. The results of this study support the hypothesis that natural selection, over multiple generations, can be successful in removing maladaptive genes from hybridized population, restoring their survival and adaptability to new environments to levels comparable to similar, non-stocked populations. Consequently, and contrary to what has been theorized (Arnold 1992, Tallmon 2004) and observed experimentally (Swindell and Bouzat 2006, Lucek et al. 2010), there was no strong indication that the adaptability, measured as phenotypic plasticity following environmental change, is affected in admixed populations either positively or negatively.

Survival of admixed populations

Survival in a new environment, the measure of fitness employed by this study, was by far greatest for a previously stocked population, Charles, which experienced only negligible introgression following past stocking efforts and which is considered to be essentially a wild population. Both the heavily (Welcome, ~ 70%) and the moderately (Shallnot, ~30%) admixed population did not differ significantly in survival from the non-stocked wild population (Dickson). This result contradicts the third hypothesis outlined in the introduction, that maladaptive traits persist in admixed populations, resulting in reduced fitness over prolonged periods, as has been observed in some situations following intraspecific hybridization (Johnson et al. 2010). That the hatchery source experienced

reduced survival in all three environments compared to both wild and admixed populations supports the idea that the admixed populations have reverted to more closely resemble their wild ancestors rather than their hatchery ancestors. This appears to be true even for Welcome, who more closely resembles their hatchery ancestors.

A possible explanation for the survival levels of admixed populations more closely resembling wild than hatchery sources is that the ability of brook trout to adapt to environmental change is not negatively affected by hybridization with a hatchery strain as it is in other salmonid species (Allendorf et al. 2001, Araki et al 2007, Hansen and Mensberg 2009). This is unlikely however as previous work done on the yields from matched plantings of pure wild, hatchery and F_1 brook trout in Algonquin Park found that hybrid yields were intermediate, falling in between those of wild populations (albeit originating from outside the park) who produced the highest yields and hatchery fish who produced the lowest returns (Fraser 1981). If admixture indeed had no effect on fitness and adaptability of brook trout we would have expected the F_1 to fare as well as the wild populations. A more likely explanation is that proposed as the primary prediction of this study: that natural selection is capable of removing low-fitness gene combinations from hybridized brook trout populations, restoring them to fitness levels similar to pre-stocking conditions. This hypothesis is supported by the general lack of elevated phenotypic variance among admixed populations, suggesting that hybridized populations have been exposed to sufficient directional/stabilizing selection to remove less fit genotypes/phenotypes from admixed populations (Jordan 1991, Nagy 1997).

PHENOTYPIC PLASTICITY

The equality in phenotypic variances among populations mentioned above goes on to further contradict the hypothesis that admixed populations possess greater long-term adaptability expressed as greater survival and increased phenotypic diversity. This lack of differences among variances and the lack of strong evidence for differences among reaction norm slopes across environments supports the idea that directional selection towards an environmental optimal has occurred. Perhaps the strongest support for this idea is that, upon review of the phenotypic parameters (figures 2.4 and 2.5), it is apparent that the admixed populations most closely resemble the two wild populations rather than the hatchery strain.

POTENTIAL CAVEATS

While it seems likely that natural selection has returned admixed populations to their local phenotypic and genotypic environmental optimum, several caveats must be mentioned. First, the original pre-stocking conditions of the study populations are unknown. We must therefore make conclusion based on comparisons to similar wild populations. The difference observed between survival among the two wild populations (Charles and Dickson) demonstrates that there are potentially inherent differences among populations within Algonquin Park, and while the two admixed populations displayed adaptability and fitness levels similar to that of the non-stocked wild population (Dickson), their original state may have been more like that of Charles, which had significantly higher survival in two of the three study lakes. In which case the current levels represent a significant reduction in adaptability and fitness as a result of admixture,

however this may not be cause for concern as the hybridized populations still fall within the range of wild populations in terms of survival in a new environment as demonstrated by their similarity to the non-stocked Dickson Lake population. A second caveat is that while our study did monitor survival over a period of the brook trout life cycle known to experience high mortality and heavily influence future population persistence (Jensen 1971), it did not encompass lifetime reproductive success, the gold standard for this type of study (Tallmon 2004). It is therefore possible that while our results suggest that admixture does not significantly reduce survival of juveniles, measuring lifetime reproductive success may still find differences in population fitness resulting from hybridization. Our results do still look promising for hybridized populations though as comparative survival through the first summer may well suggest comparative survival to a reproductive age for both wild and hybridized. A third caveat is that although we stocked each cross type into study lakes roughly 800 km from their sources and while mortality rates were sufficiently high to produce detectable and significant differences among populations it is still possible that other more cryptic fitness consequences of hybridization may expose themselves in the presence of more extreme environmental changes such as has been proposed by Allendorf et al. (2004). We can however say that within the scope of this study admixed and wild populations displayed no significant differences in fitness as a result of being transplanted into three new environments in which hatchery fish faired poorly. A final caveat to this study is that while the hatchery strain used in this study was the same strain as that which had been stocked previously into the study populations, in some cases as many as 35 years have passed since stocking ceased (Harbicht et al. unpublished data). It is therefore possible that the modern hatchery

fish no longer resemble their progenitors in terms of phenotype, adaptability or survival in new environments as a result of continued domestication in the hatchery environment. While salmonids are known to experience rapid evolution as a result of the hatchery environment (Araki et al. 2007), the Hills Lake strain of brook trout used by Ontario hatchery managers has been in the hatchery system as a brood stock with a high effective population size as far back as the 1930s (Dannzman et al. 1995). This long period of domestication in the relatively constant hatchery environment would have resulted in strong selection initially towards domestic phenotypes with the strength of that selection weakening over time as the brood stock approached an optimum. This weaker selection later on combined with the large effective population size of the brood stock means that contemporary fish probably resemble hatchery fish at the time stocking ceased in terms of phenotype and fitness in new environments.

CONCLUSIONS AND FUTURE DIRECTIONS

Future investigations into this topic should attempt to address some of the issues mentioned above. While some (like the general lack of knowledge about the admixed populations' fitness, pre-admixture) may be difficult to address, especially in a natural setting with species that possess longer generational times, others, such as the extreme environments issue, could be experimentally controlled by preferentially choosing study environments which test the limits of survival for a particular species. Addressing these questions could potentially explain in what situations the negative effects of hybridization will persist over time, and in what situations they will not. Already we have shown that populations of brook trout with moderate to high levels of hatchery admixture are capable

of returning to fitness levels equal to non-stocked populations after as few as 7 generations. Results such as these add to the considerations of policy makers and will hopefully assist in classifying the protection status or management practices regarding populations known to be admixed with hatchery strains. This knowledge also provides hope for wild populations of high ecological and economical values that are currently displaying the negative effects of hybridization with domesticated conspecifics. Our results suggest that if the incoming flow of foreign genes can be stemmed, then there is a considerable chance these populations may recover, and possibly in less time than previously thought.

Table 2.1: Environmental characteristics of source population habitat and experimental lakes used in a matched planting experiment as well as information on the hatchery admixture proportion of each source population based on estimates from STRUCTURE (Pritchard et al. 2000), the number of full and half sibling families used for matched planting and the number of families represented among the fish present in the study lake at the end of the experimental period (five months). NA represents non-applicable, as is the case with environmental characteristics for the hatchery and with the number of families formed or recovered from study lakes.

Population/ Lake	Surface Area (ha)	Mean Depth (m)	Secchi Depth (m)	Hatchery admixture	Full/half sibling families (N)	Families present during sampling (N)
Dickson	974.7	16.8	5.6	0.0	12/0	11
Charles	12.3	3.4	6.4	0.009	15/2	13
Shallnot	11.4	3	3.7	0.330	8/2	8
Welcome	469.7	7.53	4.13	0.677	11/2	10
Hatchery	NA	NA	NA	1.0	16/0	6
Penikett	3.9	4.0	2.5	NA	NA	NA
Roy Berry	2.5	6.4	5.0	NA	NA	NA
Woodside	8.7	3.3	5.5	NA	NA	NA

Table 2.2: Results of model selection using AIC values on the number of each of five populations of brook trout caught per net set in three study lakes north of Lake Huron, Ontario, Canada. Net sets varied in soak time from 15 minutes to 7 hours.

Model	**No. Parameters**	**Loglikelihood**	**AIC**	**Δ AIC**
Population * Study lake	3	-372.0637	776.1	0
Population + Study lake	2	-384.7263	785.5	9.3
Population	1	-391.0244	794.0	17.9
Study lake	1	-419.0589	846.1	70.0
Intercept	1	-421.833	847.7	71.5

Table 2.3: Results of model selection using AIC values on the phenotypic parameters measured for five populations of brook trout matched planted into three study lakes north of Lake Huron, Ontario Canada. The morphological response variables; Centroid size and Relative Warp values RW1, RW3, and RW4, were produced in tpsRelW (Rohlf 2006).

Response Variable	Model	Log Likelihood	No. Parameters	AIC	Δ AIC
Centroid Size	Population * Study lake	-771.9	3	1575.7	2.3
	Population + Study lake	**-778.7**	**2**	**1573.4**	**0.0**
	Population	-902.4	1	1816.8	243.3
	Study lake	-806.4	1	1620.9	47.5
	Intercept	-921.6	1	1847.2	273.8
RW1	Population * Study lake	1050.6	3	-2069.3	168.0
	Population + Study lake	1043.0	2	-2069.9	167.3
	Population	906.9	1	-1801.8	435.5
	Study lake	1035.3	1	-2062.7	174.6
	Intercept	891.2	1	-1778.4	458.9
	Population * Study lake + Centroid	**1135.6**	**4**	**-2237.3**	**0.0**
	Population + Study lake + Centroid	1127.4	3	-2236.8	0.4
	Population + Centroid	1039.8	2	-2065.6	171.6
	Study lake + Centroid	1118.4	2	-2226.7	10.5
	Centroid	1030.8	1	-2055.5	181.7

Table 2.3 continued

RW3	Population * Study lake	1091.7	3	-2151.4	5.7
	Population + Study lake	1083.6	2	-2151.1	5.9
	Population	1080.9	1	-2149.7	7.3
	Study lake	1060.9	1	-2113.7	43.3
	Intercept	1059.1	1	-2114.3	42.8
	Population * Study lake + Centroid	**1095.5**	**4**	**-2157.1**	**0.0**
	Population + Study lake + Centroid	1086.9	3	-2155.9	1.2
	Population + Centroid	1083.8	2	-2153.5	3.6
	Study lake + Centroid	1061.1	2	-2112.2	44.9
	Centroid	1059.3	1	-2112.6	44.5
RW4	Population * Study lake	1156.1	3	-2280.2	53.4
	Population + Study lake	1152.1	2	-2288.2	45.4
	Population	1151.7	1	-2291.4	42.2
	Study lake	1098.2	1	-2188.4	145.2
	Intercept	1096.2	1	-2188.4	145.2
	Population * Study lake + Centroid	1179.5	4	-2325.0	8.6
	Population + Study lake + Centroid	**1175.8**	**3**	**-2333.6**	**0.0**
	Population + Centroid	1165.3	2	-2316.6	17.0
	Study lake + Centroid	1129.0	2	-2248.0	85.6
	Centroid	1103.4	1	-2200.8	132.8

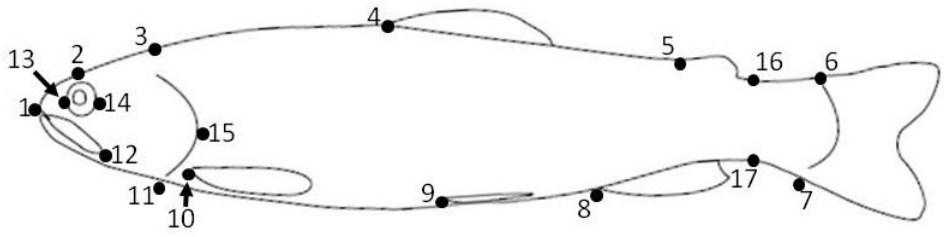

Figure 2.1: Landmarks for geomorphometric analysis on brook trout: 1, the most anterior part of body; 2, the head directly above midpoint of the eye; 3, the head directly above dorsal limit of operculum; 4, the anterior insertion point for dorsal fin; 5, the anterior limit of adipose fin; 6, the dorsal terminus of the caudal flexure; 7, the ventral terminus of the caudal flexure; 8, the anterior insertion point of the anal fin; 9, the anterior insertion point for the left pelvic fin; 10, the anterior insertion point for the left pectoral fin; 11, the meeting point of the gill plate and the ventral midline; 12, the most posterior point on upper mandible; 13, the most anterior point on the eye; 14, the most posterior point on the eye; 15, the most posterior point on the operculum; 16, the dorsal position above the thinnest part of the caudal peduncle; 17, the ventral position below the thinnest part of the caudal peduncle.

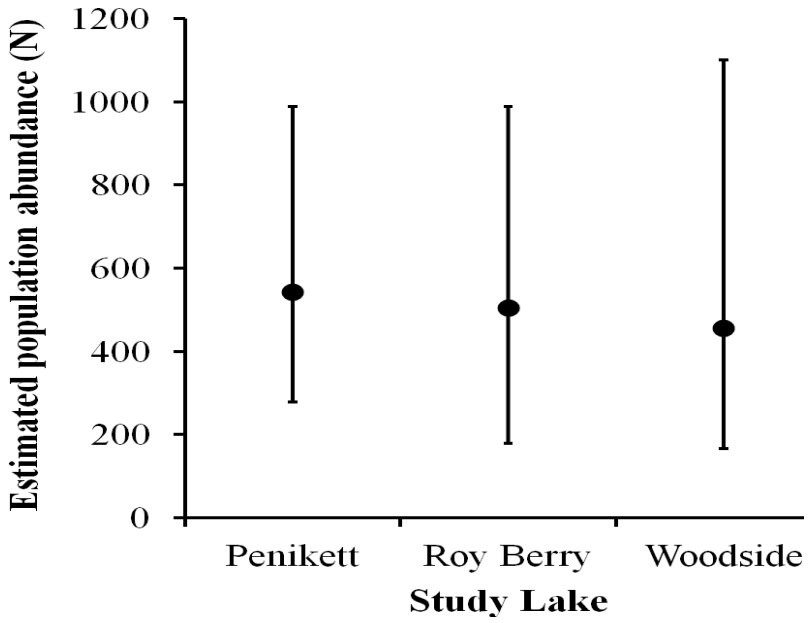

Figure 2.2: Estimated population abundances and 95% confidence limits in three study lakes north of Lake Huron, Ontario, Canada, five month post stocking. Estimates were made using the Schnabel method with the Chapman modification and confidence intervals were based on the Poisson distribution.

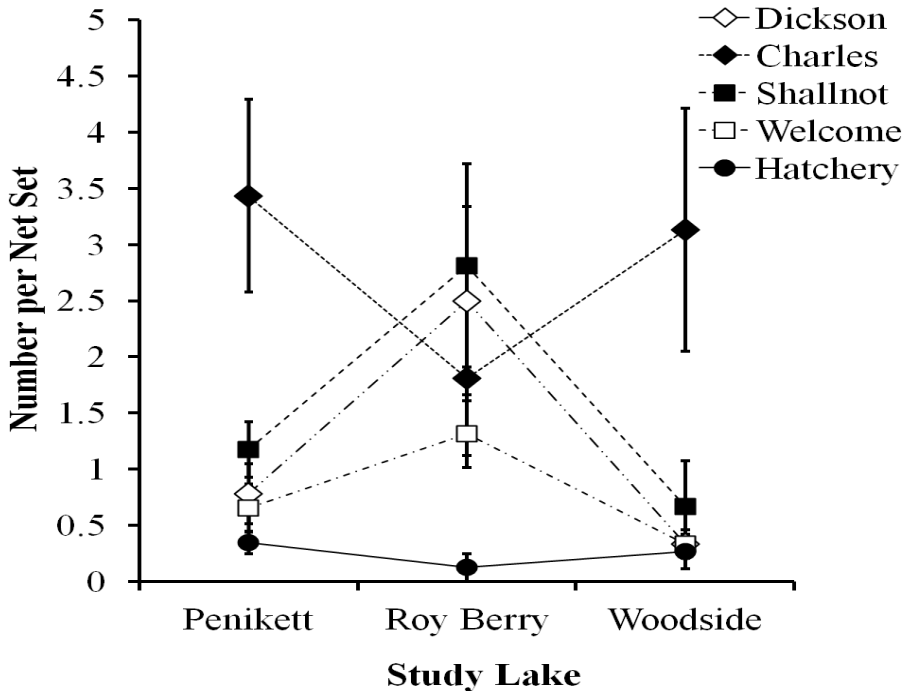

Figure 2.3: Mean (± SE) number of fish from each of five populations caught per net set (ranging in soak time from 15 min to 7 hours) in three study lakes north of Lake Huron, Ontario, Canada.

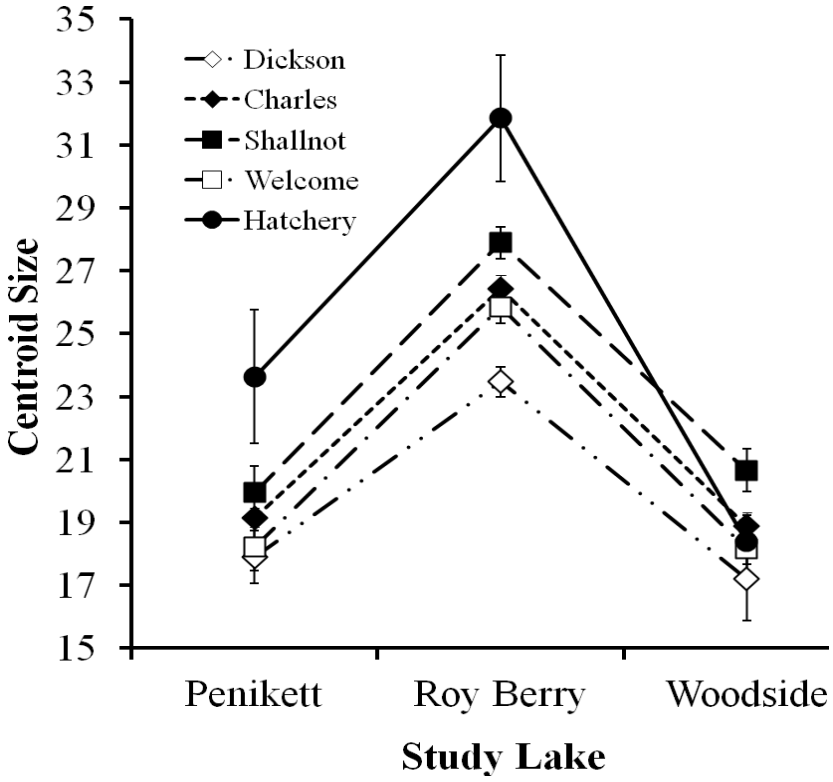

Figure 2.4: Mean (± SE) centroid size for five populations of brook trout in three study lakes north of Lake Huron, Ontario, Canada. Centroid sizes were calculated using tpsRelW by Rolph (2006) and photographs of the left side of fish captured using short-set gill net sampling.

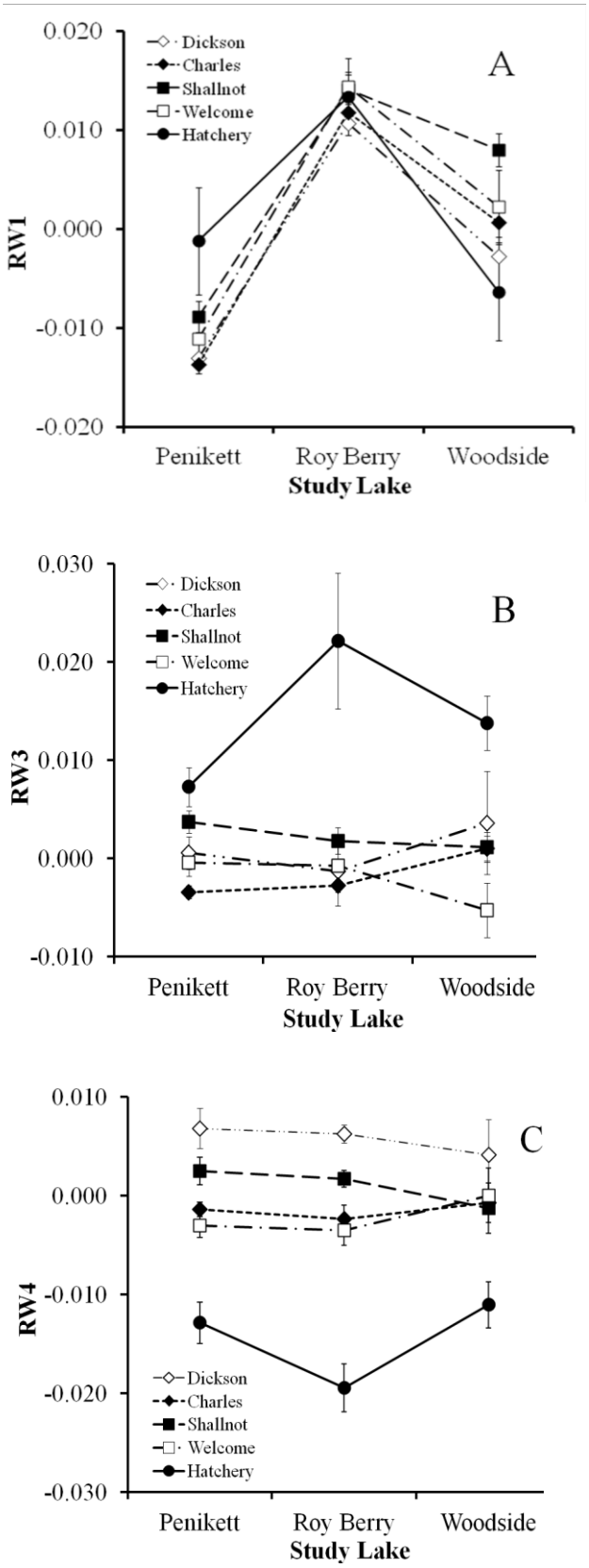

Figure 2.5: Mean (± SE) relative warp values for five populations of brook trout in three study lakes north of Lake Huron, Ontario, Canada. Relative warp values were produced using the program tpsRelW by Rolph (2006) and photographs of the left side of fish captured using short-set gill net sampling. Relative warp 1 (A) corresponds to deeper bodies at higher values, relative warp 3 (B) corresponds to shorter stockier fish higher values, and relative warp 4 (C) corresponds a lengthening of the caudal.

GENERAL CONCLUSIONS

After reading the above two chapters it should be apparent that situations where hybrid populations are involved are rarely clean and clear cut. It is perhaps for this reason that many conservation and management organizations prefer to omit hybridized populations entirely when considering species or populations that are known to hybridize with conspecifics. But with continued study it may be possible one day for such organizations to make clear regulations that can be applied in all cases related to hybrid populations. For the time being it may be prudent to handle situations where hybrids are involved on a case-by-case basis as suggested by Allendorf et al. (2004). The results of this project should at least provide management and decision makers with a more positive outlook on such cases.

The discovery that readily available information about an environment in which unintentional or undesirable hybridization can occur may help predict whether a hybridization event will result in extensive admixture between native and introduced populations shows great promise for the management of such situations. The relationship proposed by this study to explain the correlation between the environmental variables; pH, elevation, and mean depth, and their effect on hatchery admixture, namely that more favorable environments (higher pH, lower elevation and shallower mean depth) support wild populations at higher densities that are more resistant to admixture with hatchery sources is not entirely new. Suggestions previously made regarding risk management practices in aquaculture (Naylor et al. 2005) have included avoiding placing aquaculture

facilities near small populations of wild conspecifics as they stand a greater risk of being swamped genetically following the escape of a large number of conspecifics, as often occurs in the aquaculture industry (McGinnity et al. 2003). The new insight offered by this project is that resistance to admixture with domestic or hatchery conspecifics may be predictable based on the environmental and anthropogenic influences on wild population densities and that the driver of resistance to admixture may be density dependent fitness effects on the introduced individuals. The relationship between stocking events and admixture supports the idea proposed by Hansen (2002) that it might be a reliable predictor of whether extensive admixture may result from hybridization. Our study goes one step further to suggest that the number of stocking events may be a better indicator than the total number of hatchery fish stocked in predicting the genetic outcome of hybridization. We also found evidence to support the hypothesis of Evans and Willox (1991) that stocking and fishing pressure work together to increase the genetic effects of stocking hatchery fish into wild populations. These results may have considerable implications for the management of hybridizing populations.

But all hope may not be lost for admixed populations. Chapter two of this study found no evidence that hatchery-wild admixture among brook trout populations has any negative effect on their survival or adaptability to environmental change. This has significant implications for the management of hybrid or hybridizing populations by lending support to some of the current policies while calling into question the validity of others. Currently, conservation efforts are being expended searching for remnant wild populations among regions heavily affected by hatchery stocking with the intention of

using creating brood stocks for restorative purposes (e.g. Meraner et al. 2010). Our results suggest such remnant populations likely represent an excellent source of wild genes as any negative effects that slight introgression may have had on their fitness appear to be short lived. Furthermore, the negative effects typically experienced by wild populations hybridizing with hatchery or domestic sources appear to be fairly short lived as well (as little as 7 generations) among the brook trout of Algonquin Park. This has promising implications for changes to regulations regarding the potential protection status of hybridized populations. Currently, in Canada, policies regarding hybrid populations state that when hybridization is the direct result of human activities, the affected populations will be exempt from inclusion among a species or populations being assessed for protection status and may even be considered a threat (COSEWIC 2011). Our results support the theory that natural selection may return such hybrid populations to their previous fitness levels in a relatively short period of time. This suggests that in some cases, hybridized populations may return to performing their ecological role in the local environment as well as non-hybridized populations after a short period of fitness and demographic decline, during which natural selection removes unfit genes. There is also no evidence that such populations possess a reduced chance of persistence in the face of continued anthropogenically driven habitat alterations. It is my hope therefore that studies such as this one will eventually change our outlook towards hybridized populations from one of general negativity, to one more consistent with a temporary departure from 'normal'.

REFERENCES:

Addison, P. A., and C. C. Wilson. 2010. Matching management to biological scale: connectivity among lacustrine brook trout populations. North American Journal of Fisheries Management **30:** 1132–1142.

Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg. 2001. The problems with hybrids: setting conservation guidelines. Trends in Ecology and Evolution **16:** 613-622.

Allendorf, F. W., R. W. Leary, N. P. Hitt, K. L. Knudsen, L. L. Lundquist, and P. Spruell. 2004. Intercrosses and the U . S . Endangered Species Act : Should Hybridized Populations be Included as Westslope Cutthroat Trout? Conservation Biology **18:** 1203-1213.

Almodovar, A., G. G. Nicola, B. Elvira, and J. L. García-Marín. 2006. Introgression variability among Iberian brown trout Evolutionary Significant Units: the influence of local management and environmental features. Freshwater Biology **51:** 1175–1187.

Angers, B., L. Bernatchez, A. Angers, and L. Desgroseillers. 1995. Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographic scale. Journal of Fish Biology **47:** 177-185.

Araki, H., B. Cooper, and M. S. Blouin. 2007. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science **318:** 100-103.

Arnold, M. L. 1992. Natural hybridization as an evolutionary process. Annual Review of Ecology and Systematics **23:** 237–261.

Aprahamian, M. W., K. Martin Smith, P. McGinnity, S. McKelvey, and J. Taylor. 2003. Restocking of salmonids – opportunities and limitations. Fisheries Research **62:** 211–227.

Baric, S., A. Riedl, A. Meraner, N. Medgyesy, R. Lackner, B. Pelster, and J. D. Via. 2010. Alpine headwater streams as reservoirs of remnant populations of the Danubian clade of brown trout. Freshwater Biology **55:** 866-880.

Biro, P. A., J. R. Post, and E. A. Parkinson. 2003. Density-dependent mortality is mediated by foraging activity for prey fish in whole-lake experiments. Journal of Animal Ecology **72:** 546-555.

Biro, P. A., M. V. Abrahams, J. R. Post, and E. A. Parkinson. 2004. Predators select against high growth rates and risk-taking behaviour in domestic trout populations. Proceedings of the Royal Society of London, Series B: Biological Sciences **271:** 2233-2237.

Blanchfield, P. J., and M. S. Ridgway. 2005. The relative influence of breeding competition and habitat quality on female reproductive success in lacustrine brook trout

(*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences **62:** 2694–2705.

Bookstein, F. L. 1989. Principal warps: thin-plate splines and the decomposition of deformations. IEEE Transactions on pattern analysis and machine intelligence **11:** 567-585.

Bookstein, F. L. 1991. Morphometric tools for landmark data. Cambridge University Press, New York, USA.

Burke, J. M., and M. L. Arnold. 2001. Genetics and the fitness of hybrids. Annual Review of Genetics **35:** 31-52.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multi-model inference: a practical information-theoretic approach. Second edition. Springer, New York, USA.

Castric, V., L. Bernatchez, K. Belkhir, and F. Bonhomme. 2002. Heterozygote deficiencies in small lacustrine populations of brook charr *Salvelinus fontinalis* Mitchill (Pisces, Salmonidae): a test of alternative hypotheses. Heredity **89:** 27-35.

Champigneulle, A., and S. Cachera. 2003. Evaluation of large-scale stocking of early stages of brown trout, *Salmo trutta*, to angler catches in the French-Swiss part of the River Doubs. Fisheries Management and Ecology **10:** 79–85.

Chapman, D. G. 1954. The estimation of biological populations. Annals of mathematics and statistics **25:** 1-15.

Codbout, L., and R. H. Peters. 1988. Determinants of stable catch in the brook trout *Salvelinus fontinalis* sport fishery in Quebec. Canadian Journal of Fisheries and Aquatic Sciences **45:** 1771-1778.

COSEWIC. 2011. Committee on the Status of Endangered Wildlife in Canada. Guidelines on Manipulated Populations. Web site:http://www.cosewic.gc.ca/eng/sct0/rpt/rpt_csar_e.cfm [accessed 22 July 2012]

Cote, D., B. K. Adams, K. D. Clarke, and M. Langdon. 2011. Salmonid biomass and habitat relationships for small lakes. Environmental Biology of Fishes **92:** 351-360.

Cronin, M. A., L. Renecher, B. J. Pierson, and J. C. Patton. 1995. Genetic variation in domestic reindeer and wild caribou in Alaska. Animal Genetics **26:** 427-434.

Danzmann, R. G., and P. E. Ihssen. 1995. A phylogeographic survey of brook charr (*Salvelinus fontinalis*) in Algonquin Park, Ontario based upon mitochondrial DNA variation. Molecular Ecology **4:** 681–697.

Danzmann, R. G. 1997. Probmax: a computer program for assigning unknown parentage in pedigree analysis from known genotypic pools of parents and progeny. The Journal of heredity **88:** 333.

Edmands, S. 1999. Heterosis and Outbreeding Depression in Interpopulation Crosses Spanning a Wide Range of Divergence. Evolution **53:** 1757-1768.

Edmands, S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. Molecular Ecology **16:** 463-75.

Einum, S., and I. A. Fleming. 1997. Genetic divergence and interactions in the wild among native, farmed and hybrid Atlantic salmon. Journal of Fish Biology **50:** 634–651.

Ellstrand, N. C. and K. A. Schierenbeck. 2006. Hybridization as a stimulus for the evolution of invasiveness in plants? Euphytica **148**: 35–46.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology **14:** 2611–2620.

Evans, D. O., and C. C. Willox. 1991. Loss of exploited, indigenous populations of lake trout, *Salvelinus namaycush*, by stocking of non-native stocks. Canadian Journal of Fisheries and Aquatic Sciences **48**: 134-147.

Fleming, I. A., and M. R. Gross. 1993. Breeding success of hatchery and wild Coho salmon (*Oncorhynchus kisutch*) in competition. Ecological Applications **3:** 230–245.

Fraser, J. M. 1981. Comparative survival and growth of planted wild, hybrid, and domestic strains of brook trout (*Salvelinus fontinalis*) in Ontario lakes. Canadian Journal of Fisheries and Aquatic Sciences **38:** 1672-1684.

Fraser, D. J., A. M. Cook, J. D. Eddington, P. Bentzen, and J. A. Hutchings. 2008. Mixed evidence for reduced local adaptation in wild salmon resulting from interbreeding with escaped farmed salmon: complexities in hybrid fitness. Evolutionary Applications **1:** 501-512.

Fraser, D. J., A. L. S. Houde, P. V. Debes, P. O'Reilly, J. D. Eddington, and J. A. Hutchings. 2010a. Consequences of farmed-wild hybridization across divergent wild populations and multiple traits in salmon. Ecological Applications **20:** 935-953.

Fraser, D. J., C. Minto, A. M. Calvert, J. D. Eddington, and J. A. Hutchings. 2010b. Potential for domesticated-wild interbreeding to induce maladaptive phenology across

multiple populations of wild Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences **67:** 1768-1775.

García-Marín, J. L., N. Sanz, and C. Pla. 1998. Proportions of native and introduced brown trout in adjacent fished and unfished Spanish rivers. Conservation Biology **12:** 313 – 319.

Hails, R. S., and K. Morley. 2005. Genes invading new populations: a risk assessment perspective. Trends in Ecology and Evolution **20:** 245-252.

Halbisen, M. A. and C. C. Wilson. 2009. Variable introgression from supplemental stocking in southern Ontario populations of lake trout. Transactions of the American Fisheries Society **137:** 699–719.

Haldane, J. B. S. 1930. A mathematical theory of natural and artificial selection. Proceedings of the Cambridge Philosophy Society **26:** 220-230.

Hansen, M.M. 2002. Estimating the long-term effects of stocking domesticated trout into wild brown trout (*Salmo trutta*) populations: an approach using microsatellite DNA analysis of historical and contemporary samples. Molecular Ecology **11:** 1003–1015.

Hansen, M.M., D. J. Fraser, K. Meier, and K. L. D. Mensberg. 2009. Sixty years of anthropogenic pressure: a spatio-temporal genetic analysis of brown trout populations subject to stocking and population declines. Molecular Ecology **18:** 2549–2562.

Hansen, M. M., and K. L. D. Mensberg. 2009. Admixture analysis of stocked brown trout populations using mapped microsatellite DNA markers: indigenous trout persist in introgressed populations. Biology letters **5:** 656-659.

Heath, D., C. M. Bettles, and D. Roff. 2010. Environmental factors associated with reproductive barrier breakdown in sympatric trout populations on Vancouver Island. Evolutionary Applications **3:** 77–90.

Hindar, K., N. Ryman, and F. M. Utter. 1991. Genetic effects of cultured fish on natural fish populations. Canadian Journal of Fisheries and Aquatic Sciences **48:** 945–957.

Hogg, J. T., S. H. Forbes, B. M. Steele, and G. Luikart. 2006. Genetic rescue of an insular population of large mammals. Proceedings of the Royal Society Series B: Biological Sciences **273:** 1491-1499.

Hunt, L. M., and N. Lester. 2009. The effect of forestry roads on access to remote fishing lakes in northern Ontario, Canada. North American Journal of Fisheries Management **29:** 586-597.

Hutchings, J. A., and D. J. Fraser. 2008. The nature of fisheries- and farming-induced evolution. Molecular Ecology **17:** 294-313.

Jensen, A. L. 1971. The effect of increased mortality on the young in a population of brook trout, a theoretical analysis. Transactions of the American Fisheries Society **100:** 456 – 459.

Johnson, J. R., B. M. Fitzpatrick, and H. B. Shaffer. 2010. Retention of low-fitness genotypes over six decades of admixture between native and introduced tiger salamanders. BMC Evolutionary Biology **10:** 147 - 161.

Jordan, N. 1991. Multivariate analysis of selection in experimental populations derived from hybridization of two ecotypes of the annual plant *Diodia teres* W. (Rubiaceae). Evolution **45:** 1760-1772.

Karlsson, J., A. Jonsson, and M. Jansson. 2005. Productivity of high-latitude lakes: climate effect inferred from altitude gradient. Global Change Biology **11:** 710–715.

Kaufman, S. D., E. Snucins, J. M. Gunn, and W. Selinger. 2009. Impacts of road access on lake trout (*Salvelinus namaycush*) populations: regional scale effects of overexploitation and the introduction of smallmouth bass (*Micropterus dolomieu*). Canadian Journal of Fisheries and Aquatic Sciences **66:** 212-223.

Kerr, S. J. 2000. Brook trout stocking: An annotated bibliography and literature review with an emphasis on Ontario waters. Fish and Wildlife Branch. Ontario Ministry of Natural Resources. Peterborough, Ontario.

Kerr, S. J. 2006. An historical review of fish culture, stocking and fish transfers in Ontario, 1865-2004. Fish and Wildlife Branch. Ontario Ministry of Natural Resources. Peterborough, Ontario. 154 p. + appendices.

Krebs, C. J. 1999. Ecological Methodology. Second edition, Harper and Row publishing, New York, USA.

Krueger, C. C., and T. F. Waters. 1983. Annual production of macroinvertebrates in three streams of different water quality. Ecology **64:** 840-850.

Kwak, T. J., and T. F. Waters. 1997. Trout production dynamics and water quality in Minnesota Streams. Transactions of the American Fisheries Society **126:** 35–48.

Lek, S., A. Belaud, P. Baran, I. Dimopoulos, and M. Delacoste. 1996. Role of some environmental variables in trout abundance models using neural networks. Aquatic Living Resources **9:** 23-29.

Liskauskas, A. P., and M. M. Ferguson. 1991. Genetic variation and fitness: a test in a naturalized population of brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences **48:** 2152-2162.

Loomis, J., and P. Fix. 1998. Testing the importance of fish stocking as a determinant of the demand for fishing licenses and fishing effort in Colorado. Human Dimensions of Wildlife **3:** 46-61.

Lucek, K., D. Roy, E. Bezault, A. Sivasundar, and O. Seehausen. 2010. Hybridization between distant lineages increases adaptive variation during a biological invasion: stickleback in Switzerland. Molecular Ecology **19:** 3995-4011.

Matala, A. P., S. Marx, and T. G. Wise. 2008. A genetically distinct wild redband trout (*Oncorhynchus mykiss* Gairdneri) population in Crane Prairie Reservoir, Oregon, persists despite extensive stocking of hatchery rainbow trout (O. m. Irideus). Conservation Genetics **9:** 1643-1652.

Marie, A. D., L. Bernatchez, and D. Garant. 2010. Loss of genetic integrity correlates with stocking intensity in brook charr (*Salvelinus fontinalis*). Molecular Ecology **19:** 2025–2037.

Marie, A. D., L. Bernatchez, and D. Garant. 2012. Environmental factors correlate with hybridization in stocked brook charr (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences **69:** 884-893.

McGinnity, P., P. Prodöhl, A. Ferguson, R. A. Hynes, N. O. Maoiléidigh, N. Baker, D. Cotter, B. O'Hea, D. Cooke, G. Rogan, J. Taggart, and T. Cross. 2003. Fitness reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a result of interactions with escaped farm salmon. Proceedings of the Royal Society of London. Series B: Biological Sciences **270:** 2443-2450.

Meraner, A., S. Baric, and J. Dalla Via. 2008. The selection of the "wild": A combined molecular approach for the identification of pure indigenous fish from hybridised populations. Comparative Biochemistry and Physiology. Part D **3:** 36-42.

Mezzera, M., and C. R. Largiader. 2001. Evidence for selective angling of introduced trout and their hybrids in a stocked brown trout population. Journal of Fish Biology **59:** 287–301.

Nagy, E. S. 1997. Selection for native characters in hybrids between two locally adapted plant subspecies. Evolution **51:** 1469-1480.

Naylor, R., K. Hindar, I. A. Fleming, R. Goldburg, S. Williams, J. Volpe, F. Whoriskey, J. Eagle, D. Kelso, and M. Mangel. 2005. Fugitive Salmon: Assessing the risks of escaped fish from net-pen aquaculture. BioScience **55:** 427-437.

Nislow, K. H., and W. H. Lowe. 2003. Influences of logging history and stream pH on brook trout abundance in first-order streams in New Hampshire. Transactions of the American Fisheries Society **132:** 166–171.

OMNR (Ontario Ministry of Natural Resources). 1999. Stocks catalogue. Fish Culture Section, Fish and Wildlife Branch, Peterborough, Ontario.

Peakall, R., and P. E. Smouse. 2006. GenAlEx 6: Genetic Analysis in Excel, population genetic software for teaching and research. Molecular Ecology Notes **6:** 288–295.

Pierpaoli, M., Z. S. Biro, M. Herrmann, K. Hupe, M. Fernandes, B. Ragni, L. Szemethy, and E. Randi. 2003. Genetic distinction of wildcat (*Felis silvestris*) populations in Europe, and hybridization with domestic cats in Hungary. Molecular Ecology **12:** 2585-2598.

Post, J. R., L. Persson, and E. A. Parkinson. 2008. Angler numerical response across landscapes and the collapse of freshwater fisheries. Ecological Applications **18:** 1038-1049.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics **155:** 945–959.

Quinn, N. W. S. 1995. General features of brook trout, *Salvelinus fontinalis*, spawning sites in lakes in Algonquin Provincial Park, Ontario. Canadian Field-Naturalist **109:** 205 – 209.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Heredity **86:** 248-249.

Rhymer, J. M., and D. Simberloff. 1996. Extinction by hybridization and introgression. Annual Review of Ecology and Systematics **27:** 83-109.

Rieseberg, L. H., M. A. Archer, and R. K. Wayne. 1999. Transgressive segregation, adaptation and speciation. Heredity **83:** 363-372.

Rohlf, F. J. 2006. tpsRelw, Relative Warp Analysis, version 1.44. Department of Ecology and Evolution, State University of New York at Stony Brook.

Ryman, N. 1991. Conservation genetics considerations in fishery management. Journal of Fish Biology **39:** 211-224.

Swindell, W. R., and J. L. Bouzat. 2006. Gene flow and adaptive potential in *Drosophila melanogaster*. Conservation Genetics **7:** 79-89.

Tallmon, D. A., G. Luikart, and R. S. Waples. 2004. The alluring simplicity and complex reality of genetic rescue. Trends in Ecology and Evolution **19:** 489-96.

Taylor, E. B. (1991). A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. Aquaculture **98:** 185–207.

Thorstad, E.B., I. A. Fleming, P. McGinnity, D. Soto, V. Wennevik, and F. Whoriskey. 2008. Incidence and impacts of escaped farmed Atlantic salmon *Salmo salar* in nature. Norwegian Institute for Nature Research (NINA) and World Wildlife Fund Inc., Tungasletta, Norway.

Utter, F. M. 2000. Patterns of subspecific anthropogenic introgression in two salmonid genera. Reviews in Fish Biology and Fisheries **10:** 265–279.

Wahlund, S. 1928. Zusammensetzung von populationen und korrelationserscheinungen vom standpunkt der vererbungslehre aus betrachtet. Hereditas **11:** 65-106.

Warren, D. R., S. D. Sebestyen, D. C. Josephson, J. M. Lepak, and C. E. Kraft. 2005. Acidic groundwater discharge and in situ egg survival in redds of lake-spawning brook trout. Transactions of the American Fisheries Society **134**: 1193–1201.

Webster, D. A., and W. A. Flick. 1981. Performance of indigenous, exotic, and hybrid strains of brook trout (*Salvelinus fontinalis*) in waters of the Adirondack Mountains, New York. Canadian Journal of Fisheries and Aquatic Sciences **38:** 1701–1707.

Westemeier, R. L., J. D. Brawn, S. A. Simpson, T. L. Esker, R. W. Jansen, W. W. Jeffery, E. L. Kershner, J. L. Bouzat, and K. N. Paige. 1998. Tracking the long-term decline and recovery of an isolated population. Science **282:** 1695-1698.

APPENDIX 1

ESTIMATION OF HATCHERY-WILD ADMIXTURE

Non-stocked lakes within the park were assumed to approximate the local genetics of fish within the previously stocked lakes included in this study and were used as reference samples for the program STRUCTURE. Modern hatchery Hills Lake brood stock and Lake Nipigon brood stock were used as reference samples to approximate the hatchery fish that had previously been stocked in the park. The number of genetic clusters present within the reference samples was first estimated separately for the unstocked Algonquin Park samples and the hatchery samples. Ten replicate STRUCTURE trials of $K = 1$ through $K = n+3$ where n is the actual number of sampled populations present in each dataset. Admixture was allowed between populations and no prior population information was provided to the program. A burn-in period of 50 000 replicates was used as well as 50 000 Markov chain Monte Carlo (MCMC) reactions. A K value of 2 was determined to be the appropriate value following the procedure of Evanno et al. (2007) for both the Algonquin Park and hatchery reference samples and was used in future analyses. The same procedure was used for the two hatchery samples to insure that they clustered separately. Replicate trials from $K = 1$ to $K = 4$ resulted in $K = 2$ being the most appropriate number of clusters. The admixture proportions for each lake were then estimated.

To estimate the amount of hatchery admixture present in the study lakes two datasets were first constructed. The first contained all study lakes for which the stocking records indicated that only the Hills Lake strain had previously been stocked. The second

contained all lakes that had previously been stocked with both the Hills Lake strain and the Lake Nipigon strains. Along with the first dataset STRUCTURE was given the Algonquin park reference samples and the Hills Lake sample to use as learning samples to assist in clustering. $K = 3$ was used along with a burn in period of 100 000 followed by 500 000 MCMC steps. Admixture was allowed among the study lakes and allele frequencies were treated as being correlated. The second data set was tested in the same manner, only the Lake Nipigon reference samples were also provided to STRUCTURE as well as the Hills Lake samples and $K = 4$ was used. To arrive at mean lake admixture values, the individual admixture proportions were averaged for each lake.