LONG-TERM EVIDENCE THAT PRECOCIOUS PARR CAN SIGNIFICANTLY INCREASE THE EFFECTIVE SIZE OF A POPULATION OF ATLANTIC SALMON (*SALMO SALAR*)

by

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Abstract

I describe temporal changes in the genetic composition of a small anadromous Atlantic salmon (*Salmo salar*) population from South Newfoundland, an area where salmon populations are considered as Threatened (COSEWIC 2010). I examined the genetic variability (13 microsatellite loci) in 869 out-migrating smolt and post-spawning kelt samples, collected from 1985 to 2011 for a total of 22 annual collections and a 30 year span of assigned cohorts. I estimated the annual effective number of breeders (N_b) and the generational effective population size (N_e) through genetic methods and demographically using the anadromous sex ratio. Comparisons between genetic and demographic estimates show that the anadromous spawners inadequately explain the observed N_e estimates, suggesting that mature male parr are significantly increasing N_b and N_e over the study period. Spawning as parr appears to be a viable and important strategy in the near absence of anadromous males.

List of Abbreviations and Symbols Used

1SW One-sea-winter anadromous salmon

A Number of alleles

A_{div} Allelic diversity

A_r Allelic richness

C Correction factor used to take into account the genetic covariance among consecutive cohorts

CI Confidence interval

G Generation time

H_e Expected heterozygosity

H_o Observed heterozygosity

HWE Hardy-Weinberg Equilibrium

MSW Multi-sea-winter anadromous salmon

n Sample size

N_e Effective population size

N_b Effective number of breeders

N_c Census population size

N_f Effective number of females

N_m Effective number of males

NEBT Northeast Brook, Trepassey (the study system)

PCR Polymerase chain reaction

r Correlation coefficient

S Sample size

SR Sex ratio

t number of generations between samples (for the temporal method)

Glossary

Effective number of breeders

The effective population size of the parents that produced a single cohort. The effective number of breeders (abbreviated N_b) is typically less than the effective population size (N_e), unless generations do not overlap.

Effective population size

The size of an idealized Wright-Fisher population that would experience the same rate of genetic drift, or the same levels of inbreeding as the actual population being studied. Abbreviated N_e, the effective population size corresponds to an entire generation.

Grilse

The name given to anadromous salmon as they swim upstream in freshwater prior to spawning.

Iteroparity

The ability to spawn more than once in a lifetime.

Kelt

The name given to anadromous salmon after they have spawned and begin to return to the sea.

Parr

The primary (longest lasting) juvenile stage of Atlantic salmon. Parr are characterized by parr marks (dark spots along the sides of the fish) and remain in freshwater until they become smolts and go to sea. Parr (generally males) can sexually mature and attempt to spawn.

Precocious parr

Male individuals of the parr stage that sexually mature in freshwater and attempt to spawn.

Smolt

Juvenile (although a small percentage may have previously sexually matured as parr) salmon that are heading out of the freshwater system and out to sea for the first time. Smolts are characterized by a silvery appearance.

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Chapter 1 Introduction

A fundamental question in evolutionary biology is how within species variation in life history traits affects the diversity and demography observed within populations. Salmonids, for instance, are known to exhibit marked intra-specific variation in migratory and mating strategies (Verspoor *et al.* 2007). Populations may stay resident in their natal streams, growing to maturity, or they may adopt an anadromous strategy, whereby they spend one or several years at sea before returning to their natal stream to spawn (Gross *et al. 1988*). Many populations also exhibit alternative mating strategies that are usually specific to males, whereby individuals can go to sea before spawning, or sexually mature as juveniles in freshwater (Jones 1959; Thorpe 1975; Thorpe *et al.* 1983; Hutchings and Myers 1988; Valiente *et al.* 2005). Life history strategies strongly influence demographics, yet little is known about their long-term impact on the genetic diversity of wild populations. The need to understand these impacts is particularly relevant in small populations, where genetic diversity is potentially low and vulnerable to stochastic processes such as strong genetic drift and inbreeding, potentially leading to extirpation (Palstra and Ruzzante 2008). Knowledge of the impact of demographics on genetic diversity, here defined as the effective population size (N_e, the size of an ideal Wright-Fisher population exhibiting the same amount of genetic drift, or the same level of inbreeding as the actual population under consideration; Wright 1931) is crucial for a proper understanding of the vulnerability of populations to stochastic forces.

Estimating the ratio of contemporary N_e to census population size (N_c) is also of interest, because it can be informative about the mating system; for example, an

extremely low ratio may indicate skewed sex ratios such as in harem polygyny (Palstra and Ruzzante 2008). Additionally, in some cases there is a detectable correlation between N_e and N_c (e.g. Osborne *et al.* 2010), suggesting that estimating one parameter could adequately provide information about the other. However, the median $N_e : N_c$ ratio across taxa is low (0.11 - 0.14, though highly variable and generally larger in small populations; Frankham 1995; Palstra and Ruzzante 2008), and within species $N_e : N_c$ ratios can fluctuate markedly (Shrimpton and Heath 2003; Palstra and Fraser 2012), but is usually < 1 because of several well-known factors that can reduce N_e. These factors include skewed sex ratios, high variance in reproductive success, and fluctuations in population size through time (Nunney 1993, 1996; Vucetich *et al.*1997; Hedrick 2005). Since these factors are very common occurrences in most wild populations, it is not surprising that the $N_e : N_c$ ratio is often low.

Regardless, in some populations, the $N_e : N_c$ ratio has been reported to be relatively high (e.g. Araki *et al.* 2007; Palstra *et al.* 2009). This could occur in very small populations, for instance, as they might experience a decrease in variance of reproductive success (genetic compensation; e.g. Ardren and Kapuscinski 2003; Fraser *et al.* 2007). Errors in the estimates of N_e or N_c are also possible explanations for a high $N_e : N_c$ ratio. For example, migration from neighboring populations, if not taken into account, could lead to erroneous estimates of N_e (Wang and Whitlock 2003).

Even if migration rates are known and very low and/or accounted-for, $N_e : N_c$ estimates are meaningless without an accurate estimate of the census population size, generally defined as the total number of potential (sexually mature) breeders. For some taxa, it suffices to estimate N_c by counts or mark-recapture, but for many others these

methods do not account for all potential breeders. For instance, in anadromous salmonids, published estimates of N_c usually correspond to the anadromous run size, which ignores the reproductive potential of sexually mature juveniles that are often present. In Atlantic salmon (*Salmo salar*, Linnaeus 1758), these sexually mature juveniles are primarily male and are known as precocious parr or mature male parr. Reports of N_c tend to ignore the number of mature male parr (Verspoor *et al.* 2007), as their abundance is difficult to estimate in practice. If mature male parr actually spawn, or have the potential for successful spawning, this suggests that reports of $N_e : N_c$ based on the anadromous run would be expected to be upwardly biased, since N_c is underestimated. This bias could be especially problematic since anadromous runs often contain far fewer males than females (Dalley *et al.* 1983; Myers and Hutchings 1987). Since N_e is a function of the harmonic mean of the effective number of males and females (Wright 1931; Crow and Denniston 1988), it is expected that N_e would be weighed down by the less abundant sex. Yet, N_e is still often quite large compared to anadromous N_c (e.g. Palstra *et al.* 2009).

Although the existence of mature male parr has been documented for some time (Jones 1959), their potential contribution to the next generation as measured by the effective population size was largely ignored until L'Abée-Lund (1989) reported that they doubled N_e for one year in a very small population. Thus, it is perhaps incorrect to ignore mature male parr for estimates of N_c, or demographic estimates of N_e. Recent studies (Myers and Hutchings 1987; Martinez *et al.* 2000; Jones and Hutchings 2001, 2002; Saura *et al.* 2008) have shown experimentally that over short periods, mature male parr can breed with anadromous females even in the absence of anadromous males, and

that collectively they can significantly increase N_e, though individual reproductive success for parr is generally much lower than for anadromous males.

Despite the information provided by the studies mentioned above, there is still no information describing the contribution of mature male parr to contemporary N_e over periods spanning multiple generations, and this is the goal of my thesis. I examine the extent to which mature male parr contribute to N_e in a population over a 30 year period, corresponding to nearly six generations in Atlantic salmon.

Given the evidence of their genetic contribution in the short term, I hypothesize that precious male parr can significantly increase N_e relative to what would be expected based on the anadromous run size alone. I measure N_e and the related annual effective number of breeders (N_b) through genetic methods, predicting that genetic estimates will be larger than expected from demographic parameters that ignore parr. Estimating the extent to which mature male parr influence N_e over the long term in natural populations will help fill knowledge gaps concerning their temporal contribution to the maintenance of genetic diversity. Furthermore, it will provide insight regarding whether the anadromous run size adequately represents N_c.

Chapter 2 Methods

2.1 Study System and Samples

I conducted this study using a small anadromous population of Atlantic salmon in Northeast Brook, Trepassey (here forth referred to as NEBT), in the southeast of the Avalon Peninsula, Newfoundland, Canada (46°46' N, 53°21' W; Figure 1). The river has an approximately 21.2 km^2 drainage area and 9.4 km stream length, and has been closed to angling since 1984 (Mitchell *et al.* 2005).

Specimens from which demographic data and tissue samples were obtained were collected between 1985 and 2011 on a nearly annual basis (n = 22 years), using a convertible steel smolt-adult trap (Whelan *et al.* 1989) installed in a counting fence (Anderson and McDonald 1978) located near the mouth of the river (Figure 1). Upstream migrating anadromous adults were counted each year in the late summer/early fall and out-migrating juvenile smolts were counted during the entire run, between April and June of each year. The majority of returning, anadromous spawners were maiden one-sea-winter (1SW) salmon (grilse), and a moderate proportion (male average 8.1%; female average 10.6%) were repeat spawners. There were no maiden multi-sea-winter (MSW) salmon in any year in which collections took place (O'Connell *et al.* 2001, Mitchell *et al.* 2005).

I obtained a random sample (n = 869) consisting of smolts (n = 595) and post-spawning anadromous adults (kelts; n = 274) for DNA analysis (median = 31.5 samples/year). Smolts were sampled lethally, to determine sex and sexual maturity, while kelts were sampled non-lethally and were returned to the system. Thus smolts were

sampled under Plan II (where samples are taken out of the system before being able to pass on their genes), and kelts were sampled under Plan I (where sampling occurs non-lethally or after reproduction; Nei and Tajima 1981; Waples 1989). Each fish was sexed, measured (fork length in mm) and weighed (g).

I used a tissue sample collected from each fish for DNA analysis; these consisted either of scales (kept in dry paper envelopes) or a fin clip (stored in 95% ethanol). Scales were also taken from each fish to determine age, spawning status and time spent in freshwater and at sea. Age data was used to assign individuals to cohorts. Details on annual samples by age class and number of samples per cohort are in Table 1.

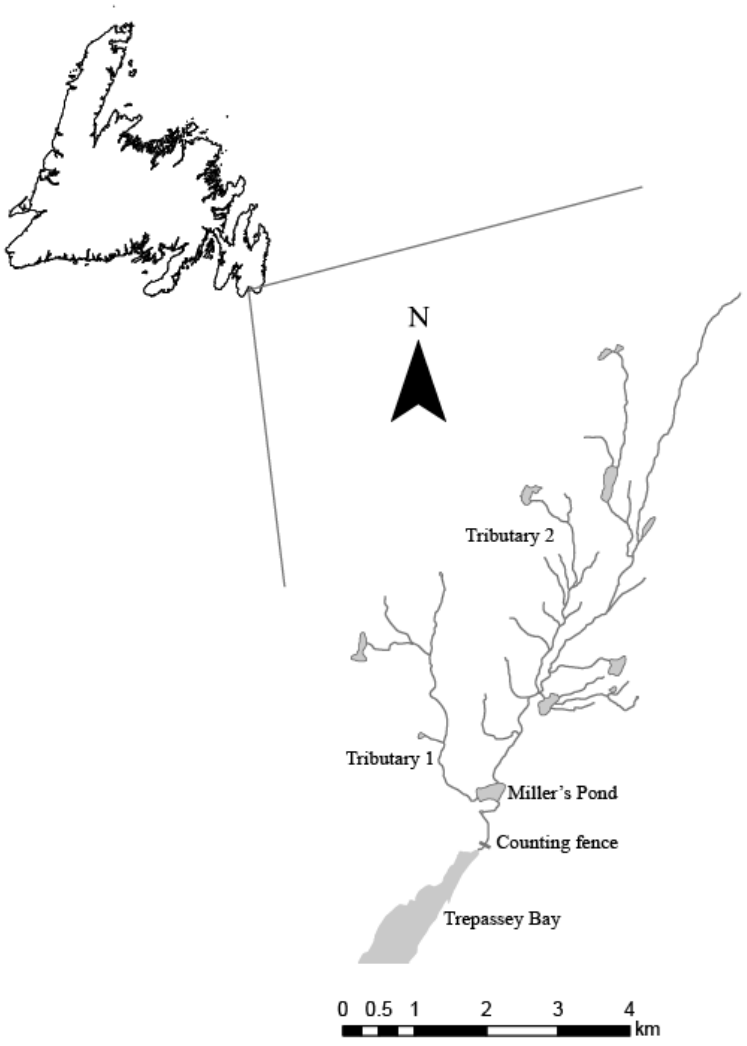

Figure 1: Map layout of Northeast Brook, Trepassey, Newfoundland. The fish counting fence was set up annually approximately 100 m above the mouth of the river, which empties into Trepassey Bay (Atlantic Ocean). Parr were sampled in the Lower Reach (between the counting fence and Miller's Pond) and just above Miller's Pond in Tributary 1 on August 18, 2011.

Table 1: Type of samples by year and number of samples in each cohort. All smolt samples were from fin clips (stored in 95% ethanol) as were all kelt samples after 1999. Earlier kelt samples were from dried scales in paper envelopes.

Year	# smolt samples	# kelt samples	Cohort (smolts)	Cohort (kelts)
1978				2
1979				4
1980				17
1981				18
1982				7
1983				
1984				2
1985		14		11
1986		14		20
1987		20		28
1988			2	
1989			3	
1990			5	7
1991		16	10	14
1992		38	14	14
1993	10	7	35	17
1994			66	13
1995	23		42	
1996			31	1
1997	57	24	30	9
1998	54	9	40	25
1999	44	32	26	31
2000	28		30	16
2001	46		30	
2002	28		41	
2003	28	24	40	
2004	26	26	32	1
2005	28	32	21	8
2006	60		39	8
2007	30		35	1
2008	30		22	
2009	30		1	
2010	33			
2011	40	18		

2.2.1 Molecular Data

I extracted DNA from samples using a glassmilk protocol (Elphinstone *et al.* 2003). For older scale samples (pre-1993) and samples that were not successfully extracted using glassmilk, I used a phenol-chloroform extraction protocol (Taggart *et al.* 1992).

I chose an initial suite of 14 microsatellite loci (Palstra *et al.* 2007, 2009; Palstra and Ruzzante 2010) for genetic analysis based on known reliability and selective neutrality in NEBT and its neighboring systems. Using these loci, I developed five multiplex protocols, enabling several loci to be amplified and analyzed simultaneously (Appendix A). Locus SSspG7 (Paterson *et al.* 2004) was run separately due to its high annealing temperature (65°C) relative to the other loci.

PCR protocols were performed using 2X HotStar Taq DNA Polymerase (Qiagen) for multiplex protocols, and using standard reagents for SSspG7. PCR conditions consisted of a 15 minute initial denaturing, followed by a three step process of 30s denaturing, 90s annealing and one minute extension (72°C) repeated 35 times, and a 30 minute final extension (60°C). Detailed protocols for each multiplex are in Appendix A.

I analyzed fragment length polymorphisms using IR 4200 and IR 4300 DNA analyzers (LI-COR Biosciences) and scored alleles using SAGA software (LI-COR Biosciences). Scoring was based on M13 PUC18 DNA ladders (Ian Paterson, unpublished). I re-analyzed sample loci that failed to amplify the first time, and at least 10% of samples were re-analyzed for each locus to assess consistency of scoring. A subset of the kelt samples had previously been used in other studies (Palstra *et al.* 2007,

2009; Palstra and Ruzzante 2010), so I compared my allele scores to theirs as another way to verify consistency.

2.2.2 Sample Statistics

For each locus and cohort as well as overall samples, I estimated allele frequencies, number of alleles, allelic richness, observed and expected heterozygosities and assessed linkage disequilibrium using FSTAT 2.9.3.2 (Goudet 1995).

I assessed departures from Hardy-Weinberg Equilibrium (HWE) in each locus and cohort using ARLEQUIN 3.5.1.3 (Excoffier *et al.* 2010), using 1 000 000 dememorization steps and 1 000 000 steps in the Markov chain.

I assessed the presence of scoring errors and artifacts (such as null alleles and large allele dropouts) using the program MICROCHECKER (van Oosterhout *et al.* 2004). I verified marker neutrality using LOSITAN (Beaumont and Nichols 1996; Antao *et al.* 2008).

2.3 Connectivity and Population Structure

Signals of allele frequency variation can arise from genetic drift as well as from sampling effects, migration or population substructure. Palstra *et al.* (2007) and Palstra and Ruzzante (2010) showed that migration between NEBT and other populations is very low and at best intermittent. NEBT has also been thoroughly sampled via electrofishing

(Mitchell *et al.* 2005), and has been found devoid of non-anadromous salmon or population substructure. Consequently, I considered NEBT as one isolated population.

2.4 Effective Population Size

I estimated N_e using a suite of methods, and used the program CREATE (Coombs *et al.* 2008) for multiple data format conversion.

The estimators I used are broadly based in two categories. First, I estimated N_e using the temporal method (Krimbas and Tsakas 1971; Nei and Tajima 1981). Secondly, I estimated the annual effective number of breeders (N_b) using several single sample methods (Waples 2006; Waples and Do 2008; Tallmon *et al.* 2008; Wang 2009) for all sampled cohorts. I then estimated N_e by multiplying the harmonic mean of these N_b estimates by generation length (estimated to be 5.2 years; Palstra *et al.* 2009) and estimated 95% CI by jackknifing over individual estimates of N_b (Patil and Lilja 2010).

2.4.1 Temporal Method Assuming Discrete Generations

Using the generation length, I combined the cohorts from 1978 to 1982 (kelts only, S = 48) into a group corresponding to approximately one generation and the cohorts from 2004 to 2008 (kelts and smolts, S = 166) into another, representing two samples separated by 26 years, corresponding to five generations. I applied the discrete temporal method (Waples 1989) using N_eEstimator (Ovenden *et al.* 2007), as well as using the maximum-likelihood method implemented in MLN_e (Wang and Whitlock 2003),

assuming a population closed to migration (Palstra *et al.* 2007; Palstra and Ruzzante 2010).

I also estimated N_e using the estimator TempoF_s (Jorde and Ryman 2007). Although all individuals in the first sample (1978 - 1982 cohorts) were kelts, I included smolts in the second sample (cohorts 2004 - 2008) to increase sample size. Although this represents a mixed Plan I - Plan II (Waples 1989) sampling design, I considered estimates using a Plan II design. I made this assumption because I considered the correction factor 1/N used for Plan I sufficiently small to ignore, given that in this case, N is the number of juveniles (Jorde and Ryman 1995; Serbezov *et al.* 2012b).

2.4.2 Temporal Method Assuming Overlapping Generations

I also applied Jorde and Ryman's (1995) cohort model for species with overlapping generations as implemented in the program GON$_e$ (Coombs *et al.* 2012). The method assumes constant age structure and compares consecutive cohorts to estimate N_e, which is expected to fluctuate around a mean value. The estimate takes into account generation length (G) and includes a correction factor C, which is a function of age class birth and death rates (Jorde and Ryman 1995). Because the method is based on estimating drift between individual cohorts, sample sizes are usually smaller than those that can be used in the discrete method. However, estimates obtained with the cohort approach are generally more reliable than those derived from the discrete method because it takes into account the genetic covariance that arises between cohorts due to age structure (Jorde and Ryman 1995). GON$_e$ (Coombs *et al.* 2012) provides separate estimates based on three

definitions of the signal of genetic drift: F_k (Pollak 1983), F_c (Nei and Tajima 1981), and F_s (Jorde and Ryman 2007).

Because there were two types of samples (Plan I for kelts, Plan II for smolts), they differ in the method of estimating N_e since Plan I samples require a correction factor for sample size. For the overlapping generation method, I only used smolt samples since they had a relatively large sample size and did not require the correction factor.

2.4.3 Single Sample Estimates (N_b)

Whereas the temporal method requires two samples to produce a single estimate of N_e, single sample methods can provide two or more estimates using the same data (Hare *et al.* 2011). In contrast to the temporal method (which estimates a harmonic mean N_e over the period roughly between sampling events; see Hare *et al.* 2011), single sample methods estimate N_e (or N_b: the effective number of breeders) of the parents that produced the sample.

Since Atlantic salmon have a life history with overlapping generations, I estimated the annual effective number of breeders (N_b) by analyzing cohorts individually. I estimated N_b using three different methods: the linkage disequilibrium method (LD) (Hill 1981; Waples 2006; Waples and Do 2008), Approximate Bayesian Computation (Tallmon *et al.* 2008) and the Sibship method (Wang 2009).

2.4.3.1 Linkage (Gametic) Disequilibrium (LD)

The linkage disequilibrium (LD) method was first developed by Hill (1981) and subsequently improved by Waples (2006) by applying a correction factor for sample size bias. The method assumes that the population is closed to migration, that alleles are neutral, that there is no subpopulation structure, and that sampling is random with respect to the entire population, though Waples and England (2011) recently showed that the method is robust to low or moderate levels of migration. Typically, it also assumes that loci are unlinked but it is more precise when using linked loci if the degree of recombination is known (Waples and Do 2010).

The LD method operates by calculating D, the difference between the expected co-occurrence of two alleles on two loci under no selection and independent segregation, and their actual co-occurrence. N_e can be calculated from the LD method using the equation:

$$\widehat{N}_e(\mathrm{D}) = \frac{1}{3(r^2 - \frac{1}{S})} \qquad \textbf{(1)}$$

where *r* is the correlation among alleles and *S* is the sample size. According to Waples (1991), the coefficient of correlation is a poor indicator of N_e, but as can be seen in *Equation 1*, the accuracy of the methods improves with increasing sample size, and especially with increases in the number of loci used.

While the LD method does not work when sampling from infinite (or very large) populations, it does work very well in small populations, particularly when N_e is much less than N_c (Bartley *et al.* 1992; Waples and Do 2010).

I estimated N_b using the program LDN_e (Waples and Do 2008), using a critical allele frequency cutoff of 0.02 and estimated confidence intervals by jackknifing over loci (Waples and Do 2010).

2.4.3.2 Approximate Bayesian Computation (ABC)

The Approximate Bayesian Computation (ABC) method (Tallmon *et al.* 2008) takes into account eight moments of information from the data, including linkage disequilibrium, requiring two or more unlinked loci, and is implemented in the web-based program ON_eSAMP 1.2 (Tallmon *et al.* 2008). For my analysis, I used 50 and 350 as the lower and upper priors, respectively. I repeated the analysis to verify consistency of each estimate. Previous work (Carrea *et al.* 2011) has shown that the method is sensitive to sample size when S is less than N_e, therefore I also analyzed the effect of sample size by comparing annual cohort sample size to the N_b estimate.

2.4.3.3 Sibship Method

The Sibship method (Wang 2009) estimates N_b based on assigning the likelihood that individuals are half or full siblings. It is theoretically more robust than other models from the perspective that it does not require the assumption of random mating. However, the base model assumes discrete generations. This method can be problematic if marker information is scarce, as it will tend to underestimate N_e. This occurs if the sample size is much smaller than N_e, if the number of loci is low, or if loci exhibit low polymorphism. Rarefaction tests have shown that sibship estimates of N_e increase with sampling effort

and therefore this method very much depends on appropriate sample size. I estimated N_b using Colony 2, (Jones and Wang 2010), and ran the data from each cohort using a medium length run, and using the full likelihood, high precision settings, assuming male and female polygamy and no inbreeding. To verify the consistency of the estimates, I repeated the analysis of a subset (3 cohorts) using a long run and 1 cohort (1994, S = 79) using an extra-long run. I also tested for sample size by plotting the N_b estimates against sample size and used a linear regression. Additionally, I selected the largest cohort (1994; S = 79) and randomly subsampled 2 replicates each for subsamples of 20, 30, 40, 50, 60, and 70 individuals. Again, I plotted the N_b estimates against subsample size and tested for a correlation using a linear regression.

2.5 Combining Estimates

Although there are many methods to estimate N_b and N_e, each method has different assumptions and biases. Consequently, Waples (2005) argued that combining estimates across methods, if done correctly, could reduce the bias found from single estimates. Therefore, I produced a combined estimate of N_e by calculating the harmonic mean estimate across all the temporal methods as well as LDN_e. I estimated 95% CI by jackknifing across the different estimates (Patil and Lilja 2010).

2.6 N_e Estimates Using the Sex Ratio in the Anadromous Run

Although all mating females come from the anadromous runs, previous work has shown that mature male parr can contribute to mating (Myers 1984; Martinez *et al.* 2000; Jones and Hutchings 2001, 2002; Saura *et al.* 2008). I therefore assessed the effective population size expected on the basis of the sex ratio in the anadromous run alone using the equation:

$$\frac{1}{\widehat{N}_{bSR}} = \frac{1}{4N_m} + \frac{1}{4N_f} \qquad \textbf{(2)}$$

(Crow and Denniston 1988), where $\widehat{N}_{\mathrm{bSR}}$ is the sex ratio-derived estimate of effective number of breeders, and N_m and N_f are the effective number of males and females, respectively. I compared these demographic estimates to the genetic estimates; their difference is expected to reflect the contribution to reproduction, and thus to the effective population size, by mature male parr. Here I made the assumption that only an unequal sex ratio would reduce N_e, with the contribution by variance in reproductive success and fluctuating population size assumed negligible (Nunney 1993, 1996; Vucetich *et al.* 1997; Hedrick 2005). It should be noted that these assumptions are unrealistic, suggesting that sex ratio-derived estimates of N_e are likely to be biased upwards. Thus, if genetic estimates are significantly greater than $\widehat{N}_{\mathrm{eSR}}$, the difference would be conservatively low.

I also made the simplifying assumption that each anadromous individual (male or female) mated ideally, *i.e.* the effective number of males and females were equal to the census number of males and females in the anadromous run respectively. Based on these

assumptions, any difference between the theoretical estimate of N_e and the corresponding genetic estimates (if the genetic estimates are larger) should reflect the influence of inequality in the number of males and females among the anadromous adults. Again, these assumptions are unrealistic, thus any difference between genetic and sex ratio-derived estimates would be conservatively underestimated.

Although sex was not determined for anadromous individuals as they swam upstream (O'Connell *et al.* 2001; Mitchell *et al.* 2005), I estimated the sex ratio and therefore the number of members of each sex for each year in three ways:

First, I determined the proportion of each sex in each kelt emigration the spring after spawning (year *X+1*) and scaled the number of each sex to the incoming anadromous run size the year before (i.e., year *X*). This method assumes that post-spawning mortality up until the sampling event was the same for both sexes, and that kelt sampling was unbiased with respect to sex. Secondly, since males are known to often have higher post-spawning mortality than females in some systems, I used the estimates of 65% male and 85% female post-spawning survival from Jonsson *et al.* (1991), to produce a corrected, but probably conservatively high estimate of the anadromous sex ratio at the time of spawning. Finally, I estimated the proportion of each sex in each incoming anadromous run by estimating the sex ratio for the smolt run the previous year (*X-1*). This assumes no sex-bias in mortality at sea or straying (Palstra *et al. 2007;* Serbezov *et al.* 2012b), unbiased sampling of smolts with respect to sex, and that anadromous fish spent one year at sea (there were no multi-sea-winter fish detected).

In each case, I inserted the numbers of males and females in each anadromous run into *Equation 2,* to produce an estimate of N_b based on the sex ratio ($\widehat{N}_{bSR}$). To facilitate comparison to temporal estimates of N_e, I estimated the harmonic mean of all the estimates of N_{bSR} within each group (kelts, kelts with mortality, smolts) and multiplied the harmonic mean by the generation length (5.2 years) to estimate the sex ratio-derived effective population size $\widehat{N}_{eSR}$. I estimated 95% confidence intervals for $\widehat{N}_{eSR}$ by jackknifing over the harmonic mean (Patil and Lilja 2010).

The estimation of N_e using the harmonic mean of N_b, automatically includes the influence of fluctuating population size. Therefore, in order to determine whether any differences between genetic and sex ratio-derived estimates of N_e were driven by the sex ratio itself or by fluctuating population size, I also separately estimated N_e based on the assumption that only fluctuating population size would decrease N_e relative to N_c. To do this, I used a sliding window technique, whereby I estimated N_c by summing the total number of anadromous spawners in five year blocks. I then calculating the harmonic mean of these N_c estimates in order to estimate N_e.

2.7 Effective Number of Parr

By applying the number of anadromous females and the genetic estimates of N_b to *Equation 2*, I solved for the effective number of males in the system. This assumed that anadromous females all mated ideally. This assumption is likely unrealistic, and thus the effective number of females is probably less than the census number of females. This implies that any estimate of N_m is likely an underestimate. By further assuming that all

anadromous males mated ideally, I then determined the effective number of parr by subtracting the number of anadromous males from N_m.

2.8 Parr Maturity

In order to verify the presence of mature male parr in the system, I sampled the system with the Department of Fisheries and Oceans August 18, 2011 (prior to spawning). I determined the percentage of male parr that were sexually mature by electrofishing two portions of the river; the lower reach above the counting fence and below Miller's pond, and the area just above Miller's pond in Tributary 1 (Figure 1). I recorded wet weight, gutted weight, and length. Parr sex and maturity were determined by gonad inspection.

2.9 Statistical Analysis

I performed statistical analyses using SigmaPlot 11 (SYSTAT) and Microsoft Excel. Annual estimates of N_{bSR} were compared to the corresponding genetic estimate from LDN_e using paired t-tests (comparisons were within but not across years). However, I only included N_b estimates where sample size was 30 or greater, and where the estimate from LDN_e was less than 200, as Waples and Do (2010) have shown that LDN_e is most accurate given these parameters.

For the estimates of N_e, I grouped the sex ratio-derived estimates (based on kelt and smolt sex ratios, respectively), and compared the mean to the grouping of all genetic

estimates of N_e. Since the corrected kelt sex ratios (assuming mortality) are not independent of the sex ratios without the correction, I performed the analysis between sex ratio-derived estimates and genetic estimates of N_e separately for the corrected and uncorrected kelt sex ratio estimates.

Chapter 3 Results

3.1 Demographics

Anadromous run sizes (small and large salmon combined) ranged from 59 (1992, 1997) to 188 (1986), and slightly but significantly declined (adjusted $r^2 = 0.351$, $p < 0.001$) over the sampling period, and this trend was more pronounced for the large salmon (adjusted $r^2 = 0.685$, $p < 0.001$) (Figure 2). Runs were mostly female-dominated (median 87.18% female from kelts, 83.87% from kelts assuming mortality, and 87.65% from smolt runs). Smolt run size ranged from 792 (1995) to 2076 (2002), but did not show any particular trend, nor did smolt productivity (O'Connell *et al.* 2001).

3.2 Sample Statistics

Due to difficulty of reliably scoring locus Ssa171 (multiple di-shifts), it was not used for further analysis, thus I used a total of 13 loci. Amplification success was high for all loci, ranging from 98.5% (SSsp2215) to 99.9% (SSsp1605), with a mean of 99.6%. The number of alleles ranged from six (Ssa12) to 20 (SSsp2215), with allelic richness ranging from 2.86 (SsaF43) to 6.2 (SSsp3016). Gene diversity ranged from low (0.273: SsaF43) to moderate (0.789: SSsp3016) (Appendix B).

Linkage disequilibrium was detected in nine out of 1872 comparisons following sequential Bonferroni correction (Rice 1989). However, eight of the nine comparisons included Ssa12. Further analysis in LOSITAN showed no sign of deviation from selective neutrality, but as a precaution I did not use Ssa12 for the linkage disequilibrium

method of estimating N_b; however it was included for all other analyses. In total, 21 of 312 HWE tests were significant at $\alpha = 0.05$ (Appendix B), but none were significant following sequential Bonferroni correction.

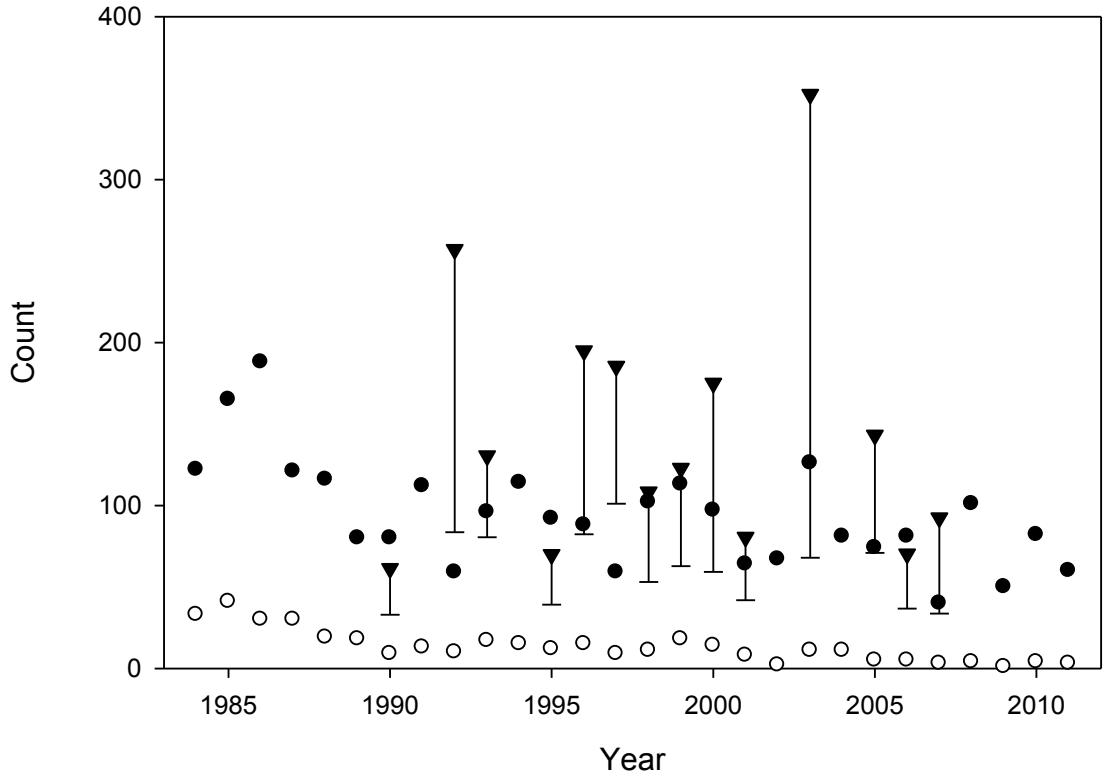

Figure 2: The annual total anadromous run size of Atlantic salmon in NEBT(filled circles), along with the annual number of large (>63cm fork length) adults (empty circles), and the corresponding estimate of effective number of breeders N_b, using LDN_e and its lower 95% confidence limit (Waples and Do 2008).

3.3 Effective Population Size

3.3.1 Temporal Method

All temporal estimates of N_e are in Table 2. Based on an estimate of five generations passing between sampling events, the maximum-likelihood estimate of N_e from MLN_e was 489 (95% CI: 268 - 1426), assuming a population closed to migration. The moment estimate from N_eEstimator was 521 (95% CI: 231 -3242). The estimate using Jorde and Ryman's (2007) TempoF_s estimator was 290 (95% CI: 133 - ∞).

Under the cohort model (Jorde and Ryman 1995), the estimate of N_e based on F_s had a mean of 443 (95% CI: 312 - 598). Given the smaller sample sizes of pairwise comparisons of consecutive cohorts, sampling error was too large to estimate F_c or F_k and the corresponding estimates of N_e.

Table 2: Temporal estimates of N_e, where *t* is the number of generations between samples and $\widehat{S}$ is the harmonic mean sample size. The cohort method (Jorde and Ryman 1995) compares consecutive cohorts, and the estimate provided is the mean estimate across all years, using GON_e (Coombs *et al.* 2012).

Method	Reference	Period	*t*	$\widehat{S}$	N_e	95% CI
MLN_e	Wang and Whitlock 2003	1978 - 2008	5	75	489	268 - 1426
N_eEstimator	Waples 1989; Ovenden *et al.* 2007	1978 - 2008	5	75	521	231 - 3242
$TempoF_s$	Jorde and Ryman 2007	1978 - 2008	5	75	290	133 - ∞
Cohort	Jorde and Ryman 1995	1988 - 2007 (consecutive cohorts)	< 1	28	443	312 - 598
Combined	Waples 2005				439	260 - 781

3.3.2 Single Sample Estimates (N_b)

LDN_e estimates of N_b (where S > 30, Waples and Do 2010) ranged from 70 (95% CI: 39 - 205) to 352 (95% CI: 68 - ∞) (Table 3). $\widehat{N_e}$, based on multiplying the harmonic mean $\widehat{N_b}$ by generation length, was 584 (95% CI: 414 - 754).

Estimates based on ON_eSAMP (Tallmon *et al.* 2008) ranged from 16 (95% CI: 14 - 21) to 99 (95% CI: 81 - 158). Estimates were generally lower than the corresponding census number of anadromous breeders; however in some years $N_e : N_c$ was > 1. Additionally, estimates were significantly related to sample size (adjusted $r^2 = 0.947$, $p < 0.001$; Appendix C). Previous work (Carrea *et al.* 2011) also showed that through random subsampling, estimates were significantly related to sample size, and that estimates from ON_eSAMP using the same settings were inconsistent. This suggests that the effective number of breeders was being underestimated and I therefore did not further consider estimates using ON_eSAMP in detail.

Sibship estimates were also low, ranging from 24 (95% CI: 13 - 46) to 73 (26 - ∞). Estimates were consistently lower than N_c, however there was a clear downward bias using this method, as N_b estimates were significantly correlated to sample size (adjusted $r^2 = 0.746$, $p < 0.001$; Appendix D), and the correlation was even more significant through rarefaction (adjusted $r^2 = 0.907$, $p < 0.001$; Appendix E). Due to this bias, I did not consider sibship estimates for further analysis.

3.4 Sex Ratio-Derived Estimates of N_e

Estimates of effective population size based on the sex ratios in kelts and smolts are given in Figure 3 and Table 3. Individual estimates of N_{bSR} based on the kelt sex ratios ranged from 11 to 121 for the direct counts and from 15 to 140, assuming post-spawning mortality (Jonsson *et al.* 1991). The corresponding N_{eSR} estimates were 179 (95% CI: 128 - 229) and 217 (95% CI: 160 - 274), respectively (Figure 4). N_{bSR} estimates using the smolt sex ratio were similar in magnitude to the estimates based on kelts, ranging from 11 to 80, with N_{eSR} estimated as 139 (95% CI: 90 - 188). Corresponding genetic estimates of N_e (LDN_e: 584; MLN_e: 489; N_eEstimator: 521; cohort method: 443; $TempoF_s$: 290) were up to 4.2 times larger than estimates from the sex ratio (Figure 4).

Individual annual estimates of N_b (LDN_e) were as much as 20.3 times larger than their corresponding estimates based on sex ratios (Table 3, Figure 3), although LDN_e estimates > 200 and very small samples are likely biased (Waples and Do 2010). Considering only years with more than 30 individuals in the samples and estimates < 200 (N = 10), estimates from LDN_e were up to 13.6 times larger than the corresponding estimates based on sex ratios.

Across all years, estimates of genetic N_b were greater than $\widehat{N}_{bSR}$ from kelts (paired $t_{1,9} = 5.970$, $p < 0.001$), kelts with mortality (paired $t_{1,9} = 5.466$, $p < 0.001$) and smolts (paired $t_{1,6} = 5.658$, $p < 0.001$). Additionally, $\widehat{N_e}$ from genetic methods was significantly greater than estimates from the sex ratios when using either the kelt sex ratio ($t_{1,5} = 3.675$, $p \leq 0.007$), or the kelt sex ratio with assumed mortality ($t_{1,5} = 3.368$, $p \leq 0.01$). The influence of fluctuating population size did not on its own significantly decrease N_e

below the genetic estimates of N_e (Figure 4), suggesting that the unequal sex ratio was the primary driver separating genetic and sex ratio-derived estimates of N_e.

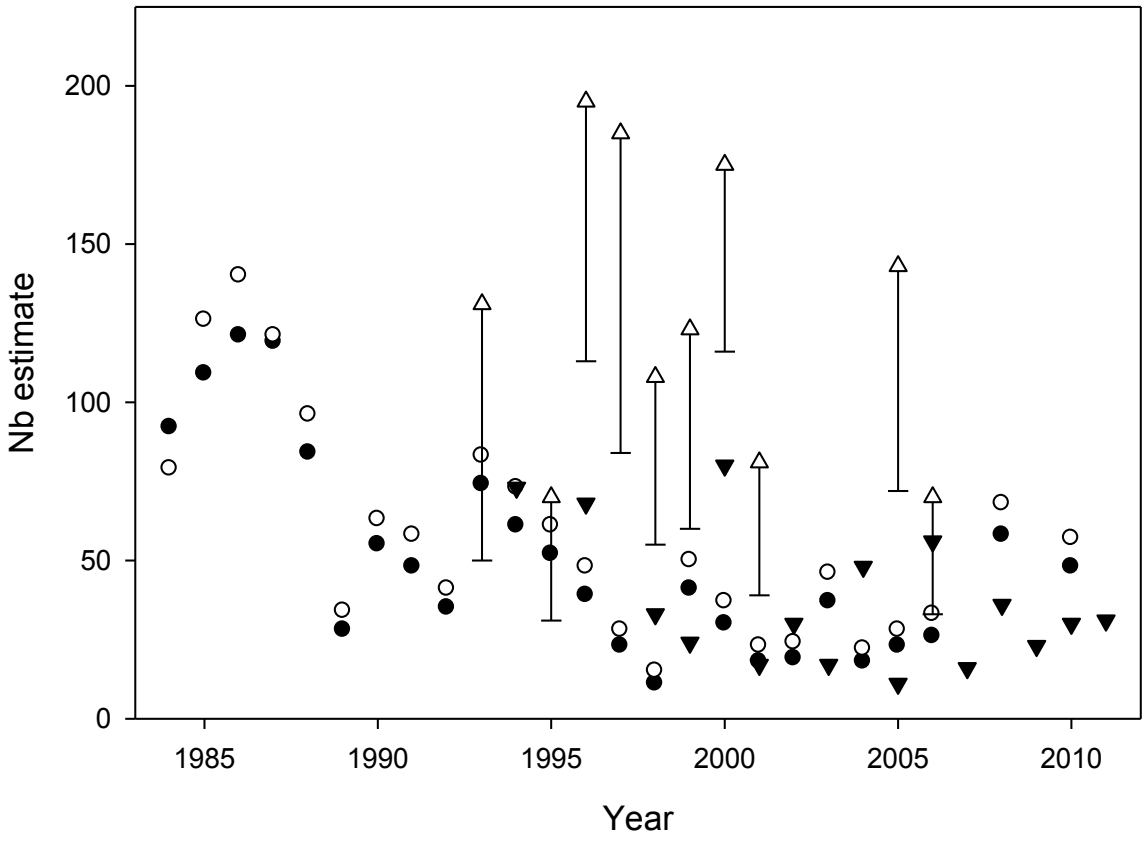

Figure 3: Annual estimates of effective number of breeders (N_b), as predicted based on the sex ratio in post-spawning kelts (filled circles), kelts with assumed freshwater mortality (empty circles) and smolts a year prior to spawning (filled triangles). Empty triangles represent the corresponding genetic estimate of N_b using LDN_e (when S > 30, only estimates < 200 included) and its lower 95% confidence limit (Waples and Do 2008). All estimates are aligned with the year in which the corresponding anadromous breeders swam upstream and spawned.

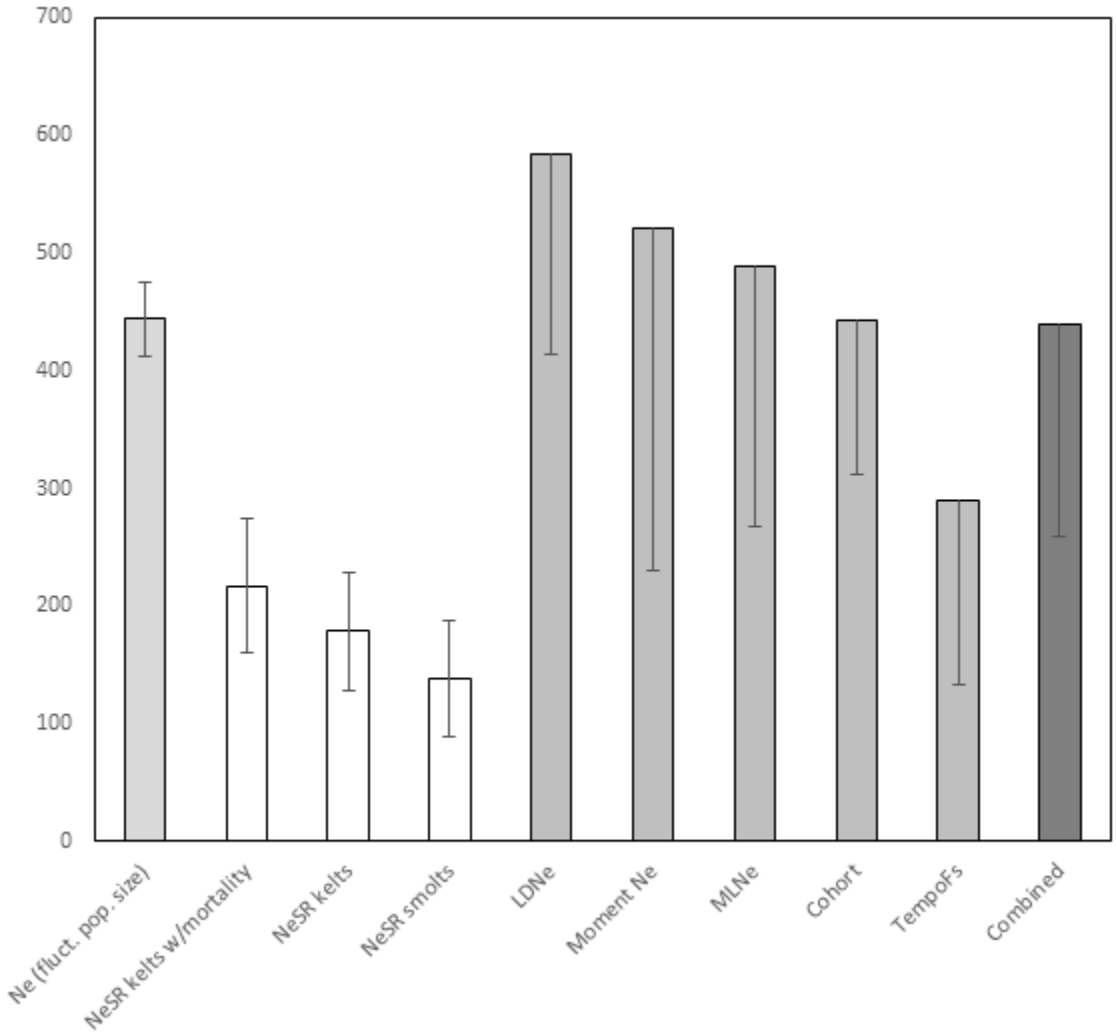

Figure 4: Estimates of effective population size using the sex ratio in kelts and smolts (N_{eSR}), and the corresponding estimates based on genetic methods. Estimates for the sex ratio-derived N_e and the LDN_e estimate correspond to the harmonic mean estimate of N_b multiplied by generation length (5.2 years; Palstra *et al.* 2009), and 95% CI are jackknifed around the harmonic mean. 'N_e (fluct. pop. size)' represents the influence of fluctuating population size alone, with jackknifed 95% confidence intervals. 'Combined' represents the combined estimate across genetic methods. For display purposes, upper 95% CI are not shown for the genetic methods, but are provided in Tables 2 and 3.

Table 3: Annual anadromous run (small and large salmon combined) and smolt run sizes, annual estimates of the effective number of breeders based on the sex ratio (given as % Female) in kelts, kelts with assumed mortality and smolts (N_{bSR}), and the corresponding genetic estimate of N_b based on LDN_e (Waples and Do 2008). Estimates of sex ratio-derived and genetic N_b are aligned with the corresponding anadromous run, however estimates from the sex ratio of the kelts and the genetic estimate are based on the year following the anadromous run (*X+1*), and the sex ratio in smolts is based on the smolt run a year prior to the anadromous run (*X-1*).

Year	Anadromous run size	Smolt run size	S Kelts	% Female (kelts)	N_{bSR} (kelts)	N_{bSR} (kelts w/mort.)	S Smolts	% Female (smolts)	N_{bSR} (smolts)	S cohort (DNA)	N_b (LDNe)	95% CI
1979										17	55	25-∞
1980										18	206	39-∞
1981										7		
1982												
1983										2		
1984	122		16	25	92	79				11	-54	42-∞
1985	165		86	79	109	126				20	10	7-16
1986	188	1117	104	80	121	140				28	-342	122-∞
1987	121	1404	23	57	119	121				2		
1988	116	1692	63	76	84	96				3		
1989	80	1708	21	90	28	34				12	21	13-41
1990	80	1902	55	78	55	63				24	61	33-231
1991	112	1911	41	88	48	58				28	-187	194-∞
1992	59	1674	33	82	35	41				52	257	84-∞
1993	96	1849	42	74	74	83				79	131	81-280
1994	114	944	44	84	61	73	10	80	73	42	-629	213-∞
1995	92	792	65	83	52	61				32	70	39-205
1996	88	1749	39	87	39	48	23	74	68	39	195	82-∞
1997	59	1829	54	89	23	28				65	185	101-689
1998	102	1727	35	97	11	15	57	91	33	57	108	53-635
1999	113	1419	80	90	41	50	54	94	24	46	123	63-654
2000	97	1740	60	92	30	37	45	71	80	30	175	59-∞
2001	64	916	52	92	18	23	28	93	17	41	81	42-323
2002	67	2076	26	92	19	24	46	87	30	40	-397	194-∞
2003	126	1064	98	92	37	46	28	96	17	33	352	68-∞
2004	81	1571	52	94	18	22	28	82	48	29	-726	81-∞
2005	74	1384	12	92	23	28	27	96	11	47	143	71-1130
2006	81	1385	56	91	26	33	27	78	56	36	70	37-271
2007	40	1777	10	100			60	88	16	22	92	34-∞
2008	101	1868	46	83	58	68	30	90	36	1		
2009	50	1600	2	50			30	87	23			
2010	82	1012	28	82	48	57	30	90	30			
2011	60	800					33	85	31			

3.5 Effective Number of Parr

The effective number of males needed to generate the estimated effective number of breeders (from LDN_e) was generally much larger than the estimated number of anadromous males, regardless of estimating the sex ratio using kelts, kelts with assumed post-spawning mortality, or smolts. The effective number of males estimated ranged from 23 to 539, corresponding to a range of effective number of parr from 4 to 531 (Table 4). This corresponded to a proportional contribution to N_m of 76% (range 16 - 98%; Table 4) by mature male parr. Therefore, mature male parr increased N_m by an average of 3.17 times, however this is likely an underestimate due to the assumptions of ideal mating by the adults.

3.6 Parr Maturity

Of the 100 parr I sampled, 48 (22 in lower reach, 26 in Tributary 1; Figure 1) were identified as males, although 35 individuals could not be sexed in the field due to small size. Of the 48 males, 38 (79.2%) were sexually mature. This proportion falls within expectations for this region and latitude (Dalley *et al.* 1983; Myers 1984; Valiente *et al.* 2005). However, most of the immature males were very small, presumably mostly young of the year. Of the parr larger than 5 g whole weight, 37 of 39 (95%) were sexually mature.

Table 4: Estimates of the effective number of males (N_m) and effective number of males that were parr (N_{parr}) required in order to generate the estimated effective number of breeders (N_b) using LDN_e, with '% parr' representing the proportion of effective males that were parr. The effective number of females (N_f) and the effective number of anadromous males ($N_m - N_{parr}$) were assumed to be equal to the census number of females and males in the anadromous runs, respectively.

Year	N_b (LDN_e)	Kelts				Kelts with mortality				Smolts				All Methods
		N_f	N_m	N_{parr}	% parr	N_f	N_m	N_{parr}	% parr	N_f	N_m	N_{parr}	% parr	% parr
1993	131	71	60	35	58	66	65	35	53					
1994														
1995	70	76	23	7	31	73	23	4	16					
1996	195	77	134	122	92	74	143	129	90	65	194	171	88	
1997	185	52	399	392	98	51	539	531	98					
1998	108	99	37	34	92	98	37	34	90	93	38	29	77	
1999	123	102	44	33	74	99	45	30	68	107	43	37	85	
2000	175	89	86	78	91	87	88	78	88	69	120	92	77	
2001	81	59	31	26	84	58	31	25	80	59	30	26	85	
2002														
2003														
2004														
2005	143	68	76	70	92	66	78	70	90	71	72	69	96	
2006	70	74	23	16	69	72	23	14	60	63	24	6	26	
Range					**31 - 98%**				**16 - 98%**				**26 - 96%**	**16 - 98%**
Mean					**78**				**73**				**76**	**76**
Median					**87**				**84**				**85**	**85**

Chapter 4 Discussion

This is the first study to show that alternative mating strategies can maintain genetic diversity over multiple generations in wild Atlantic salmon, providing results similar to those of Araki *et al.* (2007) in anadromous steelhead trout (*Oncohynchus mykiss*). I have shown that in this population, genetic estimates of contemporary N_e are higher than expected from the sex ratio of the anadromous spawning stock alone. With nil or negligible migration (Palstra and Ruzzante 2010), and in the absence of resident salmon, this suggests that mature male parr significantly increased N_e and that this contribution was maintained over multiple generations. Over a 30-year period, corresponding to roughly six generations, the mature male parr were crucial in buffering N_e and N_b despite a low number of anadromous males.

This study also provides further evidence that consideration of the anadromous run size as equivalent to the census size in salmonids is inaccurate if alternative mating strategies exist. Prior studies have often reported the $N_e : N_c$ ratio while only including the anadromous run, but this study confirms that there is little expectation or power to detect any correlation between N_e and N_c (as in Osborne *et al.*2010) under such circumstances.

The genetic estimates of N_e are much larger than what is possible given the anadromous run size and sex ratio. Furthermore, the high incidence of parr maturity in the region (Dalley *et al.* 1983; Myers 1984; this study) suggests that parr participate in spawning. Thus, I argue that the anadromous males inadequately account for the genetic estimates of N_e and that mature male parr are significantly increasing N_e and N_b. Although my data can only show that the anadromous males are insufficient in number to

account for the amount of variability (i.e.$\widehat{N_e}$) observed, I argue that mature male parr are the only explanation accounting for the difference for the following reasons:

First, this system does not host resident (non-anadromous) salmon. Thus, any potential breeders would have to come from the anadromous run, or from mature parr. Given that the counting fence was set up nearly every year to count all incoming and outgoing salmon, I am confident that I have accurately portrayed the annual anadromous run size. Thus, I conclude that mature male parr likely also make a major contribution to N_e, a result consistent with other findings from experiment (e.g. Jones and Hutchings 2001, 2002) and from the wild (Martinez *et al.* 2000; Saura *et al.* 2008).

Secondly, the Northeast Brook, Trepasssey system was chosen for this study in part because of its near complete isolation. Palstra *et al.* (2010) showed that NEBT is genetically very distinct from neighboring systems and that the differences remained stable over the 19 year timespan of their study, with gene flow estimates into and out of the system largely negligible. Because gene flow was shown to be intermittent at best, any bias in the genetic estimates of N_e due to gene flow, if it existed, would be expected to be a downward bias, suggesting my genetic estimates of N_e may actually be conservatively low.

Finally, mature male parr are likely the contributing factor buffering N_e because experiments have shown that they can increase N_e in the short term (Martinez *et al.* 2000; Jones and Hutching 2001, 2002; Saura *et al.* 2008), thus it might be expected for this to hold true over multiple generations, as my data suggests. It is also known that with decreased competition from anadromous males, the reproductive success of mature male parr can be high; for example, Moran *et al.* (1996) showed that parr can sire up to 89.3%

of the eggs in a redd (nest), although typically their success is lower (e.g. up to 23%; Hutchings and Myers 1988, 5 - 30%; Thomaz *et al.* 1997). Thus, the potential for parr majorly contributing to the next generation is high, and my data is consistent with the hypothesis that, over the roughly six generations covered by this study, mature male parr contributed significantly to reproduction and this contribution was vital for maintaining genetic diversity.

4.1 Assumptions and Caveats

4.1.1 Sex Ratio

Although sex was not determined directly in the incoming anadromous runs due to concerns regarding the potential negative consequences of handling pre-spawning anadromous salmon in a small system , counts were made as fish swam upstream through the fence. Sex was determined in both post-spawning kelts during the outmigration and outgoing smolts the year before they returned to spawn, and in both cases the sex ratio was female-biased, which is consistent with many salmon populations in Newfoundland (Dalley *et al.* 1983). However, each method implies a certain set of assumptions. In the case of the kelts, I assumed that the survival rate between the spawning period and the spring emigration period was equal between the sexes, and that kelt sampling was unbiased regarding sex. To test the influence of post-spawning mortality, I also applied Jonsson *et al.*'s (1991) estimates of freshwater survival for Norwegian kelts. Although this mortality adjustment increased $\widehat{N}_{\mathrm{bSR}}$, the genetic estimates were still significantly greater. Moreover, the estimate of N_{bSR} assuming mortality may be conservatively high,

since male kelt mortality due to aggression is probably low in NEBT, given the low abundance of adult competitors compared to the system used in Jonsson *et al.*'s (1991) study. In the case of smolts, I assumed that mortality and straying at sea does not differ between sexes, or at least that female mortality and straying was not higher than the mortality of males. Both methods (before and after spawning) produced similar estimates of the sex ratio, suggesting that male grilse are indeed comparably less abundant than females.

4.1.2 The Influence of an Unequal Sex Ratio

The effective size of a population is dependent on the sex ratio, and is thus affected more heavily by the less abundant sex. Consequently, an unequal sex ratio results in a decrease in the effective population size compared to the total number of breeders (Nunney 1993). Based on the sex ratios in the anadromous runs, it is clear that N_e should be much less than the genetic estimates I found if parr played no reproductive role. Thus it is evident that parr are contributing, but it is difficult to accurately determine the actual number of reproductive parr in the system because of the unknown variance in parr reproductive success amongst parr in this specific system. Furthermore, here I made the idealized assumption that the effective number of each sex was represented by its census number in the anadromous run. However, in the wild, the effective number of each sex is generally lower than its census count (Serbezov *et al.* 2012a); thus my sex ratio-derived, demographic estimates of effective population size are likely upwardly biased. This bias may also be exacerbated by the fact that I used an estimate of 5.2 years for the generation length. The life history table that produced this estimate (not shown)

predicted more anadromous males, and fewer mature parr than observed in the system. Thus, the average age of males is likely lower than the model predicts, and thus the generation length is likely shorter than 5.2 years. If the generation length is shorter than the estimate I used to calculate GN_b, then the GN_b estimates of N_{eSR} are biased upwards. Nevertheless, there was a significant difference between the N_{eSR} estimates and the genetic estimates of N_e, which further demonstrates the magnitude of contribution to N_e by mature male parr.

4.2 Other Factors Influencing N_e

Variance in reproductive success, as well as fluctuations in population size, can also significantly decrease N_e (Nunney 1996; Vucetich *et al.* 1997). In the present study I primarily considered the effect of the sex ratio in the anadromous run, although the influence of fluctuating population size did not appear to significantly decrease N_e relative to N_c (Figure4). Because I treated every anadromous individual as otherwise mating ideally (Poisson variance in reproductive success), I would expect that my estimates of N_b and N_e from the sex ratios are actually overestimates. For example, in a wild population of the ecologically similar brown trout (*Salmo trutta*), variance in reproductive success reduced N_e : N_c to 0.16 - 0.28 (Serbezov *et al.* 2012a). Furthermore, I ignored the presence of repeat spawners (iteroparity), which can decrease N_e by increasing variance in reproductive success, but can also bias the conversions of N_b to N_e. Serbezov *et al.* (2012a), showed that GN_b is greater than N_e when there is iteroparity, further suggesting that my conversions of estimated N_{bSR} to N_{eSR} are overestimates. Despite this, I showed that the genetically derived effective population size estimates are

higher than those expected given the anadromous sex ratios. The genetic estimates operate on signals that include the consequences of all factors that could decrease N_e relative to N_c. Therefore, the fact that I still detected a significant difference between genetic and sex-ratio derived estimates suggests mature parr play a major role in the maintenance of genetic diversity in this system. Had I also included the influence of variance in reproductive success, the difference between genetic and expected estimates would likely have been even greater. Although there is literature on the variance of reproductive success in Atlantic salmon (e.g. Jones and Hutchings 2002; Weir *et al.* 2012), I did not incorporate this data into the analysis. This was because there appear to be several factors that can influence variance in reproductive success, leading to vastly different estimates even between populations of the same species. For example, Belmar-Lucero *et al.* (2012) found a significant difference in the individual variance of reproductive success between two populations of brook trout (*Salvelinus fontinalis*) separated by less than a km, owing to differences in stream characteristics and number and size of fish. Thus, without additional data on the characteristics of NEBT, it may be biologically unrealistic to apply estimates of variance in reproductive success from a different system or from experiments.

Another consideration is that the estimates of N_e based on the temporal method may be underestimates. If the assumed generation length of 5.2 years is indeed an overestimate, then using a shorter generation length in the temporal models would result in more generations having passed between temporal samples, thus leading to higher N_e estimates than those presented. I thus suspect not only that $\widehat{N}_{bSR}$ was overestimated, but also that the temporal genetic estimates of N_e may be underestimates.

4.3 Comparison Among Genetic Estimates

Most of the genetic estimates of N_e were similar to each other (Figure 4). This suggests first that sample sizes are adequate to accurately estimate N_e. Secondly, the fact that most of the discrete generation estimates of N_e are in line with Jorde and Ryman's (1995) cohort model estimate provides further empirical evidence that the assumption of discrete generations is reasonably unbiased when several generations have passed between sampling events. Furthermore, my results provide more evidence that the TempoF$_s$ method (Jorde and Ryman 2007) tends to be less precise than the other methods (Table 3). It should be noted however that the models assuming discrete generations can be upwardly biased due to skewed allele frequencies, which TempoF$_s$ accounts for, likely explaining the lower estimate from this method. However, the cohort model (Jorde and Ryman 1995) used the same F-statistic (F_s) and found a higher estimate than the TempoF$_s$ method, suggesting that failing to take age-structure into account also possibly downwardly biased the discrete methods. Finally, the cohort method itself could be underestimating N_e because it assumes that there is no correlation between reproduction and future survival (Serbezov *et al.* 2012b) when there likely is, since spawning is known to increase mortality in parr (Leyzerovich 1973; Myers 1984) and is also very energetically taxing on adults (Verspoor *et al.* 2007).

Combining estimates of N_e across temporal methods is expected to reduce bias (Waples 2005; Waples and Do 2010), and in this case, the combined estimate was 2.02 - 3.16 times higher than the theoretical estimates from the sex ratios, even though it puts more weight on the smaller estimates. This again confirms that the anadromous males are inadequately accounting for the observed N_e.

Although I did not further consider N_b or N_e estimates from ON_eSAMP (Tallmon *et al.* 2008) or Colony 2 (Jones and Wang 2010) due to sample size correlation issues, the data have still been informative. Firstly, the results provide further evidence that both methods are sensitive to sample size if sample size is less than N_e. Secondly, neither method had reached a plateau at the largest sample size (1994 cohort, S = 79), suggesting that N_b is on average likely higher than the 1994 estimate of N_b (99, 95% CI: 81 - 158 for ON_eSAMP; 54, 95% CI: 36 - 80 for Colony 2), which correspond to N_e estimates of 514 and 280, respectively. Even with insufficient sample size, both methods estimate N_e to be greater than expected from the sex ratios, thus further supporting the results from the temporal methods and LDN_e.

4.4 Implications and Conclusion

Maturity in male parr is driven by a number of factors. For example, maturation in male salmonids can have a genetic component (e.g. Thorpe *et al.* 1998, Heath *et al.* 2002; Garant *et al.* 2003), parr maturity varies inversely with latitude (Valiente *et al.*2005), can be explained by achievement of a threshold size (Myers *et al.* 1986), can be artificially induced (Henry *et al.* 1998), and has been experimentally found to be related to winter temperatures and feeding regimes (Herbinger and Frias 1992). This evidence suggests that maturity is likely an important strategy that plastically responds to environmental conditions. Thus the pervasiveness of mature parr in NEBT is likely because it is favoured given the environmental conditions. Sexually maturing as a parr presents a trade-off however; since parr maturation generally translates into higher mortality early in life (Myers 1984), it follows that high incidences of parr maturation could lead to a

decrease in the abundance of returning anadromous males. The dynamics between system productivity, incidence of mature parr and the number of returning anadromous males is not yet fully understood, but could be crucial for predicting the demographic response to a changing freshwater environment. My results are in agreement with those of Martinez *et al.* (2000) and Saura et al. (2008), but extend the evidence that spawning as mature parr is a viable strategy over multiple generations. The similar results between studies show that parr play a key role in systems on both sides of the Atlantic Ocean.

From a conservation perspective, it is interesting to note that the effective population size of NEBT has not declined despite a decrease in the census population of anadromous adults over the same timespan (Palstra *et al.* 2009; Figure 2), agreeing with the theoretical findings of Hansen *et al.* (2009). Furthermore, the smolt run size and smolt productivity do not appear to have significantly changed (O'Connell *et al.* 2001). Thus, over the period studied, mature male parr appear to have buffered the effective population size despite a lack of mature anadromous males. However, the decline of anadromous females could still present a problem for the long term persistence of this population, especially given the significant decline of the largest (most fecund) adults (Figure 2).

This study shows that mature male parr can buffer the effective population size over multiple generations, compensating (or possibly a cause) for a skewed sex ratio in the anadromous adults. Thus, alternative life history strategies can play a key role in maintaining long term evolutionary potential.

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Appendix A PCR Protocols

Table A1: Multiplex panels, with listed annealing temperatures and typical volumes of reagents for 58 reactions (6 ladders, 1 gel). SSspG7 is run in simplex. Numbers next to the forward primers (700 or 800) denote tags for use in LI-COR IR 4200 and IR 4300 gel analyzers. Volumes used correspond to 10µM primer working solutions. T_m denotes the annealing temperature used for each reaction.

Panel 1			Volume
Tm: 58°C			(µl)
Primer mix	SSsp2210	F-700	2.9
	SSsp2210	R	2.9
	SSsp2215	F-800	2.9
	SSsp2215	R	2.9
		Per rxn vol.	
M.M	2x MM	2.5	145
	Primer mix	0.2	11.6
	dd Water	0.8	46.4
	Q-solution	0.5	29
	Total	4	232
	DNA	1	

Panel 4			Volume
Tm: 58°C			(µl)
Primer mix	SSsp2216	F-800	4.97
	SSsp2216	R	4.97
	SsaA86b	F-700	3.65
	SsaA86b	R	3.65
		Per rxn vol.	
M.M	2x MM	2.5	145
	Primer mix	0.3	17.24
	dd Water	0.7	40.76
	Q-solution	0.5	29
	Total	4	232
	DNA	1	

Panel 5			Volume
Tm: 58°C			(µl)
Primer mix	SsaF43	F-800	2.65
	SsaF43	R	2.65
	SsaD486	F-700	3.48
	SsaD486	R	3.48
		Per rxn vol.	
M.M	2x MM	2.5	145
	Primer mix	0.21	12.26
	dd Water	0.79	45.7
	Q-solution	0.5	29
	Total	4	232
	DNA	1	

Panel 2			Volume
Tm: 58°C			(µl)
Primer mix	Ssa 85	F-700	1.66
	Ssa 85	R	1.66
	Ssa A124	F-800	3.65
	Ssa A124	R	3.65
	Ssa171	F-700	4.62
	Ssa171	R	4.62
		Per rxn vol.	
M.M	2x MM	2.5	145
	Primer mix	0.34	19.86
	dd Water	0.66	38.15
	Q-solution	0.5	29
	Total	4	232
	DNA	1	

Panel 3			Volume
Tm: 57°C			(µl)
Primer mix	Ssa12	F-800	2.82
	Ssa12	R	2.82
	Ssa197	F-800	3.31
	Ssa197	R	3.31
	SSsp3016	F-700	2.98
	SSsp3016	R	2.98
	SSsp1605	F-700	4.14
	SSsp1605	R	4.14
		Per rxn vol.	
M.M	2x MM	2.5	145
	Primer mix	0.46	26.5
	dd Water	0.54	31.5
	Q-solution	0.5	29
	Total	4	232
	DNA	1	

SSspG7			Volume
Tm: 65°C			(µl)
	10x buffer		29
	dNTPs		29
	$MgCl_2$		29
	SSspG7	F-700	2.9
	SSspG7	R	2.9
	Taq pol.		2.9
	Water		136.3
	Total		232

Appendix B Basic Descriptives

Table A2: Basic descriptive statistics for each cohort used 1980-2008. Information includes the number of alleles (A), allelic diversity (A_{div}), allelic richness (A_r), observed and expected heterozygosities (H_o and H_e), and the number of individuals in each cohort that were amplified at each locus.

Locus		1980	1981	1985	1986	1987	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	TOTAL
SSsp2216	A	8	7	4	9	9	7	5	11	9	11	10	9	11	12	11	10	9	13	8	10	8	8	9	7	16
	Adiv	0.781	0.733	0.564	0.659	0.709	0.716	0.659	0.761	0.686	0.729	0.711	0.667	0.771	0.710	0.712	0.652	0.707	0.749	0.684	0.681	0.658	0.640	0.661	0.667	0.694
	Ar	6.776	6.176	4.000	7.199	5.950	6.743	4.270	7.095	5.035	6.469	5.898	6.047	7.094	6.613	5.962	5.187	5.908	6.958	5.603	5.663	4.997	5.600	5.599	5.052	5.981
	Ho	0.706	0.824	0.727	0.650	0.679	0.750	0.583	0.750	0.750	0.734	0.786	0.750	0.744	0.738	0.737	0.630	0.600	0.683	0.650	0.606	0.586	0.596	0.543	0.667	0.686
	He	0.779	0.736	0.571	0.659	0.708	0.717	0.658	0.761	0.687	0.729	0.712	0.668	0.771	0.710	0.712	0.651	0.706	0.749	0.684	0.680	0.656	0.640	0.660	0.667	0.695
	n	17	17	11	20	28	12	24	28	52	79	42	32	39	65	57	46	30	41	40	33	29	47	35	21	845
SsaA86b	A	4	4	4	5	4	4	3	5	5	6	5	4	6	4	5	5	4	5	5	5	6	5	5	4	10
	Adiv	0.272	0.495	0.405	0.463	0.361	0.477	0.259	0.400	0.378	0.439	0.318	0.378	0.410	0.403	0.434	0.406	0.457	0.469	0.332	0.347	0.623	0.490	0.485	0.373	0.411
	Ar	3.412	3.833	4.000	4.053	3.022	3.913	2.440	4.014	3.422	3.813	3.401	3.543	3.691	3.543	3.565	3.735	3.323	3.488	3.537	3.467	4.738	3.358	3.920	3.406	3.624
	Ho	0.294	0.556	0.364	**0.300**	0.393	0.583	0.292	0.429	0.404	0.494	0.333	0.438	0.410	0.415	0.456	0.413	0.500	0.488	0.325	0.303	0.793	0.426	0.457	0.333	0.425
	He	0.273	0.497	0.403	**0.459**	0.362	0.482	0.260	0.401	0.378	0.440	0.318	0.378	0.410	0.403	0.434	0.406	0.458	0.469	0.332	0.346	0.626	0.489	0.484	0.372	0.412
	n	17	18	11	20	28	12	24	28	52	79	42	32	39	65	57	46	30	41	40	33	29	47	35	21	846
SsaD486	A	5	5	6	6	5	5	5	5	6	5	6	5	6	6	5	6	6	6	5	6	5	5	5	5	8
	Adiv	0.794	0.744	0.827	0.821	0.623	0.765	0.782	0.696	0.759	0.752	0.727	0.763	0.764	0.765	0.751	0.722	0.733	0.733	0.746	0.729	0.743	0.710	0.656	0.766	0.745
	Ar	4.960	4.975	6.000	5.515	4.383	4.917	4.913	4.706	5.222	4.836	5.110	4.868	5.066	5.042	4.852	4.947	5.238	4.859	4.873	5.055	4.601	4.762	4.654	4.830	4.938
	Ho	0.706	0.824	0.909	0.700	0.536	0.833	0.750	**0.571**	0.692	0.756	0.762	0.750	0.795	0.862	0.737	0.761	0.800	0.707	0.750	0.758	0.690	0.696	0.556	0.773	0.736
	He	0.791	0.747	0.831	0.818	0.621	0.768	0.781	**0.694**	0.758	0.752	0.727	0.763	0.765	0.766	0.751	0.722	0.734	0.733	0.747	0.730	0.742	0.710	0.655	0.766	0.745
	n	17	17	11	20	28	12	24	28	52	78	42	32	39	65	57	46	30	41	40	33	29	46	36	22	845
SsaF43	A	3	4	5	5	3	2	4	3	4	6	3	5	4	8	5	4	3	4	4	4	5	3	5	5	13
	Adiv	0.221	0.348	0.336	0.351	0.229	0.083	0.323	0.226	0.225	0.284	0.093	0.258	0.213	0.344	0.308	0.267	0.270	0.306	0.209	0.314	0.256	0.402	0.350	0.325	0.273
	Ar	2.765	3.660	5.000	3.637	2.563	1.917	2.908	2.351	2.360	2.529	1.915	3.149	2.643	3.341	3.073	2.449	2.698	3.181	2.617	3.129	3.239	2.660	2.909	3.476	2.863
	Ho	0.235	0.333	0.364	0.350	0.250	0.083	0.375	0.250	0.212	0.278	0.095	0.281	0.231	0.338	0.333	0.261	0.233	**0.220**	0.200	0.355	**0.207**	0.426	0.361	0.273	0.273
	He	0.221	0.348	0.338	0.351	0.229	0.083	0.324	0.227	0.225	0.284	0.093	0.258	0.213	0.344	0.308	0.267	0.269	**0.305**	0.209	0.315	**0.255**	0.403	0.350	0.323	0.273
	n	17	18	11	20	28	12	24	28	52	79	42	32	39	65	57	46	30	41	40	31	29	47	36	22	846
SSspG7	A	6	8	4	5	6	6	7	7	11	9	8	7	7	7	9	7	7	7	8	8	8	8	5	7	15
	Adiv	0.489	0.708	0.409	0.425	0.542	0.545	0.488	0.596	0.429	0.360	0.539	0.447	0.469	0.529	0.566	0.412	0.595	0.310	0.571	0.575	0.480	0.520	0.475	0.469	0.498
	Ar	2.765	3.660	5.000	3.637	2.563	1.917	2.908	2.351	2.360	2.529	1.915	3.149	2.643	3.341	3.073	2.449	2.698	3.181	2.617	3.129	3.239	2.660	2.909	3.476	2.863
	Ho	0.471	0.611	0.273	0.500	0.607	0.667	0.542	0.679	0.423	0.380	0.571	0.469	0.487	0.531	0.544	0.391	0.533	0.268	0.550	0.485	0.429	0.617	0.528	0.455	0.500
	He	0.488	0.705	0.403	0.427	0.543	0.551	0.489	0.597	0.429	0.360	0.539	0.447	0.469	0.529	0.566	0.411	0.594	0.310	0.571	0.573	0.479	0.521	0.476	0.468	0.498
	n	17	18	11	20	28	12	24	28	52	79	42	32	39	64	57	46	30	41	40	33	28	47	36	22	846

Locus		1980	1981	1985	1986	1987	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	Total
SSsp2210	A	4	5	3	4	5	4	6	5	5	5	6	5	6	5	5	5	5	6	5	5	5	5	5	4	7
	Adiv	0.693	0.706	0.673	0.684	0.663	0.674	0.763	0.678	0.733	0.704	0.731	0.718	0.737	0.651	0.753	0.666	0.674	0.714	0.689	0.708	0.696	0.700	0.723	0.646	0.699
	Ar	3.882	4.822	3.000	3.990	4.416	3.917	5.158	4.028	4.297	4.255	4.819	4.545	4.955	4.032	4.723	3.853	4.204	4.674	4.207	4.420	4.292	4.654	4.834	3.879	4.415
	Ho	0.706	0.722	0.727	0.600	0.643	0.583	0.750	0.714	0.808	0.772	0.643	0.656	0.821	0.569	**0.877**	0.622	0.700	0.659	0.750	0.727	0.793	0.702	0.543	0.773	0.703
	He	0.693	0.706	0.675	0.682	0.662	0.670	0.762	0.679	0.734	0.704	0.729	0.717	0.738	0.651	**0.754**	0.666	0.674	0.714	0.690	0.709	0.698	0.700	0.720	0.649	0.699
	n	17	18	11	20	28	12	24	28	52	79	42	32	39	65	57	45	30	41	40	33	29	47	35	22	846
SSsp2215	A	6	6	3	7	5	6	5	6	6	8	7	9	15	15	9	9	6	6	5	6	6	8	6	5	20
	Adiv	0.551	0.598	0.382	0.437	0.556	0.629	0.440	0.509	0.535	0.626	0.552	0.646	0.843	0.749	0.600	0.577	0.509	0.553	0.553	0.560	0.518	0.651	0.606	0.526	0.571
	Ar	4.823	5.026	3.000	5.035	3.570	5.746	4.186	4.291	4.294	5.062	4.237	5.595	9.076	7.676	4.901	5.143	4.145	4.211	3.541	5.039	4.531	5.328	4.171	3.827	5.279
	Ho	**0.471**	0.444	0.455	**0.350**	0.536	0.667	0.478	0.536	0.519	0.646	0.524	0.656	**0.769**	**0.672**	0.561	0.591	0.552	0.550	0.579	0.613	0.621	**0.574**	0.714	0.571	0.569
	He	**0.549**	0.594	0.385	**0.435**	0.556	0.630	0.441	0.510	0.535	0.626	0.552	0.646	**0.842**	**0.749**	0.600	0.577	0.509	0.553	0.553	0.561	0.520	**0.650**	0.608	0.527	0.571
	n	17	18	11	20	28	12	23	28	52	79	42	32	39	64	57	44	29	40	38	31	29	47	35	21	843
SsaA124	A	4	4	3	6	5	3	5	7	5	4	4	5	4	5	5	5	5	7	4	4	4	6	4	5	10
	Adiv	0.594	0.523	0.573	0.628	0.582	0.538	0.586	0.584	0.558	0.540	0.581	0.580	0.561	0.572	0.564	0.603	0.624	0.666	0.551	0.577	0.572	0.564	0.533	0.570	0.576
	Ar	3.529	3.467	3.000	4.454	3.902	2.917	3.629	4.445	3.192	2.964	3.314	3.448	3.271	3.361	3.225	3.689	3.714	4.728	3.271	3.168	3.148	3.691	2.611	3.500	3.468
	Ho	0.706	0.611	**0.909**	0.550	0.750	0.583	0.583	0.643	0.481	0.506	0.619	**0.742**	0.615	0.646	0.571	0.578	0.667	0.610	0.564	**0.375**	0.517	**0.574**	0.500	0.591	0.604
	He	0.597	0.525	**0.589**	0.626	0.585	0.540	0.586	0.585	0.557	0.540	0.581	**0.583**	0.562	0.572	0.564	0.603	0.625	0.665	0.551	**0.574**	0.571	**0.564**	0.533	0.571	0.577
	n	17	18	11	20	28	12	24	28	52	79	42	31	39	65	56	45	30	41	39	32	29	47	36	22	843
Ssa85	A	7	5	5	7	6	6	7	6	9	7	7	6	6	6	7	7	7	6	7	6	7	6	6	8	13
	Adiv	0.625	0.636	0.536	0.697	0.657	0.765	0.605	0.743	0.742	0.665	0.648	0.622	0.675	0.600	0.602	0.629	0.694	0.571	0.638	0.620	0.539	0.621	0.615	0.680	0.643
	Ar	5.786	4.694	5.000	5.562	5.171	5.830	5.397	5.600	6.115	5.319	5.358	5.423	5.329	5.062	5.135	5.207	5.839	4.958	5.397	5.373	4.670	4.986	5.205	6.409	5.297
	Ho	0.706	0.833	0.364	0.700	0.643	0.833	0.667	0.714	0.731	0.633	0.738	0.656	0.703	0.631	0.667	0.644	0.767	0.610	0.600	0.548	0.655	0.596	0.639	0.636	0.663
	He	0.627	0.641	0.528	0.697	0.656	0.768	0.606	0.742	0.742	0.665	0.649	0.623	0.675	0.600	0.602	0.629	0.695	0.572	0.637	0.619	0.541	0.621	0.615	0.679	0.643
	n	17	18	11	20	28	12	24	28	52	79	42	32	37	65	57	45	30	41	40	31	29	47	36	22	843
SSsp1605	A	6	6	7	8	7	6	7	7	6	7	7	7	6	8	7	6	6	6	7	6	7	7	8	6	11
	Adiv	0.770	0.758	0.805	0.808	0.795	0.795	0.807	0.843	0.803	0.802	0.792	0.817	0.807	0.826	0.810	0.791	0.775	0.777	0.794	0.783	0.799	0.807	0.806	0.794	0.799
	Ar	5.725	5.415	7.000	6.450	6.015	5.913	5.829	6.301	5.577	5.815	5.855	6.040	5.563	6.326	5.925	5.501	5.509	5.312	5.934	5.566	5.811	6.169	6.309	5.501	5.889
	Ho	0.765	0.778	0.727	0.850	0.786	0.833	0.750	0.750	0.904	0.747	0.786	0.750	0.821	**0.785**	0.860	0.739	0.767	0.878	0.700	0.758	0.621	0.935	0.861	**0.773**	0.788
	He	0.770	0.759	0.801	0.809	0.795	0.797	0.806	0.842	0.804	0.802	0.792	0.815	0.807	**0.825**	0.811	0.790	0.775	0.779	0.792	0.782	0.796	0.809	0.807	**0.794**	0.798
	n	17	18	11	20	28	12	24	28	52	79	42	32	39	65	57	46	30	41	40	33	29	46	36	22	847
SSsp3016	A	5	5	6	7	7	8	7	8	8	10	7	8	9	9	10	9	8	8	9	8	8	8	8	8	12
	Adiv	0.785	0.775	0.782	0.749	0.750	0.833	0.842	0.786	0.741	0.775	0.798	0.818	0.830	0.775	0.805	0.818	0.789	0.784	0.769	0.804	0.802	0.786	0.742	0.796	0.789
	Ar	4.960	4.932	6.000	5.977	5.954	7.743	6.524	6.281	6.030	5.852	5.902	6.543	6.629	6.088	7.051	6.387	5.918	5.960	6.469	6.276	6.026	5.992	5.930	6.931	6.200
	Ho	0.882	0.667	0.818	**0.650**	0.857	0.833	0.667	0.778	0.731	0.772	0.738	0.781	0.923	0.754	0.754	0.826	0.667	**0.707**	0.775	0.697	0.793	0.783	0.750	0.857	0.769
	He	0.788	0.771	0.784	**0.746**	0.752	0.833	0.839	0.785	0.741	0.775	0.798	0.817	0.831	0.775	0.804	0.818	0.787	**0.783**	0.769	0.802	0.802	0.786	0.742	0.798	0.789
	n	17	18	11	20	28	12	24	27	52	79	42	32	39	65	57	46	30	41	40	33	29	46	36	21	845
Ssa12	A	3	4	5	6	4	3	3	4	4	4	5	4	4	5	4	4	3	4	5	4	5	4	5	5	6
	Adiv	0.542	0.525	0.664	0.662	0.532	0.527	0.531	0.511	0.528	0.525	0.548	0.581	0.530	0.534	0.555	0.572	0.553	0.516	0.587	0.477	0.595	0.556	0.575	0.584	0.555
	Ar	2.647	3.467	5.000	4.821	3.029	2.917	2.458	3.177	2.981	2.897	3.713	3.548	3.025	3.436	3.024	3.239	2.849	2.989	3.763	3.297	3.676	3.239	3.675	3.756	3.253
	Ho	0.471	0.556	0.727	0.550	0.571	0.333	0.458	0.536	0.462	0.570	0.548	**0.469**	0.487	0.569	0.544	0.696	0.700	0.512	0.525	0.469	0.759	0.587	0.639	0.727	0.561
	He	0.540	0.525	0.667	0.659	0.533	0.518	0.529	0.511	0.528	0.525	0.548	**0.579**	0.530	0.535	0.555	0.573	0.555	0.516	0.586	0.477	0.598	0.556	0.576	0.588	0.554
	n	17	18	11	20	28	12	24	28	52	79	42	32	39	65	57	46	30	41	40	32	29	46	36	22	846
Ssa197	A	9	5	6	8	6	5	5	7	9	10	10	6	7	9	9	9	9	9	9	9	6	10	8	7	13
	Adiv	0.658	0.678	0.641	0.632	0.660	0.534	0.703	0.687	0.691	0.739	0.704	0.622	0.713	0.722	0.709	0.671	0.599	0.615	0.714	0.700	0.647	0.720	0.679	0.658	0.671
	Ar	7.034	4.222	6.000	6.509	4.929	4.830	4.560	5.619	5.526	6.440	5.977	4.505	5.143	5.633	5.646	5.820	5.807	5.086	6.164	6.289	5.049	6.479	5.241	5.499	5.615
	Ho	0.688	0.722	0.636	0.500	0.607	0.667	**0.708**	0.643	0.712	0.810	0.762	0.656	0.718	0.754	0.807	0.761	0.567	0.610	0.650	**0.563**	0.586	0.761	0.639	0.773	0.679
	He	0.659	0.679	0.641	0.628	0.659	0.540	**0.703**	0.686	0.691	0.740	0.705	0.623	0.713	0.722	0.710	0.672	0.598	0.615	0.713	**0.698**	0.646	0.721	0.678	0.661	0.671
	n	16	18	11	20	28	12	24	28	52	79	42	32	39	65	57	46	30	41	40	32	29	46	36	22	845

Appendix C ONeSAMP Results

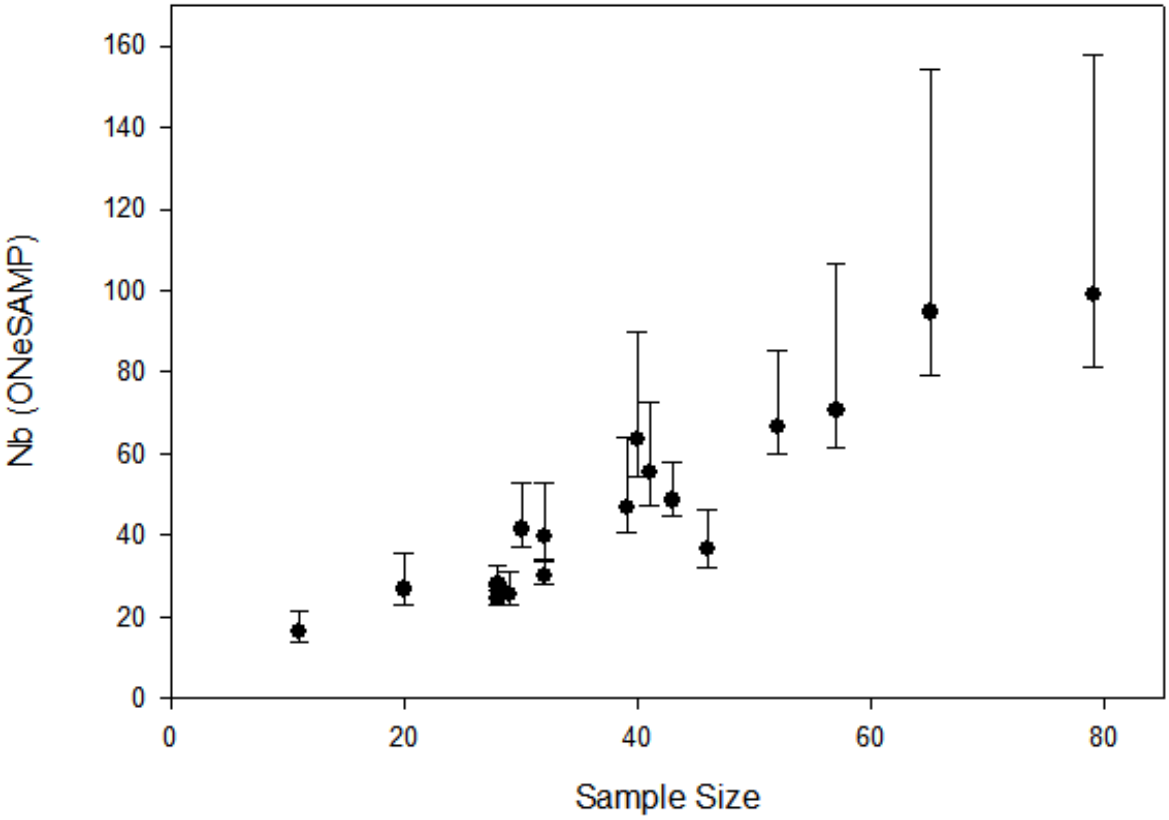

Figure A1: Correlation between sample size and annual estimate of effective number of breeders (N_b) using the ABC method implemented in the program ONeSAMP (Tallmon *et al.* 2008). Plotted are the means and 95% CI. The correlation is significant (adjusted $r^2 = 0.947$, $p < 0.001$).

Appendix D Colony 2 Results

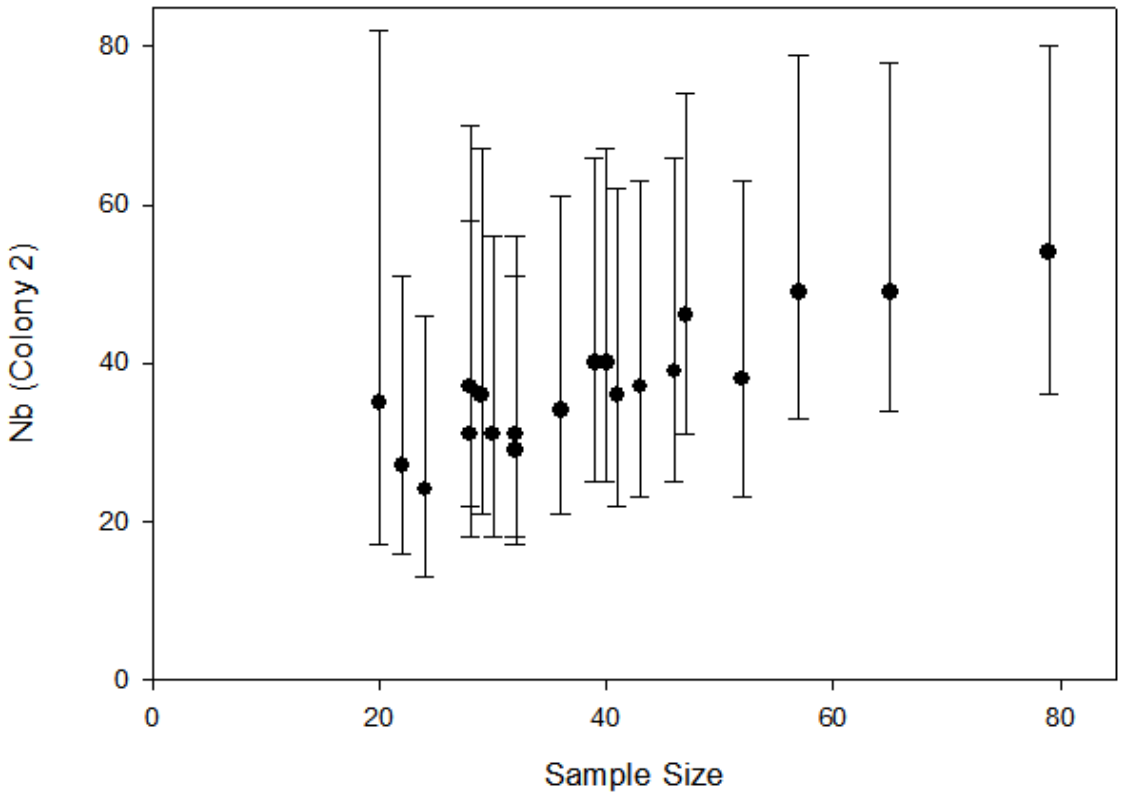

Figure A2: Correlation between sample size and annual estimate of effective number of breeders (N_b) using the sibship method implemented in the program Colony 2 (Wang *et al.* 2009; Jones and Wang 2010). Plotted are the means and 95% CI. The correlation is significant (adjusted $r^2 = 0.746$, $p < 0.001$).

Appendix E Colony 2 Subsampling Results

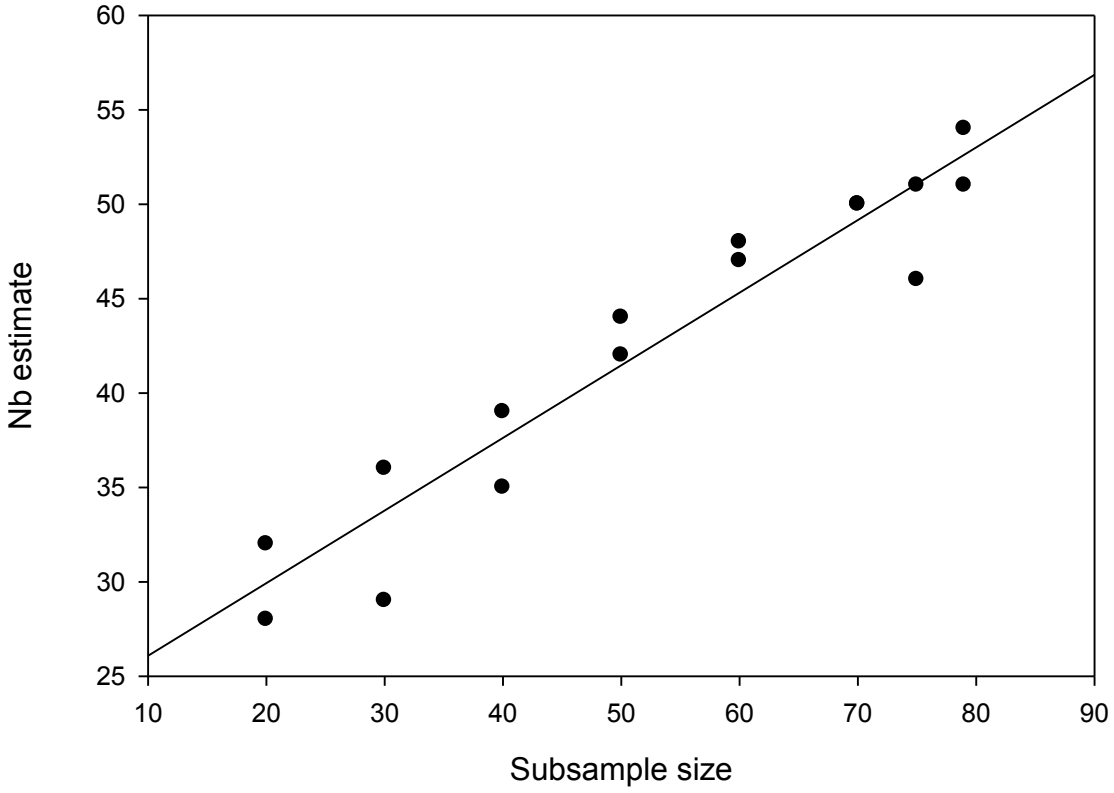

Figure A3: Correlation between subsample size and annual estimate of effective number of breeders (N_b) using the sibship method implemented in the program Colony 2 (Wang *et al.* 2009; Jones and Wang 2010). Values represent random subsamples of the largest cohort (1994; S = 79). The correlation is significant (adjusted $r^2 = 0.907$, $p < 0.001$).