

Age Structure, Changing Demography and Effective Population Size in Atlantic Salmon (*Salmo salar*)

Friso P. Palstra,^{*,1} Michael F. O'Connell[†] and Daniel E. Ruzzante^{*}

^{*}Biology Department, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada and [†]Fisheries and Oceans, St. John's, Newfoundland A1C 5X1, Canada

Manuscript received February 19, 2009

Accepted for publication June 4, 2009

ABSTRACT

Effective population size (N_e) is a central evolutionary concept, but its genetic estimation can be significantly complicated by age structure. Here we investigate N_e in Atlantic salmon (*Salmo salar*) populations that have undergone changes in demography and population dynamics, applying four different genetic estimators. For this purpose we use genetic data (14 microsatellite markers) from archived scale samples collected between 1951 and 2004. Through life table simulations we assess the genetic consequences of life history variation on N_e . Although variation in reproductive contribution by mature parr affects age structure, we find that its effect on N_e estimation may be relatively minor. A comparison of estimator models suggests that even low iteroparity may upwardly bias N_e estimates when ignored (semelparity assumed) and should thus empirically be accounted for. Our results indicate that N_e may have changed over time in relatively small populations, but otherwise remained stable. Our ability to detect changes in N_e in larger populations was, however, likely hindered by sampling limitations. An evaluation of N_e estimates in a demographic context suggests that life history diversity, density-dependent factors, and metapopulation dynamics may all affect the genetic stability of these populations.

THE effective size of a population (N_e) is an evolutionary parameter that can be informative on the strength of stochastic evolutionary processes, the relevance of which relative to deterministic forces has been debated for decades (*e.g.*, LANDE 1988). Stochastic forces include environmental, demographic, and genetic components, the latter two of which are thought to be more prominent at reduced population size, with potentially detrimental consequences for average individual fitness and population persistence (NEWMAN and PILSON 1997; SACCHERI *et al.* 1998; FRANKHAM 2005). The quantification of N_e in conservation programs is thus frequently advocated (*e.g.*, LUIKART and CORNUET 1998; SCHWARTZ *et al.* 2007), although gene flow deserves equal consideration given its countering effects on genetic stochasticity (FRANKHAM *et al.* 2003; PALSTRA and RUZZANTE 2008).

Effective population size is determined mainly by the lifetime reproductive success of individuals in a population (WRIGHT 1938; FELSENSTEIN 1971). Variance in reproductive success, sex ratio, and population size fluctuations can reduce N_e below census population size (FRANKHAM 1995). Given the difficulty in directly estimating N_e through quantification of these demo-

graphic factors (reviewed by CABALLERO 1994), efforts have been directed at inferring N_e indirectly through measurement of its genetic consequences (see LEBERG 2005, WANG 2005, and PALSTRA and RUZZANTE 2008 for reviews). Studies employing this approach have quantified historical levels of genetic diversity and genetic threats to population persistence (*e.g.*, NIELSEN *et al.* 1999b; MILLER and WAITS 2003; JOHNSON *et al.* 2004). N_e has been extensively studied in (commercially important) fish species, due to the common availability of collections of archived samples that facilitate genetic estimation using the temporal method (*e.g.*, HAUSER *et al.* 2002; SHRIMPTON and HEATH 2003; GOMEZ-UCHIDA and BANKS 2006; SAILLANT and GOLD 2006).

Most models relating N_e to a population's genetic behavior make simplifying assumptions regarding population dynamics. Chiefly among these is the assumption of discrete generations, frequently violated in practice given that most natural populations are age structured with overlapping generations. Here, theoretical predictions still apply, provided that population size and age structure are constant (FELSENSTEIN 1971; HILL 1972). Ignored age structure can introduce bias into temporal genetic methods for the estimation of N_e , especially for samples separated by time spans that are short relative to generation interval (JORDE and RYMAN 1995; WAPLES and YOKOTA 2007; PALSTRA and RUZZANTE 2008). Moreover, estimation methods that do account for age structure (*e.g.*, JORDE and RYMAN 1995) still assume this structure to be constant. Population dynamics

Supporting information is available online at <http://www.genetics.org/cgi/content/full/genetics.109.101972/DC1>.

¹Corresponding author: Biology Department, Life Sciences Centre, Dalhousie University, 1355 Oxford St., Halifax, Nova Scotia B3H 4J1, Canada. E-mail: fpalstra@dal.ca

will, however, likely be altered as population size changes, thus making precise quantifications of the genetic consequences of acute population declines difficult (NUNNEY 1993; ENGEN *et al.* 2005; WAPLES and YOKOTA 2007). This problem may be particularly relevant when declines are driven by anthropogenic impacts, such as selective harvesting regimes, that can affect age structure and N_e simultaneously (RYMAN *et al.* 1981; ALLENDORF *et al.* 2008). Demographic changes thus have broad conservation implications, as they can affect a population's sensitivity to environmental stochasticity and years of poor recruitment (WARNER and CHESSON 1985; ELLNER and HAIRSTON 1994; GAGGIOTTI and VETTER 1999). Consequently, although there is an urgent need to elucidate the genetic consequences of population declines, relatively little is understood about the behavior of N_e when population dynamics change (but see ENGEN *et al.* 2005, 2007).

Here we focus on age structure and N_e in Atlantic salmon (*Salmo salar*) river populations in Newfoundland and Labrador. The freshwater habitat in this part of the species' distribution range is relatively pristine (PARRISH *et al.* 1998), yet Atlantic salmon in this area have experienced demographic declines, associated with a commercial marine fishery, characterized by high exploitation rates (40–80% of anadromous runs; DEMPSON *et al.* 2001). A fishery moratorium was declared in 1992, with rivers displaying differential recovery patterns since then (DEMPSON *et al.* 2004b), suggesting a geographically variable impact of deterministic and stochastic factors, possibly including genetics. An evaluation of those genetic consequences thus requires accounting for potential changes in population dynamics as well as in life history. Life history in Atlantic salmon can be highly versatile (FLEMING 1996; HUTCHINGS and JONES 1998; FLEMING and REYNOLDS 2004), as exemplified by the high variation in age-at-maturity displayed among and within populations (HUTCHINGS and JONES 1998), partly reflecting high phenotypic plasticity (HUTCHINGS 2004). This diversity is particularly evident in the reproductive biology of males, which can mature as parr during juvenile freshwater stages (JONES and KING 1952; FLEMING and REYNOLDS 2004) and/or at various ages as anadromous individuals, when returning to spawn in freshwater from ocean migration. Variability in life history strategies is further augmented by iteroparity, which can be viewed as a bet-hedging strategy to deal with environmental uncertainty (*e.g.*, ORZACK and TULJAPURKAR 1989; FLEMING and REYNOLDS 2004). Life history diversity and plasticity may allow salmonid fish populations to alter and optimize their life history under changing demography and population dynamics, potentially acting to stabilize N_e . Reduced variance in individual reproductive success at low breeder abundance (genetic compensation) will achieve similar effects and might be a realistic aspect of salmonid breeding systems (ARDREN and KAPUSCINSKI 2003;

FRASER *et al.* 2007b). Little is currently known about the relationships between life history plasticity, demographic change and N_e , partly due to scarcity of the multivariate data required for these analyses.

Our objective in this article is twofold. First, we use demographic data for rivers in Newfoundland to quantify how life history variation influences age structure in Atlantic salmon and hence N_e and its empirical estimation from genetic data. We find that variation in reproductive contribution by mature parr has a much smaller effect on the estimation of N_e than is often assumed. Second, we use temporal genetic data to estimate N_e and quantify the genetic consequences of demographic changes. We attempt to account for potential sources of bias, associated with (changes in) age structure and life history, by using four different analytical models to estimate N_e : a single-sample estimator using the linkage disequilibrium method (HILL 1981), the temporal model assuming discrete generations (NEI and TAJIMA 1981; WAPLES 1989), and two temporal models for species with overlapping generations (WAPLES 1990a,b; JORDE and RYMAN 1995) that differ principally in assumptions regarding iteroparity. A comparison of results from these different estimators suggests that iteroparity may often warrant analytical consideration, even when it is presumably low. Although sometimes limited by statistical power, a quantification and comparison of temporal changes in N_e among river populations suggests a more prominent impact of demographic changes on N_e in relatively small river populations.

MATERIALS AND METHODS

Life history: Atlantic salmon life history represents the classical example of anadromy. Juveniles (called parr) typically spend their first few years in freshwater. They then undergo physiological changes (known as smoltification) and migrate out to sea (at which point they are called smolts). Anadromous adults, after spending one (one-sea winter or grilse) or several years (multisea winter) feeding in the ocean environment, return to spawn in freshwater, typically in the river of origin. Many die after spawning (semelparity), but some migrate out to sea again to return and breed again in future years (iteroparity). Alternately, males can mature in freshwater (as mature male parr) before undertaking any ocean migration.

Molecular genetic analyses: Atlantic salmon samples ($N = 2758$, scales or fin clips) were collected nonlethally from anadromous adult salmon runs between 1951 and 2004 in nine rivers, distributed throughout Newfoundland and in southern Labrador (Figure 1). Exceptions are samples consisting of smolts (Western Arm Brook, 2004) or postspawning adults returning to the sea (Northeast Brook Trepassey). Scales were kept in dry paper envelopes and fin clips were stored in 95% ethanol. Where possible, we pooled samples from 2–3 consecutive years in an attempt to obtain samples representative of the entire population at a given point in time (*e.g.*, WAPLES 1990a), *i.e.*, to reduce the effect of age structure on measures of temporal genetic variance and provide more accurate estimates of population allele frequencies (see supporting information, Table S1). Our sampling design thus

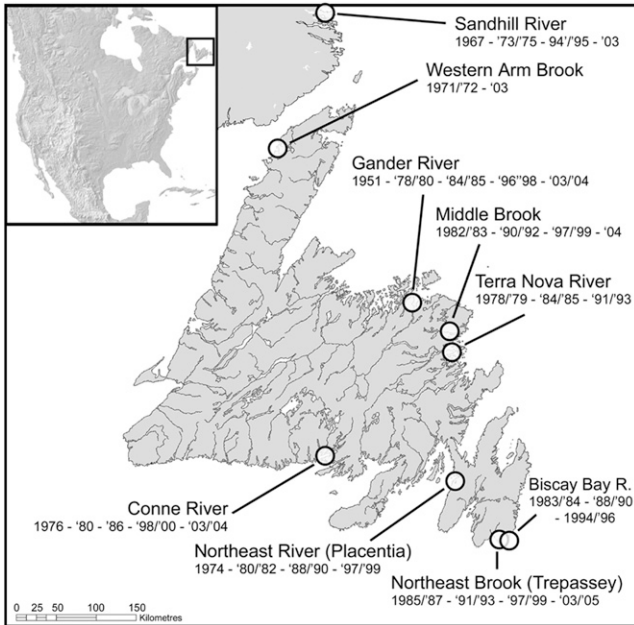


FIGURE 1.—Map of Newfoundland and Labrador, showing study river locations and years sampled. See Table S1 for sample details.

corresponds to plan I (WAPLES 1989), and temporal genetic N_e estimators involving such a design usually require a correction term inversely related to population size ($1/N$; e.g., WAPLES 1989; JORDE and RYMAN 1995), but here we assumed census population sizes were large enough for this correction to be ignored in statistical analyses.

Two different DNA extraction protocols were applied, depending on the age of the samples. For archived scale samples collected prior to 1990, DNA extraction was performed using a phenol-chloroform extraction protocol (TAGGART *et al.* 1992), followed by a concentration step using microconcentrators (Amicon, Danvers, MA) (NIELSEN *et al.* 1999a). For fin-clip samples and post-1990 scale samples, DNA was extracted following a glass milk protocol (ELPHINSTONE *et al.* 2003). Genetic polymorphism was examined in a suite of 14 microsatellite loci specific for Atlantic salmon (see PALSTRA *et al.* 2007 for details), chosen on the basis of product size range (<300 bp to reduce occurrence of large allele dropouts), scoring convenience, and variability for the region of study.

Sample statistics: Basic descriptives for each sample and locus (allele frequencies, number of alleles, and observed and expected heterozygosity) were obtained using FSTAT (version 2.9.3.2; GOUDET 1995). Temporal genetic methods for estimating N_e assume that variance in allele frequency arises solely as a consequence of genetic drift (and sampling). Technical artifacts thus need to be eliminated as a potential source of temporal genetic variance; markers should be selectively neutral and unlinked, whereas sampled individuals should represent random samples from a population. Departures from Hardy–Weinberg equilibrium (HWE) were assessed for each locus and sample by testing for significant departure of F_{is} from neutral expectations as implemented in FSTAT (based on 5600 randomizations). Fisher’s exact test (SOKAL and ROHLF 1991) was used to evaluate overall sample HWE conditions by combining probabilities from individual loci (RYMAN *et al.* 2006). The occurrence of genotyping errors [resulting from technical artifacts (null alleles) or DNA quality (large allele dropouts)], as well as linkage disequilibrium among loci, was assessed as in PALSTRA *et al.* (2007). Where

irregularities were suspected, samples were rescored and amplification procedures (if possible) repeated.

Estimating effective population size: We used four analytical approaches to estimate N_e (denoted \hat{N}_e hereafter), all based on neutral genetic marker data, while accounting for overlapping generations and age structure. These estimators require detailed knowledge of demographic parameters, such as age structure and average generation interval, which we first calculated using life table analyses.

Life table analyses: We performed life table analyses to estimate age-specific survivorship (l_x) and fecundity (b_x), information needed to account for the effects of overlapping generations and age structure. This information is used directly to estimate N_e in the analytical approach of JORDE and RYMAN (1995), but is also essential for other estimation models. Since we used demographic data on anadromous runs as a basis for life table analyses, some complications arise from uncertainties regarding freshwater survival and reproduction. Particularly, male parr maturation will affect (male) freshwater mortality and can bias the age composition of breeders when ignored. We evaluated the consequences of mature male parr reproduction in life table analyses as follows.

We estimated age-specific survival rates (s_i) by combining estimates of survival for all potential life history stages (parr, mature parr, smolts, virgin anadromous fish, and repeat spawners) present in at the previous age ($i - 1$). We ignored survival at the egg-to-fry stages, as the genetic correlations in age-structured populations depend on differences in survival among the reproductive age classes (JORDE and RYMAN 1996). Thus, for nonmature male and female parr, we assumed a constant annual freshwater survival (s_{fw}) to the smolt stage of 0.38 (median value of estimates for Canadian rivers) (SYMONS 1979; EVANS *et al.* 1984; MYERS 1984; CUNJAK and THERRIEN 1998; CUNJAK *et al.* 1998; LOCKE 1998). The majority of smolts in Newfoundland rivers spend one winter in the ocean before returning to spawn (O’CONNELL *et al.* 2006a). We therefore used information on the age composition of virgin one-sea winter adults returning to rivers to spawn (p_{anad}) (N ranging from 658 to 3689 per river) to estimate, for both sexes, percentages of individuals molting (p_{smolt}) at age i as

$$p_{smolt(i)} = p_{anad(i+1)} \left/ \left(p_{anad(i+1)} + \frac{p_{anad(i+2)}}{s_{fw}} + \frac{p_{anad(i+3)}}{s_{fw}^2} \dots \frac{p_{anad(i+n)}}{s_{fw}^{n-1}} \right) \right.$$

We used empirical values for Newfoundland rivers (DEMPSON *et al.* 2004a; O’CONNELL *et al.* 2005) of smolt-to-grilse survival (s_{smolt} , assumed to be 5% when no river-specific data were available) and iteroparity (s_{anad} , calculated from empirical data), which were both assumed to be age independent. For each year i , age-specific survival rates (s_i) were calculated for females as

$$s_{i(F)} = p_{fw(i-1)}s_{fw} + p_{smolt(i-1)}s_{smolt} + p_{anad(i-1)}s_{anad},$$

where $p_{y(i-1)}$ are the relative proportions of each life history stage y surviving from age $i - 1$ to age i .

The equation for annual survival of males requires inclusion of mature parr as an additional life history stage. Estimates of the survival of mature parr relative to nonmaturing fish (s_{rel}) range from 0.29 to 0.80 (MYERS 1984; HUTCHINGS and MYERS 1994; WHALEN and PARRISH 1999) and we assumed $s_{rel} = 0.35$ for subsequent analyses. We further used empirical estimates of mature parr incidence for rivers in this region (DALLEY *et al.* 1983; MYERS *et al.* 1986) as a basis for life table analyses. The biological plausibility of assumptions about mature parr was assessed through simulations (see File S1). Although male parr can mature at various freshwater ages (e.g., L’ABÉE-LUND

1989; HEINIMAA and ERKINARO 2004), maturation generally occurs about halfway through the freshwater stage. In Newfoundland, male parr typically mature at ages 1+ and 2+ (DALLEY *et al.* 1983; MYERS *et al.* 1986; HUTCHINGS 2002). Therefore we estimated the average age of precocious parr maturation by halving the average (female) smolt age and assumed mature parr to be present in two age classes (1+ and 2+ for the majority of rivers). Hence, age-specific survival rates for males were calculated as

$$s_{i(M)} = p_{fw(i-1)} s_{fw} + p_{mat.parr(i-1)} s_{fw} s_{rel} \\ + p_{smolt(i-1)} s_{smolt} + p_{anad(i-1)} s_{anad}$$

Age-specific survivorship values (l_x) were then calculated as $l_x = \prod_{i=1}^x s_i$, for each sex and age class x in a population.

For anadromous fish, age- and sex-specific birth rates (b_x) were estimated, using proportions of adults in the various age classes, weighted by body size (wet weight), as a proxy for relative fecundity (FLEMING *et al.* 1997; MjøLNEROD *et al.* 1998; GARANT *et al.* 2001; but see O'CONNELL *et al.* 2008). We attempted to account for the influence of male parr maturation on age-specific reproductive contribution of males as follows. In the absence of data for the rivers, we used empirical values from observations under natural (JORDAN and YOUNGSON 1992; MARTINEZ *et al.* 2000; GARANT *et al.* 2001; TAGGART *et al.* 2001) and experimental conditions (HUTCHINGS and MYERS 1988; MORAN *et al.* 1996; THOMAZ *et al.* 1997; GARCIA-VAZQUEZ *et al.* 2001; JONES and HUTCHINGS 2001, 2002). These studies suggest that mature male parr account for ~30% of the fertilizations in direct competition with anadromous fish, over a wide range of operational sex ratios. We used this average as a baseline estimate for the reproductive contribution by mature parr, the remainder (70%) being accounted for by the anadromous male year classes. We considered scenarios with larger (Northeast River Placentia, 50%) and smaller (Sandhill River, 25%) parr reproduction, on the basis of empirical estimates of parr incidence and spawning ground surveys (F. PALSTRA, personal observation). These percentages do not apply to b_x but to p_x , since they should reflect the probability that progeny genes are inherited from a male parent of age x (*cf.* FELSENSTEIN 1971). Reproduction among anadromous (male) age classes was allocated on the basis of age composition (weighted by body size). For the (two) mature parr age classes, we assumed a slight reproductive advantage for older parr (60%), since male parr size can affect reproductive success (THOMAZ *et al.* 1997; but see JONES and HUTCHINGS 2002). Using these criteria, we estimated the projected reproductive contribution of each age class as $p_x = l_x b_x$, after adjusting b_x values to generate a constant population size ($\sum l_x b_x = 1$). Age-specific values of l_x , b_x , and p_x were then used to estimate the age structure of breeders, as well as the average generation interval G (in years) following FELSENSTEIN (1971).

Temporal models assuming discrete generations: We applied a discrete temporal model to estimate N_e over the longest time span between two samples available for each river, using three different temporal genetic variance estimators. First, we used the standardized temporal allelic variance measure of POLLAK (1983) [denoted F_k and $N_{e(k)}$]. Second, we applied the unbiased, but possibly less precise measure of JORDE and RYMAN (2007) [denoted F_s and $N_{e(s)}$]. Finally, we employed the pseudo-maximum-likelihood approach of WANG (2001), assuming $N_e = 25,000$ as an upper limit. This latter method was also applied to account for gene flow, which should not *a priori* be ignored, given Atlantic salmon life history (*e.g.*, STABELL 1984; JONSSON *et al.* 2003). We therefore evaluated the impact of gene flow on effective population size by applying WANG and WHITLOCK's (2003) model incorporated in MLNE, which

jointly estimates N_e and gene flow (\hat{m}). For these latter analyses we considered all other sampled rivers in regional proximity as pooled source populations.

N_e/N ratios were calculated whenever sufficient demographic data (O'CONNELL *et al.* 2006b) were available and corresponding census sizes (N) were calculated as follows. First, age information of anadromous adults was used to estimate proportional reproductive contributions of breeders (X_i) for each cohort i in the time period to which the genetic estimates of N_e apply (WAPLES 2005). X_i values were then used to calculate census size following RYMAN and LAIKRE (1991) and intergenerational census size N (see WAPLES 2002).

Pacific salmon model assuming semelparity: Second, we applied a model developed specifically for semelparous Pacific salmonids (WAPLES 1990a,b). Under the Pacific salmon model, the signal of genetic drift (F_b , POLLAK 1983) between cohorts inversely reflects the effective annual number of breeders (N_b) in given breeding seasons. We incorporated the effects of age structure into estimation of N_e by calculating b (the slope of the regression of $2F_k$ on $1/N_b$) following TAJIMA (1992), on the basis of the age distribution of projected reproductive output (p_x values). Given uncertainties regarding what precisely is estimated when using a sample of adults derived from various cohorts (plan I, WAPLES 2005), we reconstructed age cohorts, on the basis of age determined from scale readings. Cohort samples (with $S > 10$) were then used to calculate year-specific N_b estimates using the software SALMONNb (WAPLES *et al.* 2007). Corresponding N_b/N ratios were calculated whenever annual anadromous run sizes were known. Additionally, we inferred effective population size from $N_e = g\bar{N}_b$, where \bar{N}_b is the harmonic mean of the annual N_b estimates as calculated by SALMONNb.

Cohort model for iteroparous species: Third, we applied the cohort model of JORDE and RYMAN (1995) for iteroparous species with variable age at maturity. This model assumes populations with a constant age structure, where N_e fluctuates around a mean value. For each river, we estimated cohort effective population size ($N_{e(J\&R)}$), by calculating the average standardized temporal allele frequency variance (F_b , POLLAK 1983) based on consecutive cohorts (with harmonic mean sample size $\hat{S} > 10$) as

$$N_{e(J\&R)} = \frac{C}{G^2(F - 1/\hat{S})},$$

which includes generation interval (G) and correction factor C . This C term is independent of population size and allele frequency, determined exclusively by l_x and b_x values, thus correcting for genetic covariance among cohorts attributable to age structure (JORDE and RYMAN 1995). Given uncertainty about reproductive contributions by mature parr, we used simulations to assess the sensitivity of C and G to variance in male parr maturation and reproductive contribution. On the basis of simulation results, we calculated C and G as the average of sex-specific values. A similar approach was also used to estimate N_e before and after the commercial fishery closure in 1992, as this closure may have altered population dynamics. For this purpose, life tables were adjusted on the basis of demographic data pertinent to each time period. N_e/N ratios were estimated on the basis of the harmonic mean of corresponding annual counts of anadromous fish (WAPLES 2002, 2005).

Linkage-disequilibrium method: We applied the linkage-disequilibrium method to neutral genetic data derived from single temporal samples (HILL 1981), using the program LDNe (WAPLES and Do 2008). Briefly, this method estimates the effective number of parents contributing to a sample, assuming no immigration occurred. Estimates for samples

TABLE 1

Temporal estimates of N_e assuming discrete generations, using two temporal samples (with harmonic sample size \hat{S}) separated by time t (measured in generation intervals)

River	Period	G (yr)	t	\hat{S}	\bar{F}_k	$\hat{N}_{e(k)}$	95% C.I.	\bar{F}_s	$\hat{N}_{e(s)}$	95% C.I.
Biscay Bay River	1983–1994	4.9	2.4	72	0.0159	629	(213–∞)	0.0177	331	(119–∞)
Conne River	1976–2003	4.8	5.7	57	0.0188	2547	(476–∞)	0.0177	∞	(558–∞)
Gander River	1951–2003	5.4	10.2	87	0.0141	1933	(880–18,394)	0.0138	2097	(828–∞)
	1951–1978		5.4	72	0.0203	417	(243–891)	0.0224	306	(157–6,405)
	1978–2003		4.8	80	0.0144	1305	(468–∞)	0.0152	934	(324–∞)
Middle Brook	1982–2004	5.2	3.9	84	0.0143	836	(336–∞)	0.0158	529	(208–∞)
Northeast Brook	1985–2003	5.2	3.3	52	0.0238	376	(133–∞)	0.0245	336	(174–∞)
Northeast River	1974–1997	4.5	5.3	61	0.0187	1197	(356–∞)	0.0188	1204	(279–∞)
Sandhill River	1967–2003	6.7	5.7	85	0.0152	825	(408–3,399)	0.0148	783	(321–∞)
	1967–1994		4.3	73	0.0172	628	(289–4,704)	0.0173	585	(267–∞)
	1973–2003		4.6	84	0.0147	836	(379–8,286)	0.0155	520	(235–∞)
Terra Nova River	1978–1991	5.1	2.7	76	0.0142	1371	(328–∞)	0.0142	1531	(368–∞)
Western Arm Brook	1971–2004	5.4	6.3	60	0.0185	1657	(498–∞)	0.0202	781	(295–∞)

Given are point estimates plus 95% confidence intervals using the moment-based estimators of POLLAK (1983) [\bar{F}_k and $\hat{N}_{e(k)}$] and JORDE and RYMAN (2007) [\bar{F}_s and $\hat{N}_{e(s)}$].

derived from a single cohort thus reflect N_b , whereas samples of adults of mixed ages (plan I) are intermediate between N_b and N_e (WAPLES 2005). Here, we used individual cohort samples to estimate the effective number of breeders in any given year (N_b), as well as temporal samples of consecutive years of adults pooled to estimate N_e .

Changes in demographic factors underlying N_e : We used genetic estimates of N_b (from the Pacific salmon model, referring to the number of breeders in a season) to explore the scope for temporal changes in the demographic and reproductive parameters of a population, as indirect genetic estimates can be expected to be inclusive of all the demographic properties affecting effective size. Using river-specific demographic data, the seasonal inbreeding effective female size (N_{bf}) was approximated following LANDE and BARROWCLOUGH (1988), where the average (k) and variance (σ_k^2) of progeny size for number of adult females (N) is used to estimate

$$\hat{N}_{bf} = (N\bar{k} - 1) / (\bar{k} + (\sigma_k^2 / \bar{k}) - 1).$$

Here, progeny size was approximated by a correlation between wet weight and the number of eggs (O'CONNELL *et al.* 2008). The inbreeding effective number of males (N_{bm}) in a given breeding season was then estimated as

$$N_{bm} = \frac{N_b N_{bf}}{(4N_{bf} - N_b)}$$

(WRIGHT 1938). A comparison of the relative magnitudes of N_{bm} and N_{bf} can then provide some indications of changes in reproductive biology.

RESULTS

Genetic marker characteristics: DNA was successfully extracted from 2514 of 2758 samples (91.4%). Genetic analyses on these samples yielded consistently high amplification success, ranging from 98.5% (*SSsp1605*) to 99.8% (*SSsp2215*). Locus gene diversity for these samples was high, varying from 0.64 (*Ssa12*) to 0.93 (*SSsp2216*). Gene diversity per sample ranged from

62.0% (1997, Northeast Brook) to 83% (1951, Gander; 1991, Terra Nova), with corresponding average number of alleles ranging from 7.1 (1997, Northeast Brook) to 14.8 (1991, Terra Nova). No consistent patterns of loss of genetic diversity were observed (in gene diversity or allelic richness) in any of the temporal comparisons, including that spanning over 5 decades (the longest time span available in the present study, Gander 1951–2003). Overall, 329 alleles were available for subsequent analyses, ranging from 85 (1997, Northeast Brook) to 208 (1991, Terra Nova) independent alleles per river sample (see Table S1).

Fifty-one of 1064 locus-by-sample comparisons were out of HWE, a result expected by chance alone (4.8% of comparisons significant at $\alpha = 0.05$, 0.9% of comparisons significant at $\alpha = 0.01$). None of these deviations were consistent across loci or samples. MICRO-CHECKER analyses suggested that 7 of these could be attributed to a lack of heterozygotes, possibly indicating the presence of null alleles. Only one locus (*SsaF43*) in one river (Conne) showed such signatures in multiple temporal replicates and was hence removed from further analyses involving this river. As previously shown (PALSTRA *et al.* 2007), these loci likely are unlinked and do not display strong signatures of selective sweeps in tests for selective neutrality.

Genetic estimates of effective population size: Life table simulations (see File S1 for details) suggested $s_{rel} = 0.35$ as a reasonable assumption for mature parr survival in the majority of life table analyses, along with male parr maturation rates that result in anadromous age structures compatible with empirical observations. See below for an evaluation of the genetic consequences of variation in parr maturation incidence on \hat{N}_e in the cohort model. Estimates of generation interval (G) from life table analyses (Table 1) ranged from 4.5 years

TABLE 2

Temporal estimates of N_e assuming discrete generations, considering closed ($\hat{N}_{e(\text{closed})}$) and open populations ($\hat{N}_{e(\text{open})}$), using WANG and WHITLOCK’S (2003) approach

River	Period	t	$\hat{N}_{e(\text{closed})}$	95% C.I.	$\hat{N}_{e(\text{open})}$	95% C.I.	\hat{m}	95% C.I.
Biscay Bay River	1983–1994	2.4	756	(314–∞)	404	(220–1042)	0.0135	(0.0098–0.0256)
Conne River ^a	1976–2003	5.7	1396	(524–∞)	—	—	—	—
Gander River	1951–2003	10.3	1262	(754–3531)	1038	(531–2958)	0.0200	(0.0070–0.0411)
	1951–1978	5.4	1394	(887–2627)	557	(292–1731)	0.0251	(0.0075–0.0495)
	1978–2003	4.8	5230	(1457–∞)	1026	(397–7670)	0.0169	(0.0014–0.0451)
Middle Brook	1982–2003	3.9	759	(369–∞)	730	(306–4012)	0.0126	(0.0008–0.0326)
Northeast Brook	1985–2003	3.3	493	(235–3599)	∞	(463–∞)	0.0002	(0–0.0003)
Northeast River	1974–1997	5.3	1990	(521–∞)	∞	(8083–∞)	0.0001	(0–0.0002)
Sandhill River	1967–2003	5.7	1334	(619–∞)	514	(316–1222)	0.0144	(0.0060–0.0246)
	1973–2003	4.6	702	(393–2023)	452	(244–871)	0.0164	(0.009–0.0331)
	1967–1994	4.3	1033	(438–∞)	364	(225–860)	0.0182	(0.007–0.0315)
Terra Nova River	1978–1994	2.7	∞	(673–∞)	389	(137–∞)	0.1230	(0.0083–0.4728)
Western Arm Brook	1971–2004	6.3	1329	(548–∞)	683	(327–2219)	0.0171	(0.0024–0.0252)

Given for each river are estimates of effective population sizes and incoming gene flow (\hat{m}) plus their confidence intervals, for two samples separated by time t (in generation intervals). Estimates of infinity indicate $\hat{N}_e > 2 \times 10^5$.

^a $\hat{N}_{e(\text{open})}$ was not calculated for Conne River as no putative source populations were deemed to be sampled.

[Northeast River (Placentia)] to 6.7 years (Sandhill River) and were subsequently used to scale measures of temporal genetic variance to \hat{N}_e .

Table 1 shows genetic estimates of N_e for nine rivers in Newfoundland and Labrador, assuming closed populations, characterized by discrete generations. Although these estimates display variation among methods in magnitude, patterns of relative magnitude remain fairly constant across rivers. $\hat{N}_{e(k)}$ ranged from 376 (Northeast Brook) to 2547 (Conne River), whereas $\hat{N}_{e(s)}$ ranged from 331 (Biscay Bay River) to infinity (Conne River). These results confirm that F_s may be less precise than F_b , as confidence intervals associated with $\hat{N}_{e(s)}$ were often wider (cf. JORDE and RYMAN 2007). The likelihood method of WANG (2001) gave estimates ranging from

490 (Northeast Brook) to infinity (Terra Nova River) (Table 2). For all three estimator models, corresponding confidence intervals often, but not always, included infinity. Given the relative consistency, in particular for the smaller rivers, we conclude that our results may not be strongly biased by the estimator of temporal genetic variance applied.

Considering gene flow in the likelihood method (WANG and WHITLOCK 2003), results changed in two distinct ways (Table 2). For the majority of rivers, empirical N_e estimates are reduced, with Terra Nova showing the most dramatic reduction (from ∞ to 389), suggesting the sustained influence of migration from a large source population, which masked a signal of genetic drift when ignored. Other rivers, such as Northeast

TABLE 3

Comparison of genetic N_e estimates using three different analytical models (all assuming closed populations) along with estimates of N_e for census population size N

River	Iteroparity	Discrete generations					Cohort model				Pacific salmon model				
		\hat{S}	$\hat{N}_{e(\text{discrete})}$	95% C.I.	N	\hat{N}_e/N	n	\hat{S}	$\hat{N}_{e(\text{J\&R})}$	95% C.I.	\hat{N}_e/N	n	\hat{S}	\hat{N}_b	$\hat{N}_{e(\text{Salmon})}$
Biscay Bay River	0.012	72	629	(213–∞)	3,121	0.20	7	19	1,734	(274–∞)	0.56	10	19	602	2,915
Conne River	0.053	57	2,547	(476–∞)	12,573	0.20	4	27	646	(256–∞)	0.05	9	24	167	813
Gander River	0.095	87	1,933	(880–18,394)	—	—	8	24	1,145	(194–∞)	—	10	22	759	4,117
Middle Brook	0.065	84	836	(336–∞)	2,300	0.36	7	23	429	(197–∞)	0.19	9	22	684	3,527
Northeast River	0.109	52	1,197	(356–∞)	1,262	0.95	7	23	∞	(3329–∞)	—	11	21	∞	∞
Northeast Brook	0.063	61	376	(133–∞)	400	0.94	7	19	283	(117–∞)	0.71	11	18	172	895
Sandhill River	0.095	84	825	(408–3,399)	21,971	0.04	7	24	582	(201–∞)	0.03	12	22	465	3,115
Terra Nova River	0.029	76	1,371	(328–∞)	5,243	0.26	6	21	2,970	(348–∞)	0.57	9	20	11,927	61,053

$\hat{N}_{e(\text{discrete})}$ reflects discrete generations, $\hat{N}_{e(\text{Salmon})}$ is the harmonic mean of \hat{N}_b estimates derived from SALMONNb multiplied by G , and $\hat{N}_{e(\text{J\&R})}$ estimates are derived from the model of JORDE and RYMAN (1995), on the basis of harmonic mean sample sizes \hat{S} . n refers to the number of consecutive cohort comparisons (cohort model) or to the total number of individual cohorts (Pacific salmon model). Demographic estimates of anadromous iteroparity incidence are also provided.

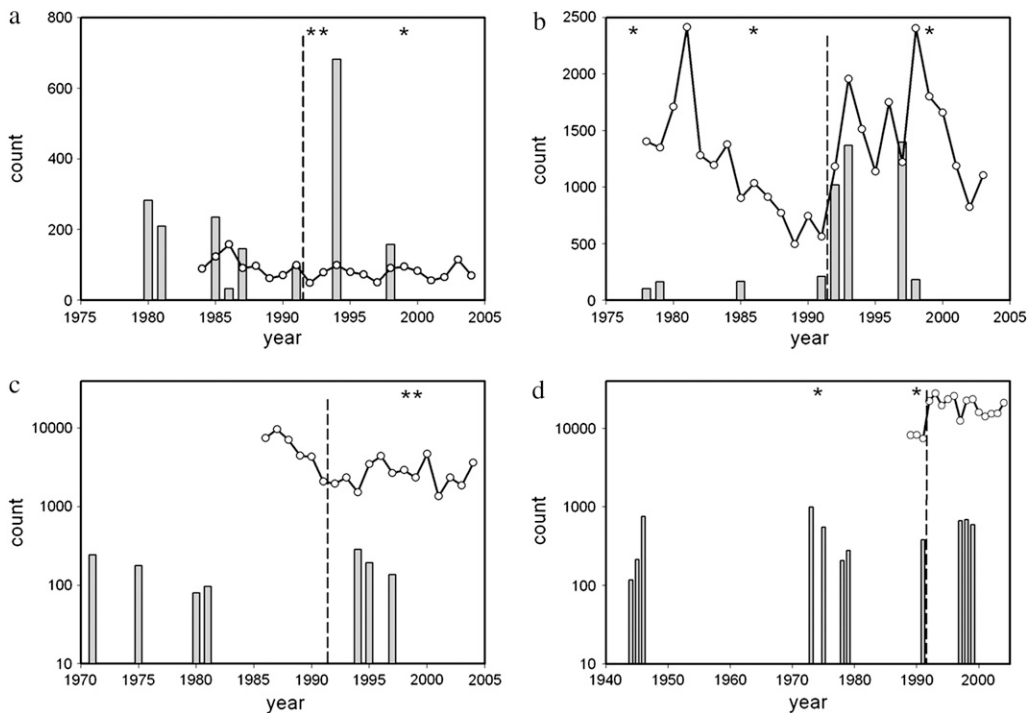


FIGURE 2.—Annual trends in anadromous run sizes (open circles, solid lines) and N_b estimates (shaded bars) for four Newfoundland rivers, (a) Northeast Brook, (b) Middle Brook, (c) Conne River, and (d) Gander River, in relation to the marine fishery closure of 1992 (dashed vertical line). Note the logarithmic scale for count in Conne River and Gander River. Annual N_b estimates were obtained with SALMONNb (WAPLES *et al.* 2007). Asterisks refer to estimates of infinity.

Brook (Trepassey) or Northeast River (Placentia) show the opposite pattern, where allowing for gene flow in the model results in an increase in N_e estimates, with very low estimates of gene flow. Such results suggest the presence of sporadic and intermittent gene flow among those rivers (see WANG and WHITLOCK 2003). In general, N_e estimates that account for gene flow appear to be smaller and (thus) more precise (narrower confidence intervals). These discrete generation N_e estimates apply to relatively long time spans (2.4–10.3 generations), over which bias due to age structure may be reduced and therefore be suitable for comparisons with N_e estimates derived from the Pacific salmon and cohort models.

The Pacific salmon model yielded N_e estimates that are large relative to estimates derived from the discrete generation model, although qualitative patterns among rivers remain mostly consistent (Table 3). Genetic estimates of the annual number of breeders (N_b) range from 172 [Northeast Brook (Trepassey)] to ∞ (Western Arm Brook). Transformation of N_b to N_e by multiplication with generation interval yields estimates ranging from 813 (Conne) to 61,053 (Terra Nova River). Year-specific N_b estimates (Figure 2) show considerable annual and river-by-river variation. Temporal trends in N_b in two comparatively small rivers [Northeast Brook (Trepassey), Figure 2a; Middle Brook, Figure 2b] suggest increases in N_b estimates after the fishery closure in 1992. These temporal trends are less apparent in larger rivers such as Conne and Gander (Figure 2, c and d), although statistical power to infer changes in these two larger rivers may have been limited (see DISCUSSION).

We employed JORDE and RYMAN'S (1995) method to estimate N_e while accounting for age structure. First, we used simulations to evaluate the consequences of varying parr maturation and reproductive contribution for model parameters C and G , *i.e.*, for the strength of genetic fluctuations for a given survival and reproduction schedule. Simulations suggest both parameters are much more sensitive to variation in reproductive contribution of mature male parr than to variation in maturation incidence. Increased male parr reproduction tends to increase the genetic covariance between cohorts (reducing C) and reduce generation interval G . However, it is the C/G ratio that determines the genetic consequences for N_e ; when considering C and G jointly (Figure 3), the effects of mature parr on N_e estimation appear limited. Mature parr reproductive contribution has a weak, buffering effect on fluctuations, with the exception of increases at very high levels of parr reproductive contribution (>90%). Although biologically plausible, these latter increases may mainly reflect a modeling artifact, related to assumptions about the age structure and reproductive contribution of the mature parr age classes. Second, the genetic consequences of age structure vary among rivers (Figure 3), reflecting differences in the river-specific age composition of adults, but the mildly buffering effect of mature parr reproduction remains fairly constant. Summarizing, these simulation results suggest that the assumption of 30% reproductive contribution by mature parr appears reasonable. Unless mature male parr reproductive success is very low (<10%) or very high (>90%), subsequent genetic N_e estimates derived from

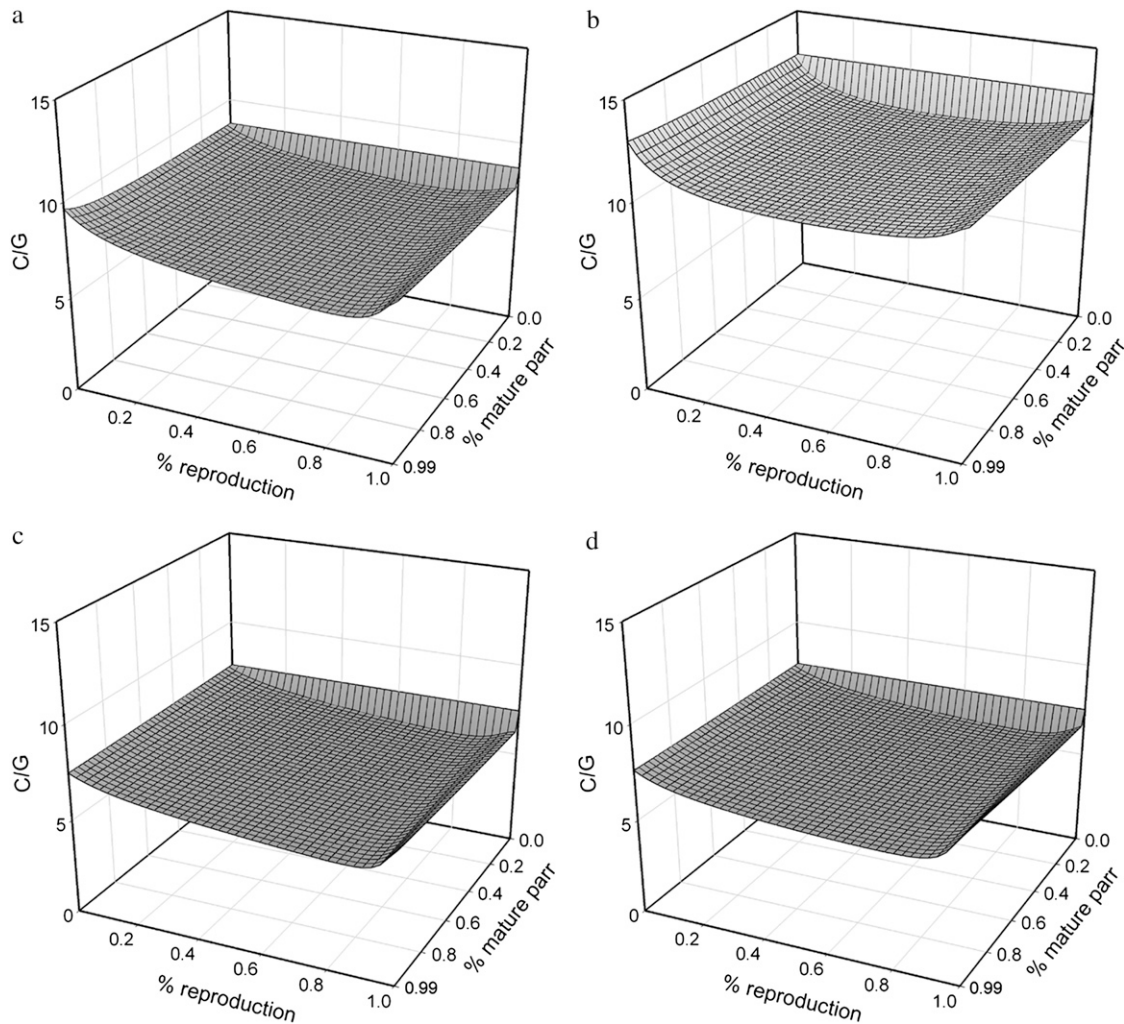


FIGURE 3.—Genetic consequences of parr maturation (% mature parr) and reproductive contribution by mature male parr (% reproduction) for the estimation of N_e from the magnitude of genetic fluctuations among consecutive cohorts, expressed as C/G , calculated following JORDE and RYMAN (1995). Results are shown for four representative rivers: (a) Northeast River, (b) Conne River, (c) Gander River, and (d) Sandhill River.

the cohort model can thus be expected to be fairly robust to variation in mature male parr reproductive contribution.

The cohort model yielded empirical \hat{N}_e values (Table 3) that are qualitatively consistent with the trends among rivers derived from the other genetic estimators. $N_{e(J\&R)}$ estimates are somewhat smaller, but usually closer to estimates from the discrete generation model, than those derived from the Pacific salmon model. The discrete model yielded smaller \hat{N}_e than the cohort model in Northeast River and Biscay Bay, which for the latter may have been caused by the comparatively short time span between temporal samples (2.4 generations). Conversely, for Conne River the discrete model yielded higher \hat{N}_e values than the other models. These results may suggest that the Pacific salmon model generally does not appear to be a good predictor of N_e for species with low iteroparity such as Atlantic salmon. Alternately, iteroparity in Atlantic salmon may be higher

than suggested by the incidence of repeat spawning in anadromous fish.

Changes in effective population size over time:

Temporal changes in effective sizes were evaluated in two different ways. First, we used single sample estimates based on linkage disequilibrium (Table 4). We find that linkage disequilibrium (LD)- \hat{N}_e can fluctuate considerably, with some indications of increases after 1992 in Middle Brook and Northeast Brook. Furthermore, this method frequently yielded N_e estimates of infinity for larger rivers such as Gander River, suggesting limitations in statistical power to infer N_e there. However, results from these analyses are difficult to interpret, as they are derived from plan I samples, reflecting a value anywhere between N_b and N_e (WAPLES 2005). Therefore, next we performed a more detailed comparison of N_b estimates on the basis of cohort samples, limited to two smaller rivers where sampling may have been more appropriate considering census sizes (Table 5). Again, these results

TABLE 4
Temporal trends in genetic N_e estimates

River	Linkage disequilibrium (LD)				Cohort model					
	Yr	\hat{S}	LD- \hat{N}_e	95% C.I.	G	C	n	\hat{S}	$\hat{N}_{e(J\&R)}$	95% C.I.
Biscay Bay	1983	64	567	(330–1,842)						
	1988	73	467	(307–933)						
	1994	81	∞	(1,203– ∞)						
Conne	1976	40	488	(205– ∞)						
	1980	45	345	(186–1,761)	4.8	53.8	1	22	804	(281– ∞)
	1986	49	3,342	(446– ∞)						
	1998	67	2,729	(582– ∞)						
	2003	96	2,008	(728– ∞)	4.8	60.9	3	29	791	(305– ∞)
Gander	1951	76	2,774	(786– ∞)						
	1978	67	1,300	(522– ∞)	5.4	33.8	6	22	1,766	(311– ∞)
	1984	76	∞	(1,409– ∞)						
	1996	68	∞	(986– ∞)	5.4	36.5	2	31	1,270	(389– ∞)
	2003	100	∞	(1,838– ∞)						
Middle Brook	1982	70	417	(269–879)	5.2	41.2	4	19	331	(147– ∞)
	1990	50	237	(159–450)						
	1997	81	498	(322–1,041)	5.1	41.7	3	27	1,616	(334– ∞)
	2004	101	318	(248–435)						
Northeast River	1974	50	∞	(1,141– ∞)						
	1980	74	400	(243–1,017)						
	1988	81	46	(42–51)						
	1997	77	149	(121–192)						
Northeast Brook	1985	50	42	(35–51)	5.1	37.9	4	17	251	(96– ∞)
	1991	65	43	(38–50)						
	1997	65	143	(101–227)	5.2	39.2	3	23	438	(142– ∞)
	2003	52	222	(127–712)						
Sandhill	1967	63	2,418	(571– ∞)	6.7	44.8	5	20	1,215	(191– ∞)
	1973	64	3,784	(688– ∞)						
	1994	85	∞	(1,855– ∞)	6.7	44.8	2	35	1,050	(390– ∞)
	2003	127	1,588	(837–11,712)						
Terra Nova	1978	71	∞	(4,464– ∞)						
	1984	74	1,020	(503–90,655)						
	1991	80	622	(402–1,317)						
Western Arm Brook	1971	42	∞	(477– ∞)						
	2004	100	∞	(1,702– ∞)						

LD- \hat{N}_e refers to estimates based on single samples using the program LDNe (WAPLES and DO 2008) and $\hat{N}_{e(J\&R)}$ reflects estimates for cohorts grouped before and after the marine fisheries closure (1992). $\hat{N}_{e(J\&R)}$ estimates are based on n cohort comparisons (and period-specific parameters G and C).

show that annual N_b estimates can fluctuate widely (*e.g.*, Middle Brook). Nevertheless, N_b estimates for Northeast Brook appear to have somewhat increased after the closure of the marine fishery (1992). In both rivers, \hat{N}_b are accompanied by wide confidence intervals.

We repeated the cohort model on cohorts grouped before and after 1992. The closure of the marine fishery in 1992 may have influenced the genetic behavior of rivers differently, with a possible relation to river size (Table 4). First, the fishery closure had little impact on life table parameters that relate age structure to N_e within rivers, suggesting that population dynamics may not have been strongly altered after 1992. However, we document small increases in C after 1992, although assumptions about mature parr reproduction were kept constant in life table analyses. Estimates derived from the cohort model suggest that N_e may have increased in

smaller census size rivers (Northeast Brook, Middle Brook), but possibly not in larger ones (Gander, Conne, Sandhill). Again, these conclusions have to be qualified by the low precision of N_e estimates: confidence intervals often include infinity. Thus, a lack of observed changes in N_e in larger rivers may have been caused by limited statistical power (sample size limitations) for detecting such changes.

Changes in demographic factors underlying N_e : Finally, an exploratory analysis of reproductive factors underlying N_b (Table 6) suggests that some temporal changes may have occurred in breeding ecology. Due to the limited availability of demographic data, these calculations were performed only for two rivers. As female fecundity was the only factor considered, N_{bf} estimates are likely biased upward; subsequent N_{bm} estimates will be biased downward and we suggest some

TABLE 5

Genetic estimates of the annual number of breeders ($LD-\hat{N}_b$) based on the linkage disequilibrium method, for samples with harmonic mean sample size (\hat{S}) as inferred with the program LDNe (WAPLES and Do 2008)

River	Yr	N	\hat{S}	$LD-\hat{N}_b$	95% C.I.	\hat{N}_b/N
Middle Brook	1977	—	12	∞	(54– ∞)	—
	1978	1403	24	∞	(203– ∞)	—
	1979	1350	24	289	(104– ∞)	0.21
	1985	904	13	437	(58– ∞)	0.48
	1986	1036	11	792	(49– ∞)	0.76
	1991	562	25	200	(94– ∞)	0.36
	1992	1182	26	457	(144– ∞)	0.39
	1993	1959	15	∞	(109– ∞)	—
	1997	1221	22	∞	(236– ∞)	—
	1998	2405	48	190	(131–329)	0.08
1999	1802	25	410	(128– ∞)	0.23	
Northeast Brook	1980	—	16	65	(28– ∞)	—
	1981	—	19	190	(48– ∞)	—
	1985	124	11	35	(15– ∞)	0.28
	1986	158	20	13	(10–17)	0.08
	1987	91	28	208	(76– ∞)	2.28
	1991	99	14	43	(19– ∞)	0.43
	1992	49	14	∞	(49– ∞)	—
	1993	79	17	773	(53– ∞)	9.78
	1994	99	13	939	(36– ∞)	9.49
	1998	91	41	196	(100–1494)	2.15
1999	95	31	435	(118– ∞)	4.58	

\hat{N}_b/N ratios are based on the size of annual anadromous runs (N).

caution in interpreting results. Demographic estimates of the effective number of females (N_{bf}) remained relatively stable over time; hence changes in genetic estimates of N_b may be mainly attributable to fluctuations in N_{bm} . Analyses suggest N_{bm} estimates may have increased after 1992, both absolutely and relative to N_{bf} ,

although relationships with census male size vary. Annual male census sizes (N_m) have remained stable or decreased in Northeast Brook, but have increased in Middle Brook, whereas N_{bm} may have increased in both rivers. Moreover, there may be a positive relation between N_{bm} and N_m in Middle Brook ($r = 0.61$, $p =$

TABLE 6

Demographic estimates of the annual effective number of male breeders (N_{bm}), derived from \hat{N}_b (Pacific salmon model), and demographic estimates of the effective number of females (N_{bf})

River	Yr	Census	N_m	N_f	\hat{N}_b	N_{bf}	N_{bm}	N_{bm}/N_{bf}
Middle Brook	1978	1403	117	1286	104	1225	27	0.02
	1979	1350	270	1080	163	1022	42	0.04
	1985	904	168	736	167	700	44	0.06
	1991	562	241	321	210	285	64	0.23
	1992	1182	220	962	1019	919	353	0.38
	1993	1959	440	1519	1369	1423	451	0.32
	1997	1221	234	987	1396	931	559	0.60
	1998	2405	172	2233	180	2089	46	0.02
Northeast Brook	1985	124	21	103	235	95	154	1.62
	1986	158	35	123	33	107	9	0.08
	1987	91	37	54	145	52	119	2.27
	1991	99	11	88	97	86	34	0.39
	1992	49	9	40	2367	39	∞	∞
	1994	99	13	86	682	84	∞	∞
	1998	91	3	88	158	85	74	0.87

Results are shown only for rivers with sufficient genetic data and demographic data; the latter include census size information of annual cohorts, divided into males (N_m) and females (N_f).

0.15, $n = 8$), but not in Northeast Brook (Trepassey, $r = 0.02$, $p = 0.96$, $n = 7$).

DISCUSSION

All genetic models make simplifying assumptions about populations, but this study reemphasizes the potential complexity of life history, age structure, and demography that underlie N_e and hence affect its empirical estimation in fluctuating populations. Iteroparity, even when presumably low, may have to be accounted for in genetic estimation models. On the other hand, variance in reproductive contribution by mature male parr may affect age structure more so than \hat{N}_e estimation. Accounting for potential changes in age structure and population dynamics, we document temporal changes in N_e in some rivers. These changes may reflect abundance increases of breeders as well as breeding system alterations and possibly effects of density-dependence and metapopulation dynamics. We start by discussing the limitations arising from our sampling design.

Sampling limitations: An important element of any genetic method of estimating N_e is adequate sampling, both in terms of sample size and in terms of representation of the entire population. These concerns are especially relevant when actual effective population sizes are large, as all (temporal) methods measure a signal that scales to $1/N_e$. Our conclusions may hence be affected by sampling error: for example, the sample sizes of individual cohorts were often small (minimum $S = 12$, Table 3). Similarly, the number of consecutive cohort comparisons was often small (Table 3, Table 5), which may have affected the accuracy of the cohort model, in particular under strong demographic perturbations (JORDE and RYMAN 1995). Considerations of sampling efforts required to obtain sufficient statistical power (e.g., PALSTRA and RUZZANTE 2008) suggest that appropriate sample sizes for larger rivers (e.g., Gander, Conne) might have been well in excess of 100 individuals. Thus, we limited some of our genetic analyses to smaller river populations. Nevertheless, sampling limitations are evident in the wide confidence intervals (C.I.'s) associated with many \hat{N}_e . This affects the strength of inferences on comparisons between \hat{N}_e from different estimator methods, as well as changes in \hat{N}_e over time. Sampling efforts to reliably infer N_e and detect temporal changes may have to be (considerably) larger than those underpinning this study ($N = 2758$).

Second, genetic estimates derived from models for overlapping generations are sensitive to the confidence with which individuals could be assigned back to their cohorts, on the basis of age determination of fish scales. Random errors in this assignment will likely introduce an upward bias into \hat{N}_e , since a mixture of individuals of different ages present in the presumed "cohorts" will

dilute signals of genetic differentiation present among the actual cohorts (PALM *et al.* 2003). This source of bias may explain why genetic estimates derived from the Pacific salmon model are often larger than those from the discrete generation model, but not the differences between N_e estimates from the semelparous Pacific salmon and cohort models.

Age structure and N_e estimation: Age structure and reproductive biology are important determinants of N_e (FELSENSTEIN 1971; HILL 1972; NUNNEY 1991, 1993, 1996; FRANKHAM 1995) with consequences for its empirical estimation (JORDE and RYMAN 1995; ENGEN *et al.* 2007; WAPLES and YOKOTA 2007). The discrete generation model has so far been most commonly applied, but will be (downwardly) biased, when applied to temporal samples taken over short time spans for species with overlapping generations (WAPLES and YOKOTA 2007; PALSTRA and RUZZANTE 2008). This bias arises mainly because signals of genetic drift (related to N_e) will be strongly affected by genetic (co)variances generated by age structure (JORDE and RYMAN 1995). Under these circumstances, genetic estimators designed specifically for overlapping generations (WAPLES 1990a,b; JORDE and RYMAN 1995) will be preferable to discrete temporal methods. Previous empirical investigations of (fish) species with low iteroparity have tended to use either one of these methods (e.g., PALM *et al.* 2003; CONSUEGRA *et al.* 2005; FRASER *et al.* 2007a). This study is the first to simultaneously apply two models that differ principally in the assumption of iteroparity, facilitating an investigation of the importance of low iteroparity for N_e estimation.

On the one hand, whenever individuals can reproduce more than once, the semelparous Pacific salmon model can be expected to overestimate N_e . This upward bias will be caused by genetic contributions of iteroparous individuals that will tend to buffer genetic fluctuations among years and thus reduce the strength of interannual genetic signals on which estimates of N_e are based in the Pacific salmon model. Conversely, the cohort model may underestimate N_e when survival depends strongly on previous reproduction (and total mortality in the population is low) (JORDE and RYMAN 1995). Unfortunately, the reproductive biology of Atlantic salmon may incorporate both of these aspects. A comparison of N_e estimates derived from these two models to discrete temporal estimates (Table 3) suggests that the upward bias in the Pacific salmon model may be more serious than the risk of downward bias in the cohort model. Although $\hat{N}_{e(J\&R)}$ were often lower than $\hat{N}_{e(\text{discrete})}$, their general similarity may suggest that total mortality rates in Atlantic salmon populations are generally low enough for bias due to reproduction-dependent mortality to be small (*cf.* Figure 4 in JORDE and RYMAN 1995). On the other hand, in six of eight rivers, the Pacific salmon model results in larger N_e estimates than the other two models (Table 3).

This upward bias may arise mainly because of the calculation of $\hat{N}_{e(\text{Salmon})}$ from the harmonic mean of annual N_b estimates. Using demographic data, WAPLES (2002) found that effective population size in fluctuating salmon populations can be (much) smaller than the product $G\hat{N}_b$. This reduction (40–60%) was attributed to relative discrepancies between the size of cohorts and their reproductive contribution to a given generation. Cohort and discrete generation models directly estimate N_e and may thus be less sensitive to this source of bias. Demographic analyses (following WAPLES 2002) suggest that this reduction may be weak to moderate in the populations studied (5–59%). Nevertheless, $\hat{N}_{e(\text{Salmon})}$ remain relatively high after correcting for this bias, suggesting that even low iteroparity can bias N_e estimation in a strictly semelparous model. Extended analyses, comparing annual N_b estimates from the Pacific salmon model with discrete generation temporal estimates of N_b , tend to support this conclusion (see Table S2). Although the interpretation of N_e estimates derived from different estimator models is complicated by differing sampling efforts in space and time, as well as wide C.I.'s (Table 3), these findings thus suggest that ignoring low iteroparity is generally not recommended.

These results might also imply that iteroparity in Atlantic salmon populations is higher than suggested by estimates of iteroparity in anadromous fish (Table 3), possibly due to contributions by resident nonanadromous salmon, including mature parr. Nonanadromous salmon probably have higher rates of iteroparity than anadromous salmon and can be common in some Newfoundland systems (LEGGETT and POWER 1969; HUTCHINGS 2002). Mature male parr might also contribute to iteroparity, given that similar discrepancies between the salmon and the discrete generation model are evident in empirical studies on systems where nonanadromous salmon are presumably absent (*e.g.*, CONSUEGRA *et al.* 2005; FRASER *et al.* 2007a). A substantial mortality cost associated with parr maturation ($s_{\text{rel}} = 0.35$) does not preclude iteroparity, either again as mature parr or as anadromous adults. For example, although less abundant and often older than females, many males that previously matured as parr are present in smolt runs in Newfoundland (CHADWICK 1981; DALLEY *et al.* 1983; MYERS 1984; HUTCHINGS 1986; HUTCHINGS and MYERS 1994). This uncertainty regarding the general incidence of iteroparity in males may make the cohort model preferable over the Pacific salmon (semelparity assumed) model for N_e estimation in Atlantic salmon.

Single sample estimators of N_e may be less influenced by assumptions of iteroparity and thus prove useful, provided that careful attention is paid to what these estimates apply to (see WAPLES 2005). LD- N_e estimates based on plan I temporal samples (Table 5) should be interpreted with some caution, as variance in the magnitude of LD- \hat{N}_e will also be generated by

variable age composition of samples (see Table S1). These difficulties emphasize that, regardless of the genetic methods applied, empirical N_e studies should be founded on detailed knowledge of life history and age structure.

Consequences of gene flow: Our study adds to a growing number of empirical investigations of N_e in salmonid fish (*e.g.*, ØSTERGAARD *et al.* 2003; SHRIMPTON and HEATH 2003; LAGE and KORNFIELD 2006; FRASER *et al.* 2007b; VÄHÄ *et al.* 2008). Using various temporal genetic methods, we document relatively high estimates of N_e as well as increases in \hat{N}_e in small populations over time. Gene flow as inferred from WANG and WHITLOCK'S (2003) approach may provide one explanation for this observation, as gene flow might often be more prevalent into effectively smaller salmonid populations, thus maintaining genetic diversity (CONSUEGRA *et al.* 2005; FRASER *et al.* 2007a; HANSEN *et al.* 2007). Indeed, in many (but not all) rivers a marked decrease in \hat{N}_e was observed once gene flow was considered. The approach of WANG and WHITLOCK (2003) has been criticized on the biological reality of its migration model (HOFFMAN *et al.* 2004; FRASER *et al.* 2007b). Nevertheless, temporal estimates of gene flow (\hat{m}) agree with contemporary genetic estimates of migration into these rivers (PALSTRA *et al.* 2007) and direct estimates of migration from tagging studies in Atlantic salmon in general (STABELL 1984; JONSSON *et al.* 2003). On the other hand, our results also caution against generalizations, as we occasionally found very limited evidence for gene flow among regionally (very) proximate rivers.

It is less clear whether temporal changes in gene flow underpin those observed in \hat{N}_e in some (smaller) rivers. Current analytical limitations complicate investigating effects of gene flow on N_e over short time spans. Removal of putative immigrants from samples prior to N_e estimation may have been one solution, but would have reduced (already limited) sample sizes, particularly for the cohort and Pacific salmon models. These sampling concerns may, however, be less important for single sample estimators. As an exploratory analysis, we removed putative migrants from two temporal samples (Middle Brook, 1982 and Middle Brook, 1997), using assignment tests (PAETKAU *et al.* 2004), and then repeated linkage-disequilibrium analyses on truncated samples. For the corrected 1982 sample this results in LD- $\hat{N}_e = 384$ (95% C.I.: 235–974, $\hat{S} = 59.6$), whereas the corrected 1997 sample yields LD- $\hat{N}_e = 234$ (95% C.I.: 172–360, $\hat{S} = 63.8$). Although these assignment tests yield estimates of migration that are relatively high compared to other approaches (Table 2) or those reported in the literature (*e.g.*, STABELL 1984), the measure of interest here is relative change over time. Hence, whereas both samples show a decrease in \hat{N}_e (see Table 4) when individuals putatively identified as migrants were excluded, this decrease is stronger for the 1997 sample (52%) than for the 1982 sample (8%).

These results may thus indicate temporal changes in the strength of gene flow that might be causally linked to changes in N_e in some rivers over time.

Life history diversity and N_e : Stability of N_e in the context of demographic changes may be attributable to life history diversity, as this facilitates maximization of lifetime reproductive success under various environmental and demographic conditions. Mature male parr reproduction has been speculated to be beneficial for N_e (SAUNDERS and SCHOM 1985; L'ABÉE-LUND 1989; MARTINEZ *et al.* 2000; VALIENTE *et al.* 2005; JUANES *et al.* 2007), although mature parr may “increase” effective size only in the sense that \hat{N}_e is increased compared to when mature parr are ignored in analyses (*e.g.*, JONES and HUTCHINGS 2002; SAURA *et al.* 2008). Nevertheless, the reasoning implies that parr reproductive contribution increases N_e by reducing the variance in (male) individual reproductive success. Empirical investigations have often applied theory for discrete generations, through the influence of mature parr on sex bias (*e.g.*, L'ABÉE-LUND 1989; CONSUEGRA *et al.* 2005) or individual reproductive success in a single breeding season (*e.g.*, MARTINEZ *et al.* 2000; TAGGART *et al.* 2001). Both approaches may not correctly reflect processes in age-structured populations with overlapping generations. First, the consequences of sex bias for N_e are expected to be much reduced (NUNNEY 1991, 1993), in particular when sex bias reflects survival (rather than recruitment) differences. Second, the genetic effects of annual variance in individual reproductive success will be buffered under iteroparity, since individuals that failed to breed in one year may succeed in the future (NUNNEY 1993). Indeed, individual parr often obtain very little reproductive success in (experimental) spawning events (*e.g.*, THOMAZ *et al.* 1997; JONES and HUTCHINGS 2001, 2002; WEIR *et al.* 2005) as mature parr also establish dominance hierarchies during spawning (HUTCHINGS and MYERS 1988; FLEMING 1996). Instead, N_e may be mainly affected by the reproductive variance of anadromous males (MJØLNEROD *et al.* 1998; JONES and HUTCHINGS 2002). We find some support for this assertion through a positive relation between annual N_b estimates and the number of anadromous males in Middle Brook. Moreover, total reproductive success by mature male parr may be highest when few anadromous males are present (JONES and HUTCHINGS 2002). For example, Northeast Brook, characterized by anadromous runs with relatively few males, also has the highest N_e/N ratios observed in this study (Table 3), which might be interpreted as a positive effect of mature male parr reproduction on N_e . However, the biological relevance of N_e/N ratios is questionable when N does not accurately reflect the potential number of breeders. If this is indeed the case in Northeast Brook, then our results may simply imply that males in this population maximize their lifetime reproductive success by predominantly maturing and reproducing as parr.

Our life table simulations suggest variance in male parr reproduction has limited influence on the empirical estimation of N_e . While we very likely would have overestimated N_e had we ignored mature parr reproduction (*i.e.*, increased values of C/G , Figure 3), N_e estimation appears more sensitive to age skew in anadromous breeders. Genetic fluctuations among cohorts are already buffered by variable anadromous age at maturity. Furthermore, genetic effects of mature parr reproduction are reduced (or more precisely, halved), since half the alleles in the progeny will descend from females, which are usually obligatory anadromous. As previously reported by JUANES *et al.* (2007), mature male parr reproduction reduces the strength of genetic fluctuations (*i.e.*, increases the genetic covariance) among consecutive cohorts. That by itself does not increase N_e , because of the concomitant decrease in generation time. Thus, higher levels of mature male parr reproductive success may concentrate breeders in a given generation into a shorter time span, without strongly affecting N_e . This could, however, render such populations more sensitive to environmental stochasticity (GAGGIOTTI and VETTER 1999) and may actually increase the pace at which genetic variance is lost (*e.g.*, RYMAN *et al.* 1981; ALLENDORF *et al.* 2008). These conclusions partly depend on the cohort model assumption of constant population size ($\sum l_x b_x = 1$). Our simulations may therefore be interpreted as a quantification of the effects of parr reproduction on age structure and genetic fluctuations, for a given value of N_e . Mature parr reproduction may be more appropriately viewed as one of a few strategies utilized by male Atlantic salmon to maximize their lifetime reproductive success, the ultimate genetic contribution a stabilization of N_e rather than an absolute increase. The demographic and genetic consequences of this reproductive strategy may be more important, through changing the age structure of a population.

Demography, density dependence, and temporal trends in N_e : A major goal of this study was to evaluate temporal trends in N_e in populations that have likely undergone demographic fluctuations. Changing population size does not affect the standard, discrete-generation temporal method, which estimates the harmonic mean N_e over the time between samples (WAPLES and YOKOTA 2007), but many models for overlapping generations do assume constant population size (*e.g.*, FELSENSTEIN 1971; HILL 1972; NUNNEY 1993; JORDE and RYMAN 1995). JORDE and RYMAN (1995) discuss the analytical consequences of demographic perturbations in the cohort model and argue that it may be accounted for by monitoring a population over time, *i.e.*, by calculating F as the mean of several cohort comparisons. In our study this requirement is fulfilled by $\hat{N}_{e(J\&R)}$ for the longest time spans per river (Table 3, minimum $n = 4$). Similarly, $\hat{N}_{e(J\&R)}$ for cohorts before and after 1992 usually consist of multiple comparisons, with the exception of

Conne River (Table 4). Moreover, these latter analyses were performed using life tables based solely on demographic information pertinent to each time period, to account for potential temporal changes in age structure. Despite the various limitations arising from our analytical approach, results suggest temporal changes in N_e in some, but not all, river populations, that correlate poorly with demographic trends. Concomitantly, the observed temporal and spatial differences in \hat{N}_e provide a poor explanation for differential recovery patterns in abundance observed among rivers after the closure of the commercial fishery (DEMPSON *et al.* 2004a). Thus, genetic stochasticity has likely not been a major force impeding population recovery in these rivers, despite very high exploitation rates in the marine fishery prior to the closure (DEMPSON *et al.* 2001).

As pointed out previously, sampling limitations likely reduced the ability to detect changes in N_e in larger census size populations. Nevertheless, larger populations may be innately more resistant to changes in N_e . First, effective population size in larger systems may be more prominently influenced by metapopulation dynamics: in Atlantic salmon, larger rivers (including some in Newfoundland) often appear to be genetically substructured (*e.g.*, BEACHAM and DEMPSON 1998; VERSPOOR and COLE 2005; VÄHÄ *et al.* 2007, 2008). Under these conditions, the reproductive factors reducing N_e within a subpopulation are modulated by processes acting among subpopulations. Population subdivision likely reduces overall N_e (WHITLOCK and BARTON 1997; NUNNEY 1999), thereby also providing one explanation for lower N_e/N ratios in some large rivers (Table 3). Yet, population (sub)structuring has also been invoked to explain greater demographic stability (EINUM *et al.* 2003). Atlantic salmon in larger river systems tend to display higher life history diversity (KLEMETSEN *et al.* 2003) that may enable maximization of lifetime reproductive success under a wider range of demographic and environmental conditions, hence conveying an increased genetic stability. Whether larger populations are generally characterized by this apparent paradox of lower N_e/N ratios and higher genetic stability (*i.e.*, stable N_e) remains to be investigated.

Density dependence may affect the relationships between life history, demography, and N_e . First, density dependence (operating at the freshwater stage) can influence the expression of life history phenotypes (KOKKO and RANKIN 2006; EINUM *et al.* 2008), either directly through genetic changes or indirectly through phenotypic plasticity modulated by growth rates (GARDINER and SHACKLEY 1991; JONSSON *et al.* 1998; NORDWALL *et al.* 2001). For example, population dynamics in Northeast Brook display signs of density dependence (KLEMETSEN *et al.* 2003), and declining anadromous runs here have also tended to become more female biased. Whether plastic or genetically based (PICHE *et al.*

2008), such trends may indicate a shift to earlier maturation as male parr in this system. Second, density dependence is also a prerequisite for genetic compensation, where reduced variance in individual reproductive success underpins increased N_e/N ratios at low population size (PRAY *et al.* 1996; ARDREN and KAPUSCINSKI 2003; FRASER *et al.* 2007b; WATTS *et al.* 2007). We observed such relationships in both rivers considered in demographic detail. Finally, under endogenous population regulation, the total reproductive value of a salmonid population (and hence N_e) may depend mainly on the availability of suitable habitat, as observed in Chinook salmon (SHRIMPTON and HEATH 2003). Freshwater habitat in Newfoundland has remained relatively pristine, hence providing an additional explanation for the observed resilience of N_e in the face of population declines. When considered from this angle, increases in N_e in some rivers after the marine fishery closure may indicate that the high exploitation rates kept these populations below their carrying capacity.

Life history evolves to maximize the lifetime reproductive success of individuals in a population and this in turn is the principal determinant of effective population size. The life history plasticity evident in Atlantic salmon and other salmonid fish (HUTCHINGS 2004) may convey resilience against declines in N_e through enabling optimization of fitness under a range of environmental conditions. Age structures characterized by overlapping generations and multiple mating opportunities (iteroparity) may thus be more resistant to the detrimental genetic consequences of years with poor recruitment and low numbers of breeders (WARNER and CHESON 1985; NUNNEY 1993; ELLNER and HAIRSTON 1994; GAGGIOTTI and VETTER 1999) and render a population less sensitive to environmental stochasticity (GAGGIOTTI and VETTER 1999). Some support for this comes from empirical studies on long-lived fish species (*e.g.*, DIAZ *et al.* 2000; LIPPE *et al.* 2006). Resilience against genetic stochasticity afforded by life history may, however, not protect against other stochastic factors or against declines driven by deterministic factors. A particular value of N_e can reflect a large variety of age structures with markedly different sensitivities to environmental and genetic stochasticity (RYMAN *et al.* 1981; GAGGIOTTI and VETTER 1999). As age structures become more complex, sensitivity to environmental stochasticity and the pace at which genetic diversity is lost may both decrease, but so may the pace of a population's response to natural selection. The evolutionary implications of effective population size therefore depend on detailed knowledge of its interactions with life history and population dynamics. These insights may be attained only through carefully considering age structure in empirical investigations of N_e .

Jake Seibert and Christina Jones are thanked for lab assistance. Coilin Minto and Hannes Hochreiner are acknowledged for valuable help with simulations. The initial manuscript draft benefited greatly

from comments by Jeff Hutchings, Paul Bentzen, and Laura Weir. Robin Waples and four anonymous reviewers provided many insightful and constructive comments on a previous version of this manuscript. This work was supported by a National Sciences and Engineering Research Council Discovery Grant (D.E.R.), Dalhousie Graduate Student Scholarships (F.P.P.), a Department of Fisheries and Oceans Academic Subvention Grant, and the Dr. Patrick Lett Fund (F.P.P.).

LITERATURE CITED

- ALLENDORF, F. W., P. R. ENGLAND, G. LUIKART, P. A. RITCHIE and N. RYMAN, 2008 Genetic effects of harvest on wild animal populations. *Trends Ecol. Evol.* **23**: 327–337.
- ARDREN, W. R., and A. R. KAPUSCINSKI, 2003 Demographic and genetic estimates of effective population size (N_e) reveal genetic compensation in steelhead trout. *Mol. Ecol.* **12**: 35–49.
- BEACHAM, T. D., and J. B. DEMPSON, 1998 Population structure of Atlantic salmon from the Conne river, Newfoundland as determined from microsatellite DNA. *J. Fish Biol.* **52**: 665–676.
- CABALLERO, A., 1994 Developments in the prediction of effective population size. *Heredity* **73**: 657–679.
- CHADWICK, E. M. P., 1981 Biological characteristics of the Atlantic salmon smolts in Western Arm Brook, Newfoundland. *Can. Tech. Rep. Fish. Aquat. Sci.* **1024**: 45 pages.
- CONSUEGRA, S., E. VERSPOOR, D. KNOX and C. GARCÍA DE LEÁNIZ, 2005 Asymmetric gene flow and the evolutionary maintenance of genetic diversity in small, peripheral Atlantic salmon populations. *Conserv. Genet.* **6**: 823–842.
- CUNJAK, R. A., and J. THERRIEN, 1998 Inter-stage survival of wild juvenile Atlantic salmon, *Salmo salar* L. *Fish. Manag. Ecol.* **5**: 209–224.
- CUNJAK, R. A., T. D. PROWSE and D. L. PARRISH, 1998 Atlantic salmon (*Salmo salar*) in winter: “the season of part discontent”? *Can. J. Fish. Aquat. Sci.* **55**: 161–180.
- DALLEY, E. L., C. W. ANDREWS and J. M. GREEN, 1983 Precocious male Atlantic Salmon parr (*Salmo salar*) in insular Newfoundland. *Can. J. Fish. Aquat. Sci.* **40**: 647–652.
- DEMPSON, J. B., C. J. SCHWARZ, D. G. REDDIN, M. F. O’CONNELL, C. C. MULLINS *et al.*, 2001 Estimation of marine exploitation rates in Atlantic salmon (*Salmo salar* L.) stocks in Newfoundland, Canada. *ICES J. Mar. Sci.* **58**: 331–341.
- DEMPSON, J. B., G. FUREY and M. BLOOM, 2004a Status of Atlantic salmon, *Salmo salar*, in Conne River, SFA 11, Newfoundland, 2003. 2004/057. *Can. Sci. Advis. Secr. Res. Doc. Fisheries and Oceans*, Ottawa.
- DEMPSON, J. B., M. F. O’CONNELL and C. J. SCHWARZ, 2004b Spatial and temporal trends in abundance of Atlantic salmon, *Salmo salar*, in Newfoundland with emphasis on impacts of the 1992 closure of the commercial fishery. *Fish. Manag. Ecol.* **11**: 387–402.
- DIAZ, M., D. WETHEY, J. BULAK and B. ELY, 2000 Effect of harvest and effective population size on genetic diversity in a striped bass population. *Trans. Am. Fish. Soc.* **129**: 1367–1372.
- EINUM, S., I. A. FLEMING, I. M. COTE and J. D. REYNOLDS, 2003 Population stability in salmon species: effects of population size and female reproductive allocation. *J. Anim. Ecol.* **72**: 811–821.
- EINUM, S., G. ROBERTSEN and I. A. FLEMING, 2008 Adaptive landscapes and density-dependent selection in declining salmonid populations: going beyond numerical responses to human disturbance. *Evol. Appl.* **1**: 239–251.
- ELLNER, S., and N. G. HAIRSTON, 1994 Role of overlapping generations in maintaining genetic variation in a fluctuating environment. *Am. Nat.* **143**: 403–417.
- ELPHINSTONE, M. S., G. N. HINTEN, M. J. ANDERSON and C. J. NOCK, 2003 An inexpensive and high-throughput procedure to extract and purify total genomic DNA for population studies. *Mol. Ecol. Notes* **3**: 317–320.
- ENGEN, S., R. LANDE and B.-E. SÆTHER, 2005 Effective size of a fluctuating age-structured population. *Genetics* **170**: 941–954.
- ENGEN, S., T. H. RINGSBY, B.-E. SÆTHER, R. LANDE, H. JENSEN *et al.*, 2007 Effective size of fluctuating populations with two sexes and overlapping generations. *Evolution* **61**: 1873–1885.
- EVANS, G. T., J. C. RICE and E. M. P. CHADWICK, 1984 Patterns in growth and smolting of Atlantic salmon (*Salmo salar*) parr. *Can. J. Fish. Aquat. Sci.* **41**: 783–797.
- FELSENSTEIN, J., 1971 Inbreeding and variance effective numbers in populations with overlapping generations. *Genetics* **68**: 581–597.
- FLEMING, I. A., 1996 Reproductive strategies of Atlantic salmon: ecology and evolution. *Rev. Fish Biol. Fish.* **6**: 379–416.
- FLEMING, I. A., and J. D. REYNOLDS, 2004 Salmonid breeding systems, pp. 264–293 in *Evolution Illuminated: Salmon and Their Relatives*, edited by A. P. HENDRY and S. C. STEARNS. Oxford University Press, Oxford.
- FLEMING, I. A., A. LAMBERG and B. JONSSON, 1997 Effects of early experience on the reproductive performance of Atlantic salmon. *Behav. Ecol.* **8**: 470–480.
- FRANKHAM, R., 1995 Effective population size adult population size ratios in wildlife—a review. *Genet. Res.* **66**: 95–107.
- FRANKHAM, R., 2005 Genetics and extinction. *Biol. Conserv.* **126**: 131–140.
- FRANKHAM, R., J. D. BALLOU and D. A. BRISCOE, 2003 *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge, UK.
- FRASER, D. J., M. M. HANSEN, S. ØSTERGAARD, N. TESSIER, M. LEGAULT *et al.*, 2007a Comparative estimation of effective population sizes and temporal gene flow in two contrasting population systems. *Mol. Ecol.* **16**: 3866–3889.
- FRASER, D. J., M. W. JONES, T. L. MCPARLAND and J. A. HUTCHINGS, 2007b Loss of historical immigration and the unsuccessful rehabilitation of extirpated salmon populations. *Conserv. Genet.* **8**: 527–546.
- GAGGIOTTI, O. E., and R. D. VETTER, 1999 Effect of life history strategy, environmental variability, and overexploitation on the genetic diversity of pelagic fish populations. *Can. J. Fish. Aquat. Sci.* **56**: 1376–1388.
- GARANT, D., J. J. DODSON and L. BERNATCHEZ, 2001 A genetic evaluation of mating system and determinants of individual reproductive success in Atlantic salmon (*Salmo salar* L.). *J. Hered.* **92**: 137–145.
- GARCIA-VAZQUEZ, E., P. MORAN, J. L. MARTINEZ, J. PEREZ, B. DE GAUDEMAR *et al.*, 2001 Alternative mating strategies in Atlantic salmon and brown trout. *J. Hered.* **92**: 146–149.
- GARDINER, R., and P. SHACKLEY, 1991 Stock and recruitment and inversely density-dependent growth of salmon, *Salmo salar* L., in a Scottish stream. *J. Fish Biol.* **38**: 691–696.
- GOMEZ-UCHIDA, D., and M. A. BANKS, 2006 Estimation of effective population size for the long-lived darkblotched rockfish *Sebastes crameri*. *J. Hered.* **97**: 603–606.
- GOUDET, J., 1995 FSTAT (version 1.2): a computer program to calculate F-statistics. *J. Hered.* **86**: 485–486.
- HANSEN, M. M., O. SKAALA, L. F. JENSEN, D. BEKKEVOLD and K. L. D. MENSBERG, 2007 Gene flow, effective population size and selection at major histocompatibility complex genes: brown trout in the Hardanger Flord, Norway. *Mol. Ecol.* **16**: 1413–1425.
- HAUSER, L., G. J. ADCOCK, P. J. SMITH, J. H. B. RAMIREZ and G. R. CARVALHO, 2002 Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proc. Natl. Acad. Sci. USA* **99**: 11742–11747.
- HEINIMAA, S., and J. ERKINARO, 2004 Characteristics of mature male parr in the northernmost Atlantic salmon populations. *J. Fish Biol.* **64**: 219–226.
- HILL, W. G., 1972 Effective size of populations with overlapping generations. *Theor. Popul. Biol.* **3**: 278–289.
- HILL, W. G., 1981 Estimation of effective population size from data on linkage disequilibrium. *Genet. Res.* **38**: 209–216.
- HOFFMAN, E. A., F. W. SCHUELER and M. S. BLOUIN, 2004 Effective population sizes and temporal stability of genetic structure in *Rana pipiens*, the northern leopard frog. *Evolution* **58**: 2536–2545.
- HUTCHINGS, J. A., 1986 Lakeward migrations by juvenile Atlantic salmon, *Salmo salar*. *Can. J. Fish. Aquat. Sci.* **43**: 732–741.
- HUTCHINGS, J. A., 2002 Sustaining Atlantic salmon in the Northwest Atlantic: considerations from a life history perspective, pp. 33–60

- in *Sustaining North American Salmon: Perspectives Across Regions and Disciplines*, edited by K. D. LYNCH, M. L. JONES and W. W. TAYLOR. American Fisheries Society, Bethesda, Maryland.
- HUTCHINGS, J. A., 2004 Norms of reaction and phenotypic plasticity, pp. 154–174 in *Evolution Illuminated: Salmon and Their Relatives*, edited by A. P. HENDRY and S. C. STEARNS. Oxford University Press, Oxford.
- HUTCHINGS, J. A., and M. E. B. JONES, 1998 Life history variation and growth rate thresholds for maturity in Atlantic salmon, *Salmo salar*. *Can. J. Fish. Aquat. Sci.* **55**: 22–47.
- HUTCHINGS, J. A., and R. A. MYERS, 1988 Mating success of alternative maturation phenotypes in male Atlantic salmon, *Salmo salar*. *Oecologia* **75**: 169–174.
- HUTCHINGS, J. A., and R. A. MYERS, 1994 The evolution of alternative mating strategies in variable environments. *Evol. Ecol.* **8**: 256–268.
- JOHNSON, J. A., M. R. BELLINGER, J. E. TOEPFER and P. DUNN, 2004 Temporal changes in allele frequencies and low effective population size in greater prairie-chickens. *Mol. Ecol.* **13**: 2617–2630.
- JONES, J. W., and G. M. KING, 1952 The spawning of the male salmon part (*Salmo salar* Linn Juv). *Proc. Zool. Soc. Lond.* **122**: 615–619.
- JONES, M. W., and J. A. HUTCHINGS, 2001 The influence of male part body size and mate competition on fertilization success and effective population size in Atlantic salmon. *Heredity* **86**: 675–684.
- JONES, M. W., and J. A. HUTCHINGS, 2002 Individual variation in Atlantic salmon fertilization success: implications for effective population size. *Ecol. Appl.* **12**: 184–193.
- JONSSON, B., N. JONSSON and L. P. HANSEN, 2003 Atlantic salmon straying from the river Imsa. *J. Fish Biol.* **62**: 641–657.
- JONSSON, N., B. JONSSON and L. P. HANSEN, 1998 The relative role of density-dependent and density-independent survival in the life cycle of Atlantic salmon *Salmo salar*. *J. Anim. Ecol.* **67**: 751–762.
- JORDAN, W. C., and A. F. YOUNGSON, 1992 The use of genetic marking to assess the reproductive success of mature male Atlantic salmon part (*Salmo salar*; L) under natural spawning conditions. *J. Fish Biol.* **41**: 613–618.
- JORDE, P. E., and N. RYMAN, 1995 Temporal allele frequency change and estimation of effective size in populations with overlapping generations. *Genetics* **139**: 1077–1090.
- JORDE, P. E., and N. RYMAN, 1996 Demographic genetics of brown trout (*Salmo trutta*) and estimation of effective population size from temporal change of allele frequencies. *Genetics* **143**: 1369–1381.
- JORDE, P. E., and N. RYMAN, 2007 Unbiased estimator for genetic drift and effective population size. *Genetics* **177**: 927–935.
- JUANES, F., J. PEREZ and E. GARCIA-VAZQUEZ, 2007 Reproductive strategies in small populations: using Atlantic salmon as a case study. *Ecol. Freshw. Fish* **16**: 468–475.
- KLEMETSEN, A., P. A. AMUNDSEN, J. B. DEMPSON, B. JONSSON, N. JONSSON *et al.*, 2003 Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic char *Salvelinus alpinus* (L.): a review of aspects of their life histories. *Ecol. Freshw. Fish* **12**: 1–59.
- KOKKO, A., and D. J. RANKIN, 2006 Lonely hearts or sex in the city? Density dependent effects in mating systems. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **361**: 319–334.
- L'ABÉE-LUND, J. H., 1989 Significance of mature male part in a small population of Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **46**: 928–931.
- LAGE, C., and I. KORNFIELD, 2006 Reduced genetic diversity and effective population size in an endangered Atlantic salmon (*Salmo salar*) population from Maine. *Conserv. Genet.* **7**: 91–104.
- LANDE, R., 1988 Genetics and demography in biological conservation. *Science* **241**: 1455–1460.
- LANDE, R., and G. F. BARROWCLOUGH, 1988 Effective population size, genetic variation, and their use in population management, pp. 87–123 in *Viable Populations for Conservation*, edited by M. E. SOULÉ. Cambridge University Press, Cambridge, UK.
- LEBERG, P., 2005 Genetic approaches for estimating the effective size of populations. *J. Wildl. Manage.* **69**: 1385–1399.
- LEGGETT, W. C., and G. POWER, 1969 Differences between two populations of landlocked Atlantic salmon (*Salmo salar*) in Newfoundland. *J. Fish Res. Board Can.* **26**: 1585–1596.
- LIPPE, C., P. DUMONT and L. BERNATCHEZ, 2006 High genetic diversity and no inbreeding in the endangered copper redhorse, *Moxostoma hubbsi* (Catostomidae, Pisces): the positive sides of a long generation time. *Mol. Ecol.* **15**: 1769–1780.
- LOCKE, A., 1998 Modeling the effects of poststocking survival rates on the success of stocking hatchery Atlantic salmon in a New Brunswick river. *N. Am. J. Fish. Manage.* **18**: 547–560.
- LUIKART, G., and J. M. CORNUET, 1998 Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conserv. Biol.* **12**: 228–237.
- MARTINEZ, J. L., P. MORAN, J. PEREZ, B. DE GAUDEMAR, E. BEALL *et al.*, 2000 Multiple paternity increases effective size of southern Atlantic salmon populations. *Mol. Ecol.* **9**: 293–298.
- MILLER, C. R., and L. P. WAITS, 2003 The history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): implications for conservation. *Proc. Natl. Acad. Sci. USA* **100**: 4334–4339.
- MJØLNEROD, I. B., I. A. FLEMING, U. H. REFSETH and K. HINDAR, 1998 Mate and sperm competition during multiple-male spawnings of Atlantic salmon. *Can. J. Zool.* **76**: 70–75.
- MORAN, P., A. M. PENDAS, E. BEALL and E. GARCIA-VAZQUEZ, 1996 Genetic assessment of the reproductive success of Atlantic salmon precocious part by means of VNTR loci. *Heredity* **77**: 655–660.
- MYERS, R. A., 1984 Demographic consequences of precocious maturation of Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **41**: 1349–1353.
- MYERS, R. A., J. A. HUTCHINGS and R. J. GIBSON, 1986 Variation in male part maturation within and among populations of Atlantic salmon, *Salmo salar*. *Can. J. Fish. Aquat. Sci.* **43**: 1242–1248.
- NEI, M., and F. TAJIMA, 1981 Genetic drift and estimation of effective population size. *Genetics* **98**: 625–640.
- NEWMAN, D., and D. PILSON, 1997 Increased probability of extinction due to decreased genetic effective population size: experimental populations of *Clarkia pulchella*. *Evolution* **51**: 354–362.
- NIELSEN, E. E., M. M. HANSEN and V. LOESCHCKE, 1999a Analysis of DNA from old scale samples: technical aspects, applications and perspectives for conservation. *Heredity* **130**: 265–276.
- NIELSEN, E. E., M. M. HANSEN and V. LOESCHCKE, 1999b Genetic variation in time and space: microsatellite analysis of extinct and extant populations of Atlantic salmon. *Evolution* **53**: 261–268.
- NORDWALL, F., I. NASLUND and E. DEGERMAN, 2001 Intercohort competition effects on survival, movement, and growth of brown trout (*Salmo trutta*) in Swedish streams. *Can. J. Fish. Aquat. Sci.* **58**: 2298–2308.
- NUNNEY, L., 1991 The influence of age structure and fecundity on effective population size. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **246**: 71–76.
- NUNNEY, L., 1993 The influence of mating system and overlapping generations on effective population size. *Evolution* **47**: 1329–1341.
- NUNNEY, L., 1996 The influence of variation in female fecundity on effective population size. *Biol. J. Linn. Soc.* **59**: 411–425.
- NUNNEY, L., 1999 The effective size of a hierarchically structured population. *Evolution* **53**: 1–10.
- O'CONNELL, M. F., J. B. DEMPSON, C. C. MULLINS, D. G. REDDIN, C. E. BOURGEOIS *et al.*, 2005 Status of Atlantic salmon (*Salmo salar* L.) stocks of insular Newfoundland (SFAs 3–14A), 2004. 2005/064. *Can. Sci. Advis. Secr. Res. Doc.*
- O'CONNELL, M. F., J. B. DEMPSON and G. CHAPUT, 2006a Aspects of the life history, biology and population dynamics of Atlantic salmon (*Salmo salar* L.) in Eastern Canada. 2006/014. *Can. Sci. Advis. Secr. Res. Doc. Fisheries and Oceans, Ottawa.*
- O'CONNELL, M. F., J. B. DEMPSON, C. C. MULLINS, D. G. REDDIN, C. E. BOURGEOIS *et al.*, 2006b Status of Atlantic salmon (*Salmo salar* L.) stocks of insular Newfoundland (SFAs 3–14A), 2005. 2006/058. *Can. Sci. Advis. Secr. Res. Doc.*
- O'CONNELL, M. F., J. B. DEMPSON and D. G. REDDIN, 2008 Inter-annual and seasonal variability in fecundity of Atlantic salmon, *Salmo salar* L., in rivers in Newfoundland and Labrador, Canada. *Fish. Manage. Ecol.* **15**: 59–70.
- ORZACK, S. H., and S. TULJAPURKAR, 1989 Population dynamics in variable environments. VII. The demography and evolution of iteroparity. *Am. Nat.* **33**: 901–923.

- ØSTERGAARD, S., M. M. HANSEN, V. LOESCHCKE and E. E. NIELSEN, 2003 Long-term temporal changes of genetic composition in brown trout (*Salmo trutta* L.) populations inhabiting an unstable environment. *Mol. Ecol.* **12**: 3123–3135.
- PAETKAU, D., R. SLADE, M. BURDEN and A. ESTOUP, 2004 Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Mol. Ecol.* **13**: 55–65.
- PALM, S., L. LAIKRE, P. E. JORDE and N. RYMAN, 2003 Effective population size and temporal genetic change in stream resident brown trout (*Salmo trutta*, L.). *Conserv. Genet.* **4**: 249–264.
- PALSTRA, F. P., and D. E. RUZZANTE, 2008 Genetic estimates of contemporary effective population size: What can they tell us about the importance of genetic stochasticity for wild population persistence? *Mol. Ecol.* **17**: 3428–3447.
- PALSTRA, F. P., M. F. O'CONNELL and D. E. RUZZANTE, 2007 Population structure and gene flow reversals in Atlantic salmon (*Salmo salar*) over contemporary and long-term temporal scales: effects of population size and life history. *Mol. Ecol.* **16**: 4504–4522.
- PARRISH, D. L., R. J. BEHNKE, S. R. GEPHARD, S. D. MCCORMICK and G. H. REEVES, 1998 Why aren't there more Atlantic salmon (*Salmo salar*)? *Can. J. Fish. Aquat. Sci.* **55**: 281–287.
- PICHE, J., J. A. HUTCHINGS and W. BLANCHARD, 2008 Genetic variation in threshold reaction norms for alternative reproductive tactics in male Atlantic salmon, *Salmo salar*. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **275**: 1571–1575.
- POLLAK, E., 1983 A new method for estimating the effective population size from allele frequency changes. *Genetics* **104**: 531–548.
- PRAY, L. A., C. J. GOODNIGHT, L. STEVENS, J. M. SCHWARTZ and G. Y. YAN, 1996 The effect of population size on effective population size: an empirical study in the red flour beetle *Tribolium castaneum*. *Genet. Res.* **68**: 151–155.
- RYMAN, N., and L. LAIKRE, 1991 Effects of supportive breeding on the genetically effective population size. *Conserv. Biol.* **5**: 325–329.
- RYMAN, N., R. BACCUS, C. REUTERWALL and M. H. SMITH, 1981 Effective population size, generation interval, and potential loss of genetic variability in game species under different hunting regimes. *Oikos* **36**: 257–266.
- RYMAN, N., S. PALM, C. ANDRE, C. G. CARVALHO, T. G. DAHLGREN *et al.*, 2006 Power for detecting genetic divergence: differences between statistical methods and marker loci. *Mol. Ecol.* **15**: 2031–2045.
- SACCHERI, I., M. KUUSSAARI, M. KANKARE, P. VIKMAN, W. FORTELIUS *et al.*, 1998 Inbreeding and extinction in a butterfly metapopulation. *Nature* **392**: 491–494.
- SAILLANT, E., and J. R. GOLD, 2006 Population structure and variance effective size of red snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico. *Fish. Bull.* **104**: 136–148.
- SAUNDERS, R. L., and C. B. SCHOM, 1985 Importance of the variation in life-history parameters of Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **42**: 615–618.
- SAURA, M., A. CABALLERO, P. CABALLERO and P. MORAN, 2008 Impact of precocious male part on the effective size of a wild population of Atlantic salmon. *Freshw. Biol.* **53**: 2375–2384.
- SCHWARTZ, M. K., G. LUIKART and R. S. WAPLES, 2007 Genetic monitoring as a promising tool for conservation and management. *Trends Ecol. Evol.* **22**: 25–33.
- SHRIMPTON, J. M., and D. D. HEATH, 2003 Census vs. effective population size in chinook salmon: large- and small-scale environmental perturbation effects. *Mol. Ecol.* **12**: 2571–2583.
- SOKAL, R. R., and J. F. ROHLF, 1991 *Biometry*. W. H. Freeman, San Francisco.
- STABELL, O. B., 1984 Homing and olfaction in salmonids—a critical review with special reference to the Atlantic salmon. *Biol. Rev. Camb. Philos. Soc.* **59**: 333–388.
- SYMONS, P. E. K., 1979 Estimated escapement of Atlantic salmon (*Salmo salar*) for maximum smolt production in rivers of different productivity. *J. Fish. Res. Board Can.* **36**: 132–140.
- TAGGART, J. B., R. A. HYNES, P. A. PRODOHL and A. FERGUSON, 1992 A simplified protocol for routine total DNA isolation from salmonid fishes. *J. Fish Biol.* **40**: 963–965.
- TAGGART, J. B., I. S. MCLAREN, D. W. HAY, J. H. WEBB and A. F. YOUNGSON, 2001 Spawning success in Atlantic salmon (*Salmo salar* L.): a long-term DNA profiling-based study conducted in a natural stream. *Mol. Ecol.* **10**: 1047–1060.
- TAJIMA, F., 1992 Statistical method for estimating the effective population size in Pacific salmon. *J. Hered.* **83**: 309–311.
- THOMAZ, D., E. BEALL and T. BURKE, 1997 Alternative reproductive tactics in Atlantic salmon: factors affecting mature part success. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **264**: 219–226.
- VÄHÄ, J. P., J. ERKINARO, E. NIEMELA and C. R. PRIMMER, 2007 Life-history and habitat features influence the within-river genetic structure of Atlantic salmon. *Mol. Ecol.* **16**: 2638–2654.
- VÄHÄ, J. P., J. ERKINARO, E. NIEMELA and C. R. PRIMMER, 2008 Temporally stable genetic structure and low migration in an Atlantic salmon population complex: implications for conservation and management. *Evol. Appl.* **1**: 137–154.
- VALIENTE, A. G., F. JUANES and E. GARCIA-VAZQUEZ, 2005 Reproductive strategies explain genetic diversity in Atlantic salmon, *Salmo salar*. *Environ. Biol. Fish.* **74**: 323–334.
- VERSPOOR, E., and L. J. COLE, 2005 Genetic evidence for lacustrine spawning of the non-anadromous Atlantic salmon population of Little Gull Lake, Newfoundland. *J. Fish Biol.* **67**: 200–205.
- WANG, J. L., 2001 A pseudo-likelihood method for estimating effective population size from temporally spaced samples. *Genet. Res.* **78**: 243–257.
- WANG, J. L., 2005 Estimation of effective population sizes from data on genetic markers. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **360**: 1395–1409.
- WANG, J. L., and M. C. WHITLOCK, 2003 Estimating effective population size and migration rates from genetic samples over space and time. *Genetics* **163**: 429–446.
- WAPLES, R. S., 1989 A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics* **121**: 379–391.
- WAPLES, R. S., 1990a Conservation genetics of Pacific salmon. 2. Effective population size and the rate of loss of genetic variability. *J. Hered.* **81**: 267–276.
- WAPLES, R. S., 1990b Conservation genetics of Pacific salmon. 3. Estimating effective population size. *J. Hered.* **81**: 277–289.
- WAPLES, R. S., 2002 Effective size of fluctuating salmon populations. *Genetics* **161**: 783–791.
- WAPLES, R. S., 2005 Genetic estimates of contemporary effective population size: To what time periods do the estimates apply? *Mol. Ecol.* **14**: 3335–3352.
- WAPLES, R. S., and C. DO, 2008 LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Mol. Ecol. Res.* **8**: 753–756.
- WAPLES, R. S., and M. YOKOTA, 2007 Temporal estimates of effective population size in species with overlapping generations. *Genetics* **175**: 219–233.
- WAPLES, R. S., M. MASUDA and J. PELLA, 2007 SALMONNb: a program for computing cohort-specific effective population sizes (N_b) in Pacific salmon and other semelparous species using the temporal method. *Mol. Ecol. Notes* **7**: 21–24.
- WARNER, R. R., and P. L. CHESSON, 1985 Coexistence mediated by recruitment fluctuations—a field guide to the storage effect. *Am. Nat.* **125**: 769–787.
- WATTS, P. C., I. J. SACCHERI, S. J. KEMP and D. J. THOMPSON, 2007 Effective population sizes and migration rates in fragmented populations of an endangered insect (*Coenagrion mercuriale*: Odonata). *J. Anim. Ecol.* **76**(4): 737–751.
- WEIR, L. K., J. A. HUTCHINGS, I. A. FLEMING and S. EINUM, 2005 Spawning behaviour and success of mature male Atlantic salmon (*Salmo salar*) part of farmed and wild origin. *Can. J. Fish. Aquat. Sci.* **62**: 1153–1160.
- WHALEN, K. G., and D. L. PARRISH, 1999 Effect of maturation on part growth and smolt recruitment of Atlantic salmon. *Can. J. Fish. Aquat. Sci.* **56**: 79–86.
- WHITLOCK, M. C., and N. H. BARTON, 1997 The effective size of a subdivided population. *Genetics* **146**: 427–441.
- WRIGHT, S., 1938 Size of a population and breeding structure in relation to evolution. *Science* **87**: 430–431.

GENETICS

Supporting Information

<http://www.genetics.org/cgi/content/full/genetics.109.101972/DC1>

**Age Structure, Changing Demography and Effective Population
Size in Atlantic Salmon (*Salmo salar*)**

Friso P. Palstra, Michael F. O'Connell and Daniel E. Ruzzante

Copyright © 2009 by the Genetics Society of America
DOI: 10.1534/genetics.109.101972

FILE S1**Life table simulations for Atlantic salmon**

We used simulations to test the biological plausibility of our life table estimates of age specific survivorship, related to assumptions about the biology of the mature male parr. Specifically, we evaluated the consequences of the incidence of parr maturity and their relative survival for the sex and age composition of anadromous fish, by simultaneously varying the former two parameters (from 0 to 0.99). Under the assumption of no difference in ocean survival between the sexes, we compared the sex composition of simulated anadromous age groups with empirical values for each river, to obtain an indication of realistic parameter ranges for subsequent analyses.

Simulation results

Survivorship and age structure were strongly influenced by the incidence of male parr maturation and relative survival (s_{rel}). Simulation results (Figure S1) show, not surprisingly, that increases in both the incidence of mature parr maturation and their relative mortality (reduced s_{rel}) lead to reductions in the expected relative number of anadromous males in the model. For rivers such as Northeast River (Placentia), with a strong anadromous sex bias (90% female), the range of plausible parameter combinations is much narrower (Figure S1 a,b) than for rivers such as Sandhill (Figure A.1e,f), where the observed anadromous sex ratio is close to one (i.e. 50% of anadromous fish are female). The mortality costs associated with parr maturation may therefore be substantial. For example, for Gander River, with an anadromous sex ratio of 75% females (Figure S1 c,d), typical for many Newfoundland rivers, our simulations suggest that parr maturation incurs a reduction in subsequent survival of at least 40%. However, at this lower extreme all male parr are assumed to mature, which seems incompatible with empirical observations, suggesting that the mortality associated with parr maturation may often be higher. Based on these results, we used a value of $s_{rel}=0.35$ for most subsequent life table analyses, along with male parr maturation rates that result in an age structure compatible with empirical observations. An exception is Northeast River (Placentia), where the extreme sex ratio in anadromous fish (>90% female) necessitates assuming a higher mortality associated with parr maturation ($s_{rel}=0.25$). Simulations suggest that a wider range of parameter combinations of parr survival and maturation are plausible for Sandhill River (Figure S1 e,f), although mature parr maturation incidence is less than 10% for a wide range of s_{rel} . In summary, we used simulations to select biologically realistic values for mature parr incidence and survival, which were subsequently used to calculate survivorship values. These life table analyses yielded estimates of generation time (G) and correction factor C , used in the genetic estimation of \mathcal{N}_e . Resulting estimates of G range from 4.5 yrs [Northeast River (Placentia)] to 6.7 yrs (Sandhill River).

Discussion

Despite the comparative wealth of ecological and demographic data available for Newfoundland Atlantic salmon, our life table analyses required a number of important assumptions. Empirical estimates of (high) mature parr incidence (Pepper 1976; Dalley *et al.* 1983; Myers 1984; Myers *et al.* 1986) and their survival relative to non-maturing parr (Myers 1984; Hutchings and Myers 1994) were only available for a number of Newfoundland rivers. However, high parr maturation rates may generally be expected, given that the study rivers are predominantly lacustrine systems, facilitating high growth rates and productivity (O'Connell and Ash 1993). Uncertainty regarding survival at the freshwater stages (both absolute and relative between mature and non-mature male parr) thus likely has a larger impact on our results. The most critical assumptions in our analyses were 1) resident (non-anadromous) life history phenotypes do not constitute a major component of populations, 2) freshwater survival (for immature parr) is independent of age, and 3) previous maturation as parr does not reduce ocean survival of male smolts.

Resident (non-anadromous) Atlantic salmon are common in many Newfoundland river systems (Power 1958; Scott and Crossman 1964; Andrews 1966; Leggett and Power 1969, see Hutchings 2002 for a review). These are fish that never migrate out to sea and complete their entire life cycle in freshwater. In some instances, they have been reported to be genetically distinct from anadromous forms (Birt *et al.* 1991; Verspoor and Cole 2005), though they can spawn in the same locations (Hutchings 1986) and no behavioral mechanisms appear to isolate the forms from interbreeding (Hutchings and Myers 1985). In an extensive study conducted on Newfoundland systems, Adams (2007) attributed genetic differentiation between the two life history forms mainly to geographic separation, detecting little genetic differences where they co-occur. Like male parr maturation, they can therefore be considered as one of several life history strategies present in a salmon population (Hutchings and Myers 1985; Hutchings 1986). Hence we cannot rule out bias in our life table analyses in populations where non-anadromous salmon are very common, such as Terra Nova River (Andrews 1966), made more accessible to anadromous fish in the past century only (Burgeois *et al.* 2002). This river is characterized by very high genetic diversity and large estimates of N_e , which appear incompatible with what is known about the current size of the anadromous runs (O'Connell *et al.* 2006b). Based upon the limited information on the reproductive biology and age structure of non-anadromous fish (Scott and Crossman 1964; Leggett and Power 1969), we may have underestimated age-specific survival and iteroparity, but perhaps overestimated fecundity. Large non-anadromous population components likely result in more even age distribution of projected reproduction (p_x), with a reduction in genetic fluctuations among cohorts that is larger than accounted for by the cohort model. This would imply that N_e estimates based on the cohort model may be biased upwards for river systems with (unaccounted for) large components of non-anadromous salmon. Though the presence of non-anadromous life history phenotypes may generally enhance population stability, there is thus some uncertainty that this presence would also result in larger (and more stable) effective population sizes.

Secondly, freshwater survival in Atlantic salmon is likely not constant over time, with high mortality occurring from egg deposition to initiation of foraging by fry (reviewed by Hutchings and Jones 1998). However, the survivorship of relevance for N_e is that among the reproducing year classes (Nunney 1993) and we therefore ignored this initial stage in our life table analyses. Although the assumed annual survival rate corresponds to locally relevant empirical estimates (Evans *et al.* 1984), a more important consideration is whether this survival increases with age. An underestimation of freshwater survival during and after the male parr maturation ages implies that we may have overestimated the incidence of parr maturation and/or underestimated their relative survival. Although this has consequences for the age composition of adults, our simulations suggest that N_e estimation is more strongly influenced by assumptions about the age distribution of reproductive output. Furthermore, analyses of brown trout populations in lake systems in Sweden (Jorde and Ryman 1996; Palm *et al.* 2003) suggest a relative insensitivity of annual survival rate to age (i.e. type II survivorship curve). Therefore, our findings with regards to N_e may be comparatively robust to uncertainty about (relative) freshwater survival.

We further assumed that smolts that previously matured have similar ocean survival to that of non-mature fish, whereas some work suggests this may instead be reduced (Berglund *et al.* 1992 but see Skilbrei 1990). We therefore implicitly linked parr maturation with the sex bias in anadromous runs, ubiquitous in Newfoundland (Davis and Farwell 1975; Chadwick *et al.* 1978; O'Connell *et al.* 2006a). Fleming (1998) argued that increased ocean mortality, together with sex differences in (anadromous) age-at-maturity, form a better explanation for anadromous sex bias, although decreased freshwater survival was acknowledged to affect this bias. However, smolt runs in Newfoundland already display a strong bias towards females (Davis and Farwell 1975; Dalley 1978; Chadwick *et al.* 1978; Chadwick 1981; Dalley *et al.* 1983; Myers 1984; Hutchings 1986). Furthermore, anecdotal observations suggest there may be no sex difference in ocean survival for smolts in Newfoundland (Chadwick *et al.* 1978). Since our life table analyses explicitly corrected for sex differences related to anadromy, a link between parr maturation and sex bias in anadromous runs may therefore indeed exist in Newfoundland.

Finally, several lines of indirect evidence provide support for our life table analyses. Firstly, under the assumption that genetic drift is the predominant cause of temporal genetic fluctuations, allele frequency change (F) will depend on the time separating the two cohorts considered (Jorde and Ryman 1995). Low values of F are especially expected between cohorts that are born exactly one generation apart (Jorde and Ryman 1996, see also Waples and Teel 1990). A comparison of these values for the study rivers (*results not shown*) suggests that a generation time of roughly five years (as commonly derived from life table analyses) seems usually appropriate. Secondly, numerical estimates of relative parr abundance, based on these life tables, yielded numbers comparable to empirical observations. A quantification, based on the assumed survival and maturation schedules, suggests a range of 120-220 mature male parr may be present for each anadromous female in a river. These estimates are comparable to a value of 167 reported by L'Abée-Lund (1989) for a Norwegian river with similar (high) incidence of male parr maturation. These numbers are much lower (80-135 mature male parr per anadromous female), had we assumed zero survival of male parr after maturation. Despite some uncertainty remaining in life table analyses, the parameter estimates derived from them may thus provide a reasonable basis for estimating N_e .

References not included in main publication

- Adams, B.K. (2007) *Migratory strategies of Atlantic salmon (Salmo salar) In Newfoundland and Labrador*. PhD Thesis Dalhousie University, Halifax, Nova Scotia, Canada.
- Andrews, C.W. (1966) Landlocked Atlantic salmon (*Salmo salar* L.) in the Terra Nova River system, Newfoundland. *Can. Field Nat.* 80:101-109.
- Birt, T.P., Green, J.M. and W.S. Davidson (1991) Mitochondrial DNA variation reveals genetically distinct sympatric populations of anadromous and non-anadromous Atlantic salmon, *Salmo salar*. *Can. J. Fish. Aquat. Sci.* 48:577-582.
- Burgeois, C.E., Murray, J. and G. Clarke. A review of Atlantic salmon enhancement activities on the Terra Nova River (SFA 5), Newfoundland. 2002/025. 2002. Fisheries and Oceans, Science Branch. *Can. Sci. Advis. Secr. Res. Doc.*
- Chadwick, E.M.P., Porter, T.R. and P. Downton (1978) Analysis of growth of Atlantic salmon (*Salmo salar*) in a small Newfoundland river. *J. Fish Res. Board Can.* 35:60-68.
- Dalley, E.L. (1978) Studies on the biology of sexually mature male salmon parr, *Salmo salar* Linnaeus 1758, in insular Newfoundland. MSc. Thesis Memorial University of Newfoundland, St. John's, Newfoundland, Canada.
- Davis, J.P. and M.K. Farwell (1975) Exploits River and Indian River Atlantic salmon development programs, 1974. 1-75. Research Division Branch, St. John's, Newfoundland. *Can. Fish. Mar. Serv.*
- Hutchings, J.A. and R.A. Myers (1985) Mating between anadromous and non-anadromous Atlantic salmon, *Salmo salar*. *Can. J. Zool.* 63:2219-2221.
- O'Connell, M.F. and E.G.M. Ash (1993) Smolt size in relation to age at 1st maturity of Atlantic salmon (*Salmo salar*) - the role of lacustrine habitat. *J. Fish Biol.* 42:551-569.
- Pepper, V.A. (1976). Lacustrine nursery areas for Atlantic salmon in insular Newfoundland. 671. *Fish. Mar. Serv. Tech. Rep.*
- Power, G. (1958) The evolution of the freshwater races of the Atlantic salmon (*Salmo salar* L.) in eastern North America. *Arctic* 11:86-92.
- Scott, W.B. and E.J. Crossman (1964) Fishes occurring in the fresh waters of insular Newfoundland. Department of Fisheries, Queens Printer, Ottawa.
- Waples, R.S. and D.J. Teel (1990) Conservation genetics of Pacific salmon .I. Temporal changes in allele frequency. *Conservat. Biol.* 4:144-156.

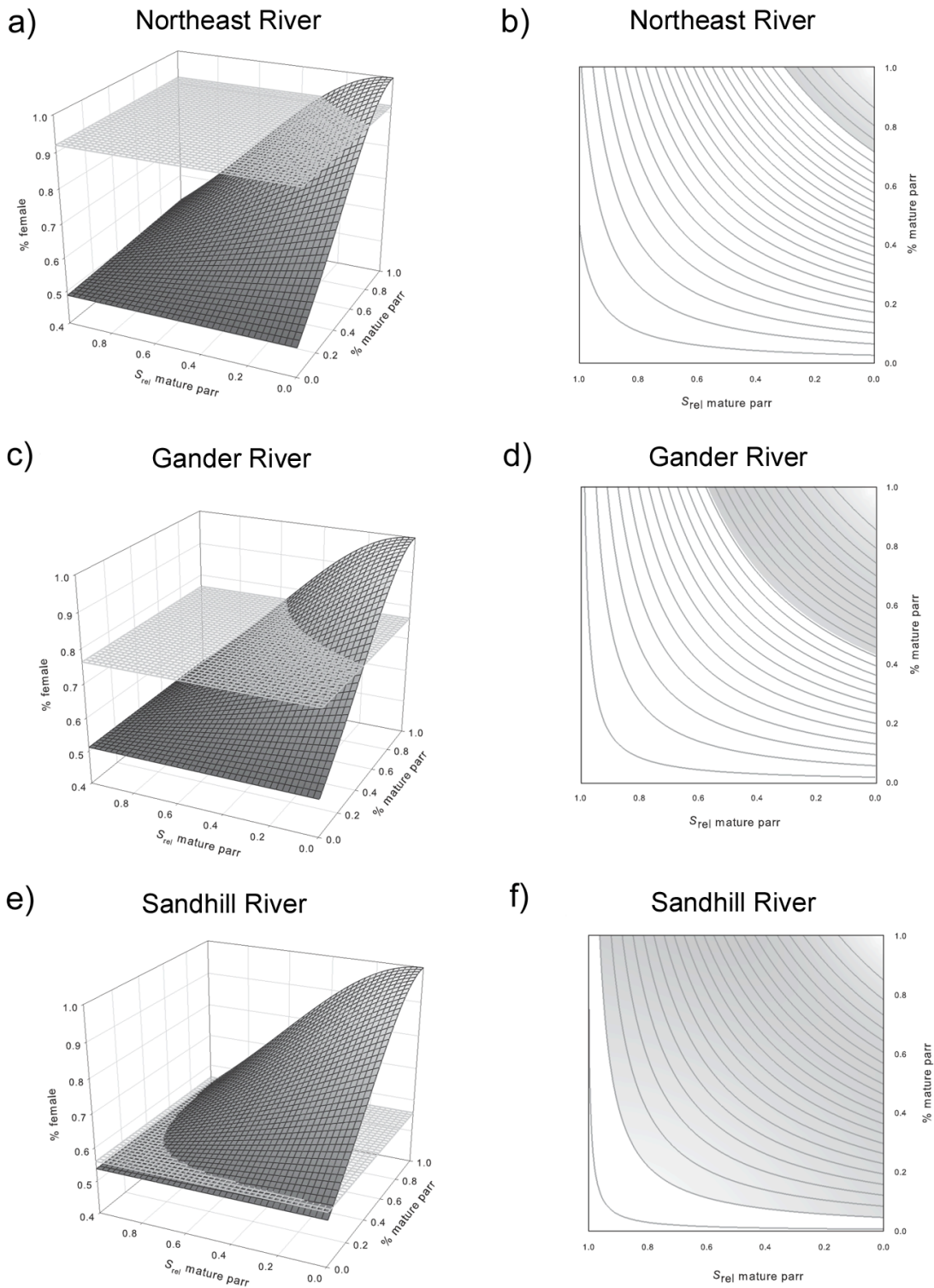


FIGURE S1.—Life table simulations for three different rivers, evaluating the consequences of mature parr incidence and mortality for anadromous sex ratios, compared to actual observed values in the specific rivers (intersecting plane). Graphs on the right illustrate the range of combinations of relative survival (S_{rel} mature parr) and maturation incidence (% mature parr) for which simulations resulted in anadromous sex ratios similar to the average of empirical values observed in the annual runs in each river.

TABLE S1**Basic descriptives of molecular markers**

Table S1 is available for download as an Excel file at <http://www.genetics.org/cgi/content/full/genetics.109.101972/DC1>.

TABLE S2**Comparison of Pacific Salmon N_b estimates to discrete generation estimates**

River	Year	\hat{S}	N_b	95% CI	N_b SalmonNb
Biscay Bay	1984	19.5	155	(29 - ∞)	97
	1990	26.7	83	(29 - ∞)	2366.5*
Conne	1980	26.5	39	(19 - 175)	207.9*
	1999	29.7	505	(48 - ∞)	279
Gander	1978	23.8	123	(31 - ∞)	180.2*
Middle Brook	1992	27.9	157	(36 - ∞)	667.6*
	1998	26.2	35	(18 - 116)	1396.4*
Northeast Brook	1987	25.3	69	(20 - ∞)	170.8*
Sandhill River	1967	33.8	196	(47 - ∞)	647.5*
Terra Nova	1978	26.7	295	(45 - ∞)	426.3*
	1979	28.3	186	(41 - ∞)	133

Comparison of Pacific Salmon N_b estimates to discrete generation estimates. Given for several rivers, where genetic data were available, is a comparison between estimates of the annual number of breeders as inferred from SALMONNb (WAPLES *et al.* 2007) and discrete generation N_b estimates. These latter estimates were calculated from the temporal genetic variance between samples of anadromous adults in a given year x and reconstructed cohorts of individuals (based on age information) born in year x , with harmonic mean sample size \hat{S} . These samples can therefore be assumed to be separated by one generation and expected to be unbiased by age structure. Asterisks indicate those Salmon N_b estimates that are larger than the discrete generation estimates. Results suggest the Pacific salmon model overestimates N_b in the majority of cases where relevant comparisons could be made.