

Demographic and genetic estimates of effective population size (N_e) reveals genetic compensation in steelhead trout

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Abstract

Estimates of effective population size (N_e) are required to predict the impacts of genetic drift and inbreeding on the evolutionary dynamics of populations. How the ratio of N_e to the number of sexually mature adults (N) varies in natural vertebrate populations has not been addressed. We examined the sensitivity of N_e/N to fluctuations of N and determined the major variables responsible for changing the ratio over a period of 17 years in a population of steelhead trout (*Oncorhynchus mykiss*) from Washington State. Demographic and genetic methods were used to estimate N_e . Genetic estimates of N_e were gained via temporal and linkage disequilibrium methods using data from eight microsatellite loci. DNA for genetic analysis was amplified from archived smolt scales. The N_e/N from 1977 to 1994, estimated using the temporal method, was 0.73 and the comprehensive demographic estimate of N_e/N over the same time period was 0.53. Demographic estimates of N_e indicated that variance in reproductive success had the most substantial impact on reducing N_e in this population, followed by fluctuations in population size. We found increased N_e/N ratios at low N , which we identified as genetic compensation. Combining the information from the demographic and genetic methods of estimating N_e allowed us to determine that a reduction in variance in reproductive success must be responsible for this compensation effect. Understanding genetic compensation in natural populations will be valuable for predicting the effects of changes in N (i.e. periods of high population density and bottlenecks) on the fitness and genetic variation of natural populations.

Keywords: effective number of breeders, genetic drift, inbreeding, microsatellite loci, *Oncorhynchus mykiss*, population productivity

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Introduction

The parameter, effective population size (N_e), incorporates relevant demographic information that determines the evolutionary consequences of members in a population contributing nonrandomly to future generations (Wright 1931, 1938). For those interested in the evolution and conservation of small populations, N_e is important because it is inversely related to the rate of loss of genetic diversity and the rate of increase in inbreeding (identity-by-descent) in a population that is finite, but otherwise randomly mating. The importance of N_e is heightened

by evidence that populations with low N_e are more susceptible to extinction (Newman & Pilson 1997; Saccheri *et al.* 1998).

Three variables are responsible for reducing N_e below the number of sexually mature adults in a population (N): fluctuations in population size (FPS), variance in individual reproductive success above binomial expectations (VRS), and an unequal sex ratio (SR). Frankham (1995) suggested that fluctuation in population size was the largest variable reducing N_e/N in natural populations. Others have argued that variance in reproductive success caused by high fecundity or polygamy is a major contributor to reducing N_e/N (Nunney 1996; Stortz *et al.* 2001).

In a review of studies that estimated N_e , Frankham (1995) found the average N_e to N ratio, across 102 species was 0.11. Nunney (1993) examined the theoretical basis of the N_e/N ratio and suggested it should be approximately 0.5

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under most natural conditions. The ratio of N_e/N in Pacific salmon (*Oncorhynchus* spp.) is thought to be close to 0.2 (R. Waples personal communication as cited in Allendorf *et al.* 1997). Only two published studies have estimated the ratio in Pacific salmonids. Bartley *et al.* (1992) estimated N_e/N to be 0.04 in a wild population of chinook salmon (*O. tshawytscha*) and Heath *et al.* (2002) reported point estimates of N_e/N ranging from 0.06 to 0.29 for three steelhead trout populations from northern British Columbia.

No studies have examined the temporal stability of the N_e/N ratio in a natural population of salmonids. It is highly possible that the salmonid N_e/N ratio could change within populations as spawner numbers fluctuate. Work with plants (Husband & Barrett 1992) and *Tribolium* (Wade 1980; Pray *et al.* 1996) indicate that the N_e/N ratio decreases as the population density increases. Biological evidence for the N_e/N ratio decreasing at high spawner numbers is provided by the work of Chebanov (1991), who found that a smaller percentage of female sockeye salmon (*O. nerka*) were able to reproduce successfully at high spawner densities. Although these experimental and theoretical findings suggest that high spawner numbers could decrease the N_e/N ratio, the impact of spawner numbers on the ratio of N_e/N in a wild population is currently unknown.

The temporal and linkage disequilibrium methods are two commonly used indirect genetic estimators of N_e in wild populations. The temporal method is based on the logic that if N_e is the only parameter needed to determine rates of change in genetic variation at neutral loci, then a measure of genetic change over time should allow the estimation of N_e (Waples 1989). The linkage disequilibrium method of estimating N_e , developed by Hill (1981) and modified by Waples (1991), is based on the principle that in closed finite populations, associations between alleles at different neutral loci are a function of the population's N_e ; therefore, measuring these associations between alleles should allow estimation of N_e .

Waples (1990a) has shown that effective population size per generation in Pacific salmon is equivalent to the effective number of breeders per year (N_b) multiplied by the generation length (g). Numerous procedures for estimating N_b from genetic data have been developed (Waples 1990b, 1991; Bartley *et al.* 1992; Tajima 1992) and, for anadromous Pacific salmonids, g corresponds closely to the average age at reproduction (Waples 1990a).

Demographic estimates of N_e and N_b use the concepts and equations introduced by Wright (1931, 1938) and later modified by others (reviewed by Caballero 1994). These equations use extensive demographic data to account for the effects of SR, VRS and FPS on the variance in allele frequency change and inbreeding in the population under consideration.

This paper presents the results of a study of historical population dynamics and associated genetic changes in an

anadromous natural population of *O. mykiss*, commonly called steelhead trout. We used selectively neutral genetic markers at microsatellite DNA loci and extensive demographic information to estimate N_b and N_e in this population over a period of 17 years. Genetic variability was assessed at three time points by analysing DNA from fish scales stored in an archival collection. The goals of the study were to: (i) estimate the ratios N_b/N and N_e/N , (ii) identify the most important variables contributing to the reduction of the ratios estimated in goal 1, (iii) determine if there is a relationship between spawner numbers and the N_b/N ratio, and (iv) compare comprehensive genetic and demographic estimates of N_e and N_b . The results of this study are the first to examine the impact of population size and density on the N_e/N ratio of a natural vertebrate population.

Materials and methods

Description of population study site

The Washington Department of Fish and Wildlife (WDFW) has operated a fish trap located 1.3 km from the mouth of Snow Creek and has monitored the winter steelhead adult and juvenile populations since 1977. Steelhead spawn above and below the trap, utilizing approximately 10 km of stream above the trap for spawning. Juveniles hatching from fertilized eggs that have incubated in redds (gravel nests) reside in Snow Creek for 1–3 years until they undergo smoltification, a physiological transformation that allows the fish to survive in salt water. Smolts then migrate down to the ocean. Steelhead in Snow Creek are native and have been designated a distinct stock on the basis of the geographical isolation of the local breeding population. The few steelhead adults of hatchery origin that have strayed into Snow Creek were captured at the trap and not released upstream. Information on the life history of steelhead in Snow Creek can be found in Johnson & Cooper (1992). No stocking has occurred in Snow Creek and fishing is prohibited.

Snow Creek flows directly into Discovery Bay on the Strait of Juan de Fuca. Elevation ranges from sea level to 1302 m on Mount Zion in Olympic National Park. The watershed supports the following species of salmonids: steelhead trout, chum salmon (*O. keta*), coho salmon (*O. kisutch*), and cutthroat trout (*O. clarki*, anadromous and resident forms). Johnson & Cooper (1992) described the Snow Creek watershed in detail.

Estimation of N and sex ratio

Estimates of the number of spawners per brood year (N) were based on data collected at the Snow Creek fish trap.

Since 1977, all adult fish released above the trap have been counted and identified by sex. The number below the trap was determined with the following equation: (number of steelhead redds) \times (0.81 females/redd) \times (1/proportion female captured in upstream trap). Redds were counted throughout the spawning season. The value, (0.81 females/redd), is an average derived at Snow Creek during a 7-year period when all redds were counted above the trap and compared with the number of adults released above the trap (Johnson & Cooper 1992).

Calculation of recruits, recruits-per-spawner and brood year contribution to the next generation

The WDFW examined growth annuli on scales from all adults sampled at the fish trap to determine the age of each individual. We used this age information to determine the number of recruits produced by each brood year. Recruits (R_i) are defined as the number of offspring produced in brood year i that return as spawners in subsequent brood years. We define R_T as the total number of spawners in the next generation produced by all the spawners in the current generation (i.e. $\sum R_i$ over a generation). The yearly spawner abundance in brood year i (N_i) and the number of recruits were used to calculate the recruits per spawner ratio (λ) for each brood year as R_i/N_i . The relative contribution of brood year i to the next generation (X_i) was calculated as R_i/R_T .

Freshwater population productivity estimates

The number of smolts produced per spawner in a brood year was used as an index of population productivity during the freshwater life phase. WDFW examined scales from all smolts sampled at the fish trap to determine the age of each individual. Once the age of each smolt was determined, all fish were assigned to their respective brood year.

Demographic estimators of N_b and N_e

We used the methods of Lande & Barrowclough (1987) for combining N_e estimates gained from each demographic variable (SR, VRS, FPS) into a single comprehensive N_e estimate. The Lande and Barrowclough estimator assumes discrete generations, we therefore used the method of Waples (2002) to estimate N_e based on FPS because this method accounts for overlapping generations with a semelparous life history characteristic of salmonids.

Unequal sex ratio

The reduction in N_b caused by an unequal sex ratio is defined as:

$$N_{b(\text{sex ratio})} = 4(N_f \times N_m)/(N_f + N_m) \quad (1)$$

where N_f represents the number of sexually mature female and N_m represents the number of male steelhead trout returning to Snow Creek in any given brood year.

Variance in reproductive success

Letting k represent the number of progeny produced by an individual during its lifetime, the effective numbers of males and females in a brood year (N_{bm}, N_{bf}) are estimated as:

$$N_{bm} = (N_m \bar{k}_m - 1)/[\bar{k}_m + (\sigma_{k_m}^2/\bar{k}_m) - 1] \quad (2)$$

$$N_{bf} = (N_f \bar{k}_f - 1)/[\bar{k}_f + (\sigma_{k_f}^2/\bar{k}_f) - 1] \quad (3)$$

The symbols N_f and N_m are as defined in equation 1 and \bar{k} and σ_k are the mean and variance of k for each sex.

Effective number of breeders in a brood year

The following equation provides an estimate of the effective number of breeders from the sampling variance in allele frequency per brood year caused by unequal SR and VRS

$$N_{b(Demo)} = \frac{4(N_{bf} * N_{bm})}{N_{bf} + N_{bm}} \quad (4)$$

Fluctuations in population size

We used the method of Waples (2002) to account for the influence of FPS on N_e . This method provides a way to incorporate the information from $N_{b(Demo)}$ estimated from equation 4 into a single-generation N_e estimate for salmon populations that fluctuate in size. The following equation provides a comprehensive estimate of effective population size over a full generation:

$$N_{e(Demo)} = 1 / \sum (X_i^2 / N_{b(Demo)i}) \quad (5)$$

where $N_{b(Demo)i}$ is the effective size of brood year i , X_i is the proportional contribution of spawners in brood year i to the next generation, and the summation is over all brood years in a generation. $N_{e(Demo)}$ is useful for evaluating the genetic effects of differential reproductive success by each of the brood years in a generation. If one assumes $N_b/N = 1$ in all brood years, equation 5 reduces to measuring the effects of FPS on N_e .

Collection of smolt samples for genetic analysis

We obtained scale samples from an archival collection maintained by the WDFW. The collection consisted of scales from steelhead adults and smolts captured at the fish

trap. All smolt scale samples were taken in a sampling regime that was stratified according to smolt size and migration time. Scales from each individual were stored separately in envelopes or gum cards that recorded information about the individual. Ward & Slaney (1988) provide a detailed description of the methods used to sample smolts. We chose three years for sampling based on two criteria: (i) availability of a large number of randomly sampled individuals from a single brood year, and (ii) separation of the samples by many years. We took samples from 2-year-old smolts migrating downstream in 1979, 1987 and 1996 to estimate the allele frequencies of the successful spawners in the 1977, 1985 and 1994 brood years.

Analysis of genetic variation at microsatellite loci

We used the following microsatellite primers to examine nuclear DNA polymorphisms: *Omy77* (Morris *et al.* 1996), *Oneu3*, *Oneu8*, *Oneu11*, and *Oneu14* (Scribner *et al.* 1996), *Str2* (Estoup *et al.* 1998), and *Ssa85* and *Ssa197* (O'Reilly *et al.* 1996). DNA was extracted from epithelial cells adherent on the fish scales by boiling the scales in a resin solution (Chelex 100, Sigma). Polymerase chain reaction conditions and scoring of polymorphisms followed the methods of Ardren *et al.* (1999).

Genetic estimators of N_b and N_e

Estimation of $N_{b(T)}$. To estimate N_b via the temporal method we used the following equation:

$$\hat{N}_{b(T)} = b / \{2[\hat{F} - 1/\bar{S} + (1/N_0)]\} \quad (6)$$

because the sampling of scales was nonlethal, which allowed individuals the opportunity to contribute to future generations (Waples 1989). We estimated the standardized variance in allele frequency change (\hat{F}), the parameter b , which is an analogue of the number of generations between samples in the discrete generation model (Waples 1990b; Tajima 1992) and $N_{0'}$ which is the spawner census size of the brood year at time 0. Sample sizes (S) were the harmonic means of the sample sizes at time 0 and t .

Estimation of F . Several methods of computing F have been proposed. We found similar results for F_c (Nei & Tajima 1981) and F_k (Pollack 1983) so we only report F_c here. Nei & Tajima's (1981) measure for one locus is:

$$\hat{F}_c = \frac{1}{L} \sum_{i=1}^L \frac{(X_{oi} - X_{ti})^2}{(X_{oi} + X_{ti})/2 - X_{oi} + X_{ti}} \quad (7)$$

where L is the number of alleles and the values of X are the frequencies of allele i ($i = 1, 2, \dots, L$) at sampling times 0 and t . For multiple loci, we computed a weighted mean \hat{F} as:

$$\hat{F}_c = \frac{\sum(L_j F_{c_j})}{\sum L_j} \quad (8)$$

where the value j is the index of the different loci (Nei & Tajima 1981). The 95% confidence limits for N_b and N_e were determined according to the methods of Waples (1989).

Estimation of b . Because steelhead do not conform to the discrete generation model, we could not directly estimate the number of generations between samples (t) based solely on the number of years between samples. We used the method developed by Waples (1990b) for estimating N_b from \hat{F} that incorporates the Pacific salmon life history. The value of b is determined by the age structure of the population and the number of years between samples. Values for b were obtained using a BASIC computer program and a simple algorithm developed by Tajima (1992). Age composition of the spawners and the generation interval (g) for Snow Creek steelhead trout were estimated based on information collected by Johnson & Cooper (1992).

Estimation of $N_{e(T)}$. We estimated N_e for the population over the time period examined by estimating N_b and multiplying it by g . This method was developed with the assumption of semelparity, but Waples (1990a) has found it to be applicable to populations of steelhead in which repeat spawning is low. In the Snow Creek local breeding population, the frequency of repeat spawners averages 10% (R. Cooper, personal communication), which is considered low enough to estimate $N_{b(T)}$ with little bias (R. S. Waples, personal communication).

Estimation of $N_{b(D)}$. We used the method developed by Hill (1981) for combining multiple pairs of unlinked loci to estimate N_e using linkage disequilibrium data. The nonrandom association of alleles at different loci is linkage disequilibrium. Disequilibrium (D) can be caused by random genetic drift, migration, epistatic selection, and founder events. The correlation of allele frequencies, $r = D/[p(1-p)q(1-q)]^{1/2}$, is a related measure of association among alleles. We used the method of Waples (1991) for combining multiple pairs of unlinked loci to estimate N_e . For unlinked loci, r is related to N_e by its expected variance, $E(r^2) = 1/(3N_e)$ (Hill 1981). Estimating r^2 by sampling from the populations produces another source of error; consequently, for sample size S , the relationship is:

$$E(\hat{r}^2) = 1/(3N_e) + 1/S \quad (9)$$

which can be rearranged as,

$$\hat{N}_{e(D)} = 1/[3(\hat{r}^2 - 1/S)] \quad (10)$$

Table 1 Characterization of microsatellite loci observed in Snow Creek steelhead trout

Locus	1977 brood year ($n = 50$)				1985 brood year ($n = 50$)				1994 brood year ($n = 50$)			
	Alleles	H_O^*	H_E^\dagger	P^\ddagger	Alleles	H_O	H_E	P	Alleles	H_O	H_E	P
<i>Omy77</i>	19	0.72	0.88	0.006	11	0.72	0.82	0.183	10	0.82	0.80	0.098
<i>Oneμ11</i>	3	0.22	0.26	0.342	3	0.40	0.36	0.830	3	0.48	0.43	0.819
<i>Oneμ14</i>	8	0.60	0.69	0.301	8	0.52	0.73	0.008	8	0.60	0.76	0.022
<i>Oneμ3</i>	14	0.76	0.89	0.081	15	0.94	0.89	0.731	10	0.72	0.83	0.547
<i>Oneμ8</i>	4	0.36	0.46	0.195	3	0.38	0.46	0.095	3	0.40	0.38	1
<i>Ssa197</i>	2	0.42	0.49	0.384	2	0.46	0.42	0.511	3	0.36	0.38	0.265
<i>Ssa85</i>	19	0.86	0.90	0.371	18	0.74	0.88	0.018	19	0.86	0.88	0.375
<i>Str2</i>	27	0.92	0.95	0.067	22	0.94	0.93	0.029	19	0.96	0.92	0.050
Over all loci	96	0.61	0.69	—	82	0.64	0.69	—	75	0.65	0.67	—

* H_O , observed heterozygosity.

† H_E , expected heterozygosity.

‡ P , probability level for Hardy–Weinberg equilibrium. None of the tests for conformance to Hardy–Weinberg expectations were significant at the $P < 0.05$ level after sequential Bonferroni adjustments for simultaneous tests.

(Hill 1981), where $N_{e(D)}$ is an estimate of N_e made by the gametic disequilibrium method. We estimated D using the method of Burrows (Cockerham & Weir 1977), using only the most common allele at every locus. Confidence intervals for $N_{e(D)}$ were estimated according Waples (1990). Each of our samples was from a brood year rather than the entire population. Therefore, the estimate in equation 10 is for the effective number of breeders in the sampled brood year ($N_{b(D)}$) rather than $N_{e(D)}$ per generation.

Estimating the relationship of N_b/N or N_e/N

We used the ratio of N_e or N_b to the arithmetic mean of N (\bar{N}) as an index of the cumulative impacts of interbrood year (FPS) and intrabrood year (VRS and SR) variables in reducing N_b/N or N_e/N . To isolate the cumulative effect of the intrabrood year variables in reducing N_b/N and N_e/N , we estimated the ratios with the harmonic mean of N (\bar{N}) in the denominator (Kalinowski & Waples 2002).

Additional approaches for detecting genetic bottlenecks

We used two additional tests to monitor the Snow Creek steelhead population for evidence of genetic change induced by a bottleneck (low N_b). The Wilcoxon signed rank test was used to determine if a significant reduction in the number of alleles per locus occurred between any of the sampling periods. We also used the graphical method of Luikart *et al.* (1998) to determine if a bottleneck-induced distortion of allele frequency distributions had occurred in this population. To examine the allele frequency distribution in each sampling period, we grouped all the alleles from the eight loci into 10 allele frequency classes

and plotted a frequency histogram. The allele frequency classes were in 0.10 increments starting with 0–0.1 and ending with 0.901–1. Evidence for a recent bottleneck was defined as the low-frequency alleles (0–0.1) becoming less abundant than the intermediate-frequency alleles (0.101–0.9).

Data analysis

All statistical analyses were performed using Microsoft EXCEL (Office 97) or SYSTAT (v.5.0). Observed and expected heterozygosities, allele frequencies, and tests for conformance of genotypic frequencies to Hardy–Weinberg expectations were calculated using TOOLS FOR POPULATION GENETICS ANALYSIS (TFPGA) v1.3 (Mark P. Miller, Northern Arizona University). Statistical significance levels (α) for conformity to Hardy–Weinberg expectations were determined using sequential Bonferroni adjustments for simultaneous tests.

Results

Genetic variation

Allelic frequencies. The number of alleles per locus over all samples ranged from two to 27, with an average of 11 (Table 1). The frequency of the most common allele at a locus ranged from 0.10 to 0.85. The mean frequency of the most common allele over all time periods was 0.43. For some loci, the most common allele changed over the 17-year study period.

Figure 1 presents the allele frequency distributions for all eight loci during each of the three brood years. All brood

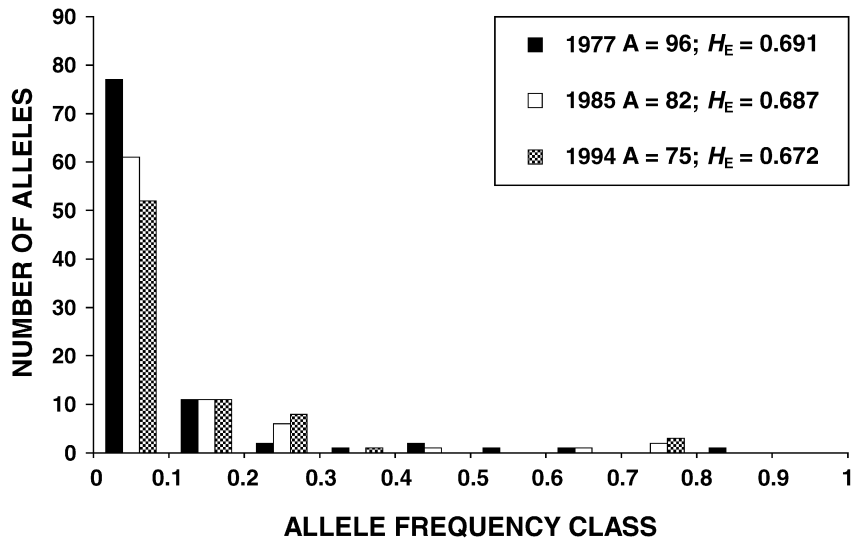


Fig. 1 Historical comparison of the allele frequency distributions at eight microsatellite loci in the Snow Creek steelhead trout population based on sampling 50 smolts for each time period. Numbers in the legend are the number of total alleles (A) found at the examined time period and the expected heterozygosity (H_E) over all loci.

years had more alleles in the low-frequency class than in the intermediate-frequency classes, indicating that this population has not recently undergone a severe genetic bottleneck. However, there was a noticeable trend in the reduction of total number of alleles observed over time, with 96, 82 and 75 alleles detected in the 1977, 1985 and 1994 brood years, respectively. The Wilcoxon signed rank test indicated that the reduction in the number of alleles per locus between the 1977 and 1994 brood years was marginally significant ($P = 0.052$).

Genotypic frequencies. Genotypic frequencies at all loci in Snow Creek were in agreement with Hardy–Weinberg expectations (Table 1). Expected heterozygosities (H_E) at individual loci ranged from 0.26 to 0.95. Estimated H_E over all loci were 0.69, 0.69 and 0.67 for the 1977, 1985 and 1994 brood years, respectively.

Demographic estimates of N_b and N_e

Number of spawners (N). Estimates of N from 1977 to 1994 ranged from 12 to 154 (Fig. 2). Over the periods 1977–85, 1985–94 and 1977–94, N averaged 113, 56 and 79 spawners, respectively.

Impact of SR on the reduction of N_b . The sex ratio accounted for a ratio, $N_b/N = 0.91$ in the Snow Creek population over the entire time period. This minor reduction in N_b was a result of the almost equal SR in most of the brood years examined (Table 2). In the 1984 brood year the sex ratio was one so SR caused no reduction in N_b/N . The largest reduction in N_b/N caused by SR occurred in the 1986 brood year when 49 females and 12 males returned to spawn resulting in a N_b/N ratio of 0.62.

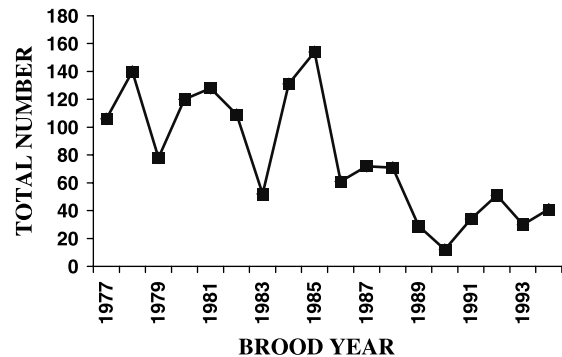


Fig. 2 Number of steelhead returning to spawn in Snow Creek from 1977 to 1994.

Impact of VRS on the reduction of N_b . Direct estimates of \bar{k} and σ_k^2 were not available for Snow Creek steelhead. We therefore used a reasonable approximation consisting of values for \bar{k} and σ_k^2 that Garant *et al.* (2001) had reported for an Atlantic salmon population of approximately the same size as the Snow Creek steelhead trout population. Garant *et al.* (2001) had determined microsatellite loci genotypes for all candidate parents and then used microsatellite genotypes of fry to assign parentage to 650 fry sampled from throughout the watershed.

Using the values of \bar{k} and σ_k^2 reported in Garant *et al.* (2001), we found that VRS resulted in an average N_b/N ratio of 0.68 in the females and 0.66 in the males. The impact of VRS on each sex can be determined within each brood year by comparing N_{bf} with the number of females and N_{bm} with the number of males in each brood year (Table 2).

Impact of FPS on the reduction of N_e . To isolate the effects of FPS on N_e , we assumed $N_b/N = 1$ in each brood year (i.e.

Table 2 Estimates of N_b for the 1977–94 Snow Creek steelhead trout brood years based on sex ratio and variance in reproductive success data

Brood year	No. of females	No. of males	$N_{b(\text{SexRatio})}^*$	$N_{b(\text{SexRatio})}/N^*$	N_{bf}^\dagger	N_{bm}^\dagger	$N_{b(\text{Demo})}^\ddagger$	$N_{b(\text{Demo})}/N^\ddagger$
1977	61	45	104	0.98	41.3	29.6	69.0	0.65
1978	57	83	135	0.96	38.5	54.6	90.4	0.65
1979	36	42	77	0.99	24.3	27.6	51.8	0.67
1980	64	56	119	0.99	43.8	36.4	79.5	0.67
1981	61	67	128	1	41.3	44.1	85.3	0.67
1982	63	46	107	0.98	42.6	30.4	70.9	0.65
1983	23	29	51	0.98	15.3	19.3	34.1	0.66
1984	65.5	65.5	131	1	44.5	42.9	87.4	0.67
1985	67	87	151	0.98	45.6	56.9	101.3	0.66
1986	49	12	38	0.62	33.5	7.6	24.9	0.41
1987	45	27	68	0.94	30.4	17.8	44.9	0.63
1988	49	22	60	0.85	33.6	14.1	39.8	0.56
1989	15	14	29	1	10.3	9.0	19.2	0.67
1990	9	3	9	0.75	6.1	1.9	5.8	0.49
1991	21	13	32	0.95	14.1	8.6	21.4	0.63
1992	34	17	46	0.90	22.8	11.4	30.4	0.60
1993	21	9	25	0.83	14.4	5.7	16.4	0.55
1994	25	16	39	0.94	17.2	10.2	25.6	0.63
Harmonic mean				0.91				0.61

* N_b based on SR information only.

† \bar{k} and σ_k^2 values taken from Garant *et al.* 2001; ($\bar{k}_f = 16.9$ and $\sigma_{kf}^2 = 151.0$), ($\bar{k}_m = 14.5$ and $\sigma_{km}^2 = 124.8$).

‡ N_b based on SR and VRS information; (\bar{k} and $\sigma_k^2 =$ values in previous two columns).

Table 3 Demographic estimates of N_e for Snow Creek Steelhead trout from 1977 to 1994; this table presents comprehensive N_e estimates and N_e estimates which isolate the effects of fluctuating population size

Generation*	Brood years	$4 \times \bar{N}^\dagger$	$4 \times \bar{N}^\ddagger$	N_e based on FPS (assuming $N_b/N = 1$)		Comprehensive N_e (assuming $N_{b(\text{Demo})}/N = 0.61$)	
				$N_{e(\text{Demo})}$ (Eqn 5)	$N_{e(\text{Demo})}/N_T$	$N_{e(\text{Demo})}$ (Eqn 5)	$N_{e(\text{Demo})}/N_T$
1	1977–80	444	424	425	0.96	259	0.59
2	1981–84	420	365	322	0.77	196	0.47
3	1985–88	358	315	299	0.84	182	0.51
4	1989–92	126	96	112	0.89	68	0.54
5	1991–94	156	150	146	0.94	89	0.57
Harmonic mean (4.5 generations)		230	197	203	0.87	124	0.53

*A generation is assumed to contain 4 brood years.

†Arithmetic mean of the number of spawners in a generation (\bar{N}) multiplied by the generation interval (4 years). This value also equals the total number of spawners within a generation (N_T).

‡Harmonic mean of the number of spawners in a generation (\bar{N}) multiplied by the generation interval (4 years).

§The ratio $N_{b(\text{Demo})}/N = 0.61$ is the harmonic mean of the $N_{b(\text{Demo})}/N$ ratios estimated for each brood year from 1977 to 1994 (see Table 2).

equal sex ratio and binomial variance in reproductive success among individuals). This allowed us to isolate the effects of demographic processes among brood years. The estimated generation interval for Snow Creek steelhead was 3.95 so we used 4-year generation blocks to analyse the data. For the 4.5 generations of data shown in Table 3, the

ratio $N_{e(\text{Demo})}/N_T$ ranged from 0.77 to 0.96. N_T is defined as the total number of spawners in a generation (i.e. ΣN_i over a generation). Over all generations examined in this study, the harmonic mean of the single generation values computed using equation 5 was 203. When considering the entire time period, FPS led to an $N_{e(\text{Demo})}/N_T$ ratio of 0.87.

Brood year	Spawners (N_i)	Recruits* (R_i)	R_i/N_i (λ_i)	N_i/N_T	R_i/R_T (X_i)
1977	106	132	1.24	0.24	0.31
1978	140	105	0.75	0.32	0.24
1979	78	63	0.81	0.18	0.15
1980	120	130	1.08	0.27	0.30
Generation 1 N_T, R_T	444	429			
1981	128	127	0.99	0.30	0.38
1982	109	70	0.64	0.26	0.21
1983	52	92	1.78	0.12	0.27
1984	131	47	0.36	0.31	0.14
Generation 2 N_T, R_T	420	337			
1985	154	32	0.21	0.43	0.26
1986	61	15	0.24	0.17	0.12
1987	72	38	0.53	0.20	0.31
1988	71	37	0.52	0.20	0.30
Generation 3 N_T, R_T	358	121			
1989	29	31	1.07	0.23	0.13
1990	12	35	2.90	0.10	0.15
1991	34	90	2.64	0.27	0.38
1992	51	83	1.62	0.40	0.35
Generation 4 N_T, R_T	126	239			
1991	34	90	2.6	0.22	0.27
1992	51	83	1.6	0.33	0.25
1993	30	86	2.9	0.19	0.26
1994	41	71	1.7	0.26	0.21
Generation 5 N_T, R_T	156	330			

*Total number of adult spawners in subsequent years produced by adults spawning in that brood year.

As Waples (2002) has pointed out, it is useful to compare N_i/N_T (the fraction of total adults in a generation that spawn in that year) with X_i (the fraction of recruits in the next generation produced by that brood year) to determine the influences of FPS on N_e . The N_e is maximized when $X_i = N_i/N_T$ for each brood year within a generation. This condition was almost met for generation 1 of the Snow Creek dataset ($N_{e(Demo)}/N_T = 0.96$ in Table 3), which had the largest N_e/N ratio. This result indicates that spawners contributed to subsequent generations at a rate that was directly proportional to their abundance within their own generation. In contrast, the largest reduction in the N_e/N ratio due to FPS occurred in generation 2 ($N_{e(Demo)}/N_T = 0.77$ in Table 3). In this case, the 1984 brood year had the highest N_i/N_T but the lowest X_i (Table 4). As a result, the 1984 brood year represented 31% of the spawners in generation 2 but only contributed 14% of the genes to the next generation.

Comprehensive demographic N_b and N_e estimates. We estimated the comprehensive $N_{e(Demo)}/N_T$ to be 0.53 as a result of the cumulative effects of VRS, FPS and SR over the entire 17-year period (Table 3). There was little variation in the

Table 4 Spawner–recruit data for 18 brood years in the Snow Creek, WA steelhead trout population (source Johnson & Cooper 1992 and T. Johnson personal communication 2002)

values of $N_{e(Demo)}/N_T$ in each of the generations examined (0.47–0.59). This was caused in large part by the low levels of variation in the intrabrood year variables that had the largest impact on reducing $N_{e(Demo)}$. The $N_{b(Demo)}/N$ estimates that combined effects of SR and VRS (Table 2), in each brood year, ranged from a high of 0.67 to a low of 0.41 and the harmonic mean of the ratio was 0.61 over the entire 17-year period. VRS had a much stronger effect in reducing the $N_{b(Demo)}/N$ ratio than SR.

When considering each of the demographic variables independently, VRS caused the largest reduction in $N_{e(Demo)}/N_T$. The impact of fluctuation in population size had the second largest impact in reducing $N_{e(Demo)}/N_T$ but was tempered by a proportional contribution of each segment of a generation to subsequent generations. Sex ratio caused a minor reduction in $N_{e(Demo)}/N_T$.

Genetic estimates of N_b and N_e

Temporal Changes in Allelic Frequency. Allele frequency changes between years at all loci were determined for three time intervals: 1977–85, 1985–94 and the entire interval 1977–94. We pooled all alleles at a locus with frequencies that

Table 5 Estimated age composition of Snow Creek steelhead spawners given by the frequency (f) of each age from 3 to 6 years (where $\sum f(x) = 1$), generation interval (g), and the parameter b that is analogous to time in generations between samples in the temporal model with discrete generations

Time period	$f(3)\ddagger$	$f(4)\ddagger$	$f(5)\ddagger$	$f(6)\ddagger$	g	b
1977–85*	0.061	0.7308	0.1923	0.0128	4.18	2.07
1985–94†	0.1429	0.7619	0.0952	—	3.95	3.93
1977–94†	0.1429	0.7619	0.0952	—	3.95	4.33

*Age composition determined from the 1985 brood year.

†Age composition determined from the 1994 brood year.

‡Data collected from the portion of the adults returning to spawn above the Snow Creek fish trap. Approximately 51% of the steelhead returning to Snow Creek spawned above the fish trap in 1985 and 1994.

averaged less than 0.02 over both sampled years into a single allele class (Waples 1990b). Magnitudes of change varied greatly among all alleles and often among time intervals for the same allele. Allele frequency changes were as small as 0.01 for multiple alleles at multiple loci over all time periods and as large as 0.17 for an allele at *Ssa197* during 1977–94.

Estimates of N_b and N_e using the temporal method. Spawner ages in the 1985 and 1994 brood years ranged from 3 years to 6 years with close to 75% of the fish spawning at 4 years of age (Table 5). The age composition of spawners resulted in a generation interval of 4.18 years for the 1977–85 interval and 3.95 years for the 1977–94 and 1985–94 intervals. The value of b ranged from 2.07 to 4.33 (Table 5).

Table 6 reports estimates of $N_{b(T)}$ and $N_{e(T)}$ based on the temporal changes in allele frequencies observed in the three time intervals. Estimates of $N_{b(T)}$ were 55 for 1977–85, 87 for 1985–94, and 58 for 1977–94. Point estimates of $N_{b(T)}$

indicated an increased N_b during the 1985–94 period. When the 95% confidence intervals (CI) were considered, there was no evidence that $N_{b(T)}$ changed over the three time periods. Multiplying N_b by g produced N_e estimates for Snow Creek. The lower bounds of all of the 95% CI of $N_{e(T)}$ were entirely above 50. The upper bounds of $N_{e(T)}$ 95% CI for the entire 17-year study period (1977–94) did not exceed 500.

To examine the effect of each locus on $N_{b(T)}$, we removed one locus at a time and recalculated estimates based on the remaining loci. The only change in $N_{b(T)}$ that was greater than 20% was caused by removing the *Oneu3* locus, which led to a 24% increase in $N_{b(T)}$ for the second interval.

Estimates of N_b using the linkage disequilibrium method. Estimates of $N_{b(D)}$ for Snow Creek (Table 7) were similar to estimates using the temporal method. We estimated $N_{b(D)}$ to be 30, 20 and 37 in 1977, 1985 and 1994, respectively.

Ratio of effective number of breeders to number of spawners per brood year. Ratios of $N_{b(T)}/\bar{N}$, $N_{b(D)}/N$, and $N_{b(T)}/\bar{N}$ were estimated for all time periods and sampling dates (Table 8). The 95% confidence intervals did not overlap for the estimates of $N_{b(T)}/\bar{N}$ from 1977 to 1985 (0.25–0.76) and 1985–94 (1.04–3.45) and the 95% confidence intervals for $N_{b(T)}/\bar{N}$ overlapped to the smallest degree from 1977 to 1985 (0.23–0.68) and 1985–94 (0.68–2.25), indicating that the ratios changed in the population across these two time periods. Estimates of $N_{b(D)}/N$ also suggested that the ratio had changed in this population. The point estimates for $N_{b(D)}/N$ in 1977 and 1985 were below 0.5 while in the 1994 the estimate climbed to 0.90.

The point estimates of $N_{b(T)}/\bar{N}$ and $N_{b(T)}/\bar{N}$ were nearly identical for the 1977–85 time period, indicating FPS had little impact on reducing the $N_{b(T)}/\bar{N}$ ratio over this interval. Alternatively, FPS appeared to play a major role in reducing the $N_{b(T)}/\bar{N}$ ratio from 1985 to 1994 as indicated

Table 6 Estimated effective number of breeders per brood year ($N_{b(T)}$) and effective population size per generation ($N_{e(T)}$) using the temporal method for Snow Creek steelhead trout

Period	F_c^*	b^\dagger	S^\ddagger	N_0^\S	K^\parallel	n^{**}	g^{++}	$N_{b(T)}$ (95% CI)	$N_{e(T)}$ (95% CI)
1977–85	0.0294	2.07	50	106	64	56	4.15	55 (26–77)	228 (108–320)
1985–94	0.0360	3.93	50	154	56	48	3.95	87 (38–125)	344 (150–494)
1977–94	0.0479	4.33	50	106	63	55	3.95	58 (30–76)	233 (119–300)

*Standardized variance of allele frequency change \hat{F}_c (Nei & Tajima 1981).

†An analogue of the number of generations elapsed between two samples in the discrete generation model; the value of b is determined by the age structure of the spawners and the number of years between sampling dates Waples 1990b.

‡Harmonic mean of sample sizes.

§Number of spawners at time 0.

¶Total number of alleles over all loci.

**Degrees of freedom = $K - \text{number of loci examined}$.

++Generation interval.

Table 7 Estimates of effective number of breeders for Snow Creek Steelhead at three time periods using the disequilibrium method ($N_{b(D)}$)

Brood Year	Statistic				
	S^*	$L†$	$r^2‡$	$N_{b(D)}$	(CI)§
1977	50	8	0.031	30	9–∞
1985	50	8	0.037	20	7–101
1994	50	8	0.029	37	10–∞

*Sample size.

†Number of loci.

‡Mean squared correlation of allele frequencies at pairs of loci.

§95% confidence interval for estimates (Waples 1991).

Table 8 Ratio of effective number of breeders to number of spawners per brood year estimated using the temporal ($N_{b(T)}$) and linkage disequilibrium ($N_{b(D)}$) methods

Time period	\bar{N}^*	$\tilde{N}†$	$N_{b(T)}/\bar{N}$ (95% CI)	$N_{b(T)}/\tilde{N}†$ (95% CI)
1977–85	113	102	0.49 (0.23–0.68)	0.54 (0.25–0.76)
1985–94	56	36	1.57 (0.68–2.25)	2.40 (1.04–3.45)
1977–94	79	50	0.73 (0.38–0.96)	1.15 (0.59–1.51)
	$N‡$		$N_{b(D)}/N‡$	
1977	106		0.28 (0.09–∞)	—
1985	154		0.16 (0.05–0.66)	—
1994	41		0.90 (0.24–∞)	—

*Arithmetic mean of spawners over the time period.

†Harmonic mean of spawners over the time period.

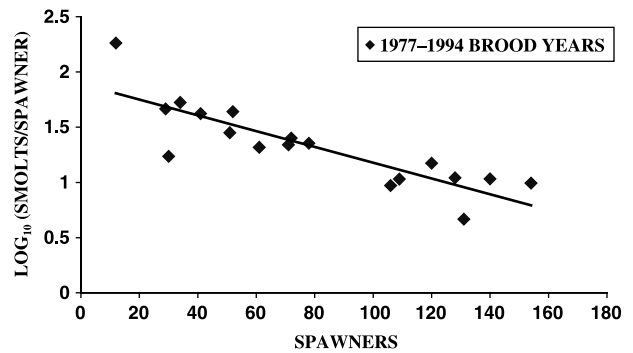
‡Spawners for the 1977, 1985 and 1994 brood years, respectively.

by the large difference between the point estimates of the $N_{b(T)}/\bar{N}$ and $N_{b(T)}/\tilde{N}$ ratios over this time period.

Comparing demographic and genetic estimates of N_b and N_e

The $N_{e(T)}$ and $N_{e(Demo)}$ are both comprehensive estimates of N_e . Estimates of $N_{e(Demo)}$ for generations 1 (1977–80; $N_e = 259$) and 2 (1981–84; $N_e = 196$) were very close to the 1977–85 $N_{e(T)}$ point estimate of 232. However, the harmonic mean of 95 for the estimates of $N_{e(Demo)}$ for generations 3 (1985–88; $N_e = 182$), 4 (1989–92; $N_e = 68$) and 5 (1991–94; $N_e = 89$) was lower than the 1985–94 $N_{e(T)}$ 95% confidence interval of 150–494.

Comprehensive intrabrood year estimates were made with $N_{b(D)}$ and the $N_{b(Demo)}$. The only point estimate of $N_{b(D)}$ with a defined upper confidence limit was for the 1985

**Fig. 3** Relationship between the number of spawners per brood year and the number of smolts produced per spawner in that brood year for Snow Creek steelhead trout. A least squares trendline was fit to the data using the equation $y = -0.0071x + 1.8933$ ($R^2 = 0.72$).

brood year. The point estimate of 20 for $N_{b(D)}$ (95% CI of 7–101) in the 1985 brood year is much lower than the $N_{b(Demo)}$ estimate of 101.3 based on SR and VRS.

N_b and productivity

There was a strong inverse relationship between the number of spawners in a brood year and the number of smolts produced per spawner in that brood (Fig. 3). When the number of spawners was low in a brood year, the number of smolts produced per spawner increased dramatically; and as the number of spawners increased, fewer smolts were produced per spawner.

Snow Creek had a higher $N_{b(T)}/\tilde{N}$ ratio in 1985–94 than in 1977–85 (Table 8). The 1985–94 period was characterized by low spawner numbers and high numbers of smolts produced per spawner while the 1977–85 period had higher spawner numbers and a lower number of smolts produced per spawner (Figs 2, 3). Ratios of $N_{b(D)}/\bar{N}$ for the three brood years sampled indicated the $N_{b(D)}/\bar{N}$ ratio was no larger than 0.30 when the number of spawners was over 100; however, when the spawners decreased to 41, the point estimate was 0.90. These results suggest that steelhead brood years with low spawner numbers had higher numbers of smolts produced per spawner and higher N_b/N ratios.

Discussion

This is the first study to document how the number of individuals in a natural vertebrate population (and presumably population density) has an impact on the N_b/N and N_e/N ratios. The four major findings of this study are summarized as follows: (i) estimates of the $N_{b(T)}/\tilde{N}$ ratio within the Snow Creek steelhead trout population established that the ratio increased during years with few

spawners, which we identified as genetic compensation; (ii) a reduction in VRS was responsible for genetic compensation in this population; (iii) when each demographic variable was examined independently, VRS resulted in the largest reduction in N_e/N , FPS resulted in the next largest reduction, and SR caused only a minor reduction in N_e/N ; and (iv) genetic and demographic estimates of N_e to N ratios from 1977 to 1994 indicated that the Snow Creek local breeding population lost genetic diversity at a rate 1.3–2 times faster per generation than predicted based on spawner censuses.

Allelic diversity and frequency distributions

Low-frequency alleles (0–0.1) were the most common allele frequency class for all three brood years examined, indicating that this population has not undergone a severe bottleneck within the recent past. Our results did indicate that the 1994 brood had undergone a marginally significant reduction in alleles per locus compared to the 1977 brood year. This reduction in allelic diversity was most likely caused by the low number of spawners in the 1990 brood year (12 total) contributing 76% of the 1994 spawners (Fig. 2 and Table 5). Long-term impacts of this reduction in allelic diversity are reduced by the steelhead life history. For example, the 1993 and 1995 brood years could have the alleles that were missing in the 1994 brood year. These transient reductions in allelic diversity within a brood year may have serious consequences within a brood year (i.e. fixation of deleterious recessive alleles). However, if allelic diversity does not remain low in surrounding brood years immediately before and after the brood year with low allelic diversity, the mixing of recruits from different brood years in subsequent generations reduces the impact of the reduction in allelic diversity in future brood years.

N_e estimates

Results indicated that Snow Creek steelhead have persisted with an $N_{e(T)}$ as low as 119 and as high as 300 between 1977 and 1994. These $N_{e(T)}$ estimates translate to an expected loss of heterozygosity over the 17-year time period (4.3 generations) of between 0.72% and 1.8%. We observed a 2.7% decline in the H_E over all loci over this time period (Table 1).

Robustness of demographic-based estimates of N_b and N_e . The complete demographic data gathered by Johnson & Cooper (1992) allowed for robust estimates of N_e and N_b based on SR and FPS. Our estimates of the effects of FPS were most likely a slight overestimate of the reduction in N_e because equation 5 assumes a semelparous life history while Snow Creek steelhead have a low rate of repeat

spawning (approximately 10%). The main effect of repeat spawning is that fish contribute offspring to different brood years. Spreading the reproductive contribution of individuals into multiple brood years reduces FPS.

Ideally, we would use estimates of \bar{k} and σ_k^2 from Snow Creek steelhead trout estimated every brood year to determine the effect of VRS on N_b and N_e but the necessary samples to estimate these variables were unavailable. We believe the estimates of these variables by Garant *et al.* (2001) for Atlantic salmon are a reasonable substitute. Atlantic salmon and steelhead trout have very similar reproductive strategies (Fleming 1998), long freshwater residence periods prior to smoltification and outmigration, and similar stream habitat use by juveniles (Volpe *et al.* 2001). Furthermore, the size of stream in the study was similar to Snow Creek. Assuming the estimates of FPS and SR are accurate, the concordant results between the comprehensive genetic and demographic estimates of N_e over the entire 17-year period suggest that the values reported by Garant *et al.* (2001) are a reasonable average for Snow Creek steelhead.

Robustness of genetic-based estimates of N_b and N_e . Consideration of the assumptions for the temporal and linkage disequilibrium methods suggests that, for the Snow Creek population, the estimates of $N_{b(T)}$ and $N_{b(D)}$ are robust and accurate.

No mutation. Given the range of mutation rates reported for microsatellite loci (Jarne & Lagoda 1996) and that only a small number of generations were sampled (fewer than five) in this study, mutation was unlikely to have played an appreciable role in changing allelic frequencies.

Alleles are selectively neutral. Numerous studies using microsatellite loci in *Oncorhynchus mykiss*, other salmonids, and other taxa indicate that most microsatellite loci are selectively neutral (Jarne & Lagoda 1996). There is still the possibility that the loci used in this study were physically or pseudolinked to loci that were under selection. Using the Kolmogorov–Smirnov test, we tested the assumption of neutrality by comparing the distribution of $n\hat{F}/\bar{F}$ with the χ^2 distribution, with degrees of freedom equal to the number of loci (e.g. Hedgecock *et al.* 1992), and found no significant differences at the 0.05 level, indicating that genetic drift alone could explain the observed allele frequency changes.

No migration from other populations. Based on modelling results, Waples (1990b) concluded that natural migration rates are probably small enough in most Pacific salmon populations that naturally occurring migration is not a serious problem for the temporal method.

We are unaware of resident (nonanadromous) *O. mykiss* in Snow Creek. Our assumption of no contribution of smolts by resident *O. mykiss* (if they are present) is supported by a reduction in the number of alleles per locus

observed in the 1994 brood year compared to the 1977 brood year. This change would be expected if the spawners in the brood year 1990 (12 total) produced 76% of the 1994 spawners.

Random mating (this assumption is only necessary for the linkage disequilibrium method). Population structure within Snow Creek steelhead was unlikely because the genotypic frequencies over all time periods were not statistically different from Hardy–Weinberg expectations. Random mating is therefore a reasonable assumption for this population.

Random sampling. The smolts were randomly sampled with stratification according to fish size and time of outmigration; therefore, the assumption of random sampling was met for the Snow Creek local breeding population.

Inbreeding N_e vs. variance N_e

The genetic-data-based $N_{e(T)}$ provides an estimate of variance N_e . The demographic-data-based $N_{e(Demo)}$ and genetic-data-based $N_{b(D)}$ provide estimates of inbreeding N_e . The variance N_e predicts the variance in allele frequency between generations and inbreeding N_e predicts the expected reduction in heterozygosity caused by inbreeding. Inbreeding N_e and variance N_e are the same in a population of constant size, but the two measures will differ when the population size is changing (Crow & Denniston 1988). Inbreeding effective size is related to the number of parents and variance effective size to the number of offspring, displacing the two measures in time. However, over the period examined in this study (nearly five generations) the N_e values estimated by the temporal method and by equation 5 will be roughly equivalent because the offspring of one generation become the parents in the next generation resulting in the long-term average of the two estimators being the essentially same (Nunney 2002).

Comparing demographic and genetic estimates of N_b and N_e

The rigorous dataset for SR and FPS indicates that these variables were not responsible for the differences in the genetic and demographic estimates of N_e and N_b observed from 1985 to 1994. A change in VRS related to the number of spawners in a brood year would explain the differences observed in the 1985–94 $N_{e(T)}$ and the estimates of $N_{e(Demo)}$ for generations 3, 4 and 5. During the 1985–94 time period, the average number of spawners was half that in the 1977–85 period. If our hypothesis of a reduction in VRS at lower spawner numbers is correct, there would be a genetic compensation consisting of less variance in allele frequency change over this period resulting in larger estimates of $N_{e(T)}$. On the other hand, $N_{e(Demo)}$ estimates

assumed a stable VRS over all brood years so the $N_{e(Demo)}$ values would be lower than $N_{e(T)}$. Additional evidence for our genetic compensation hypothesis is provided by comparing the 1985 $N_{b(D)}$ estimate of 20 (CI of 7–101) with the demographic $N_{b(Demo)}$ of 101.3. If the VRS is increased at high spawner numbers, we would predict the $N_{b(D)}/N$ -value to be lower than the $N_{b(Demo)}/N$ -value, which assumes a constant VRS.

N_b/N or N_e/N and genetic compensation

Because census sizes are the only demographic data available for most populations, the ratio of N_b/N or N_e/N is a critical parameter for monitoring changes in genetic diversity within populations. Taxon-specific N_b/N or N_e/N ratios would be useful because it would be possible to predict the rate of genetic loss in populations by simply estimating N . Our estimates of this ratio demonstrate that a constant N_b/N or N_e/N ratio cannot be assumed in wild steelhead populations.

Increased N_b/N ratios during brood years with few spawners returning to Snow Creek (Table 8) were most likely the result of a larger percentage of the total returned adults contributing to future generations or of reductions in redd superimposition, competition for spawning territory, competition for mates, embryo mortality caused by delayed spawning, or some combination of these factors. This indicates genetic compensation: a great reduction in further losses of genetic variation when the number of spawners decreases.

Estimates of $N_{b(T)}/\bar{N}$ clearly demonstrated the effect of intrabrood year variables on reducing N_b/N decreased when N was low. Because SR only deviated slightly from ideal conditions over the entire study period, the majority of intrabrood year reductions in the N_b/N ratio can be directly attributed to VRS. Additional evidence for a decrease in VRS driving genetic compensation in this population is provided by the essentially identical estimates of $N_{b(T)}/\bar{N}$ and $N_{b(D)}/\bar{N}$ when N was high (i.e. during the 1977–85-time period), indicating that VRS had the largest impact on reducing the $N_{b(T)}/\bar{N}$ ratio. Alternatively, when N was low (i.e. during the 1985–94-time period), FPS appeared to play the major role in reducing the $N_{b(T)}/\bar{N}$ (Table 8).

The observations of decreased N_b/N ratios at high spawner numbers were consistent with findings for *Tribo- lium* (Wade 1980; Pray *et al.* 1996) and *Eichhornia paniculata* (Husband & Barrett 1992). Theoretical, behavioural and physiological work on salmon stock assessment and ecology also predicts a compensatory relationship between spawner density and the number of smolts produced per spawner (Ricker 1954, 1958). Hilborn & Walters (1992) provided two biological mechanisms that could lead to this compensatory relationship: (i) females are highly territorial

and once all territories are full, subsequent females will be unable to spawn; or (ii) females randomly deposit eggs and as more females enter to spawn, they are more likely to dig up previously deposited eggs (redd superimposition).

Schroder (1981) found that high densities of female spawners increased competition for egg burial sites and inhibited some chum salmon from laying all their eggs in an experimental stream. Chebanov (1991) expanded the findings of Schroder (1981) by comparing the effects of spawning population density of sockeye salmon on the ability of females to deposit eggs and the mortality rates of embryos. Chebanov's study found high spawner densities led to an increased number of unspawned eggs, loss of spawned eggs in the redd (as a result of redd superimposition and loss of eggs to the current during spawning), and increased rates of embryo mortality. The increased mortality of embryos is thought to be indirectly caused by a delay in spawning as a result of increased spawner density. Springate *et al.* (1984) showed decreased fertility in *O. mykiss* when spawning was delayed for one or two days.

Genetic compensation and population productivity

Figure 3 illustrates the inverse relationship between the number of spawners in a brood year and the number of smolts per spawner produced by a brood year. Numerically, this relationship helps the population avoid extinction because brood years with few spawners produce as many smolts as brood years with many spawners. The genetic consequence of brood years with few spawners contributing disproportionately to the population in future generations is a reduction in effective population size (Ryman & Laikre 1991; Waples 2002). However, our results indicate that increased N_b/N -values associated with genetic compensation can lessen this long-term reduction in effective size caused by the increased productivity of brood years with few spawners. We believe the combination of genetic compensation and increased population productivity during brood years with few spawners is an important force that acts to reduce demographic and genetic risks of extinction in Pacific salmon populations.

Population viability

All finite populations lose genetic diversity and increase their rate of inbreeding at the rate of $1/2N_e$ per generation. Snow Creek had an estimated N_e per generation greater than 50 but below 500. At these levels, the population is not losing diversity at a rate fast enough to warrant immediate concerns about inbreeding or loss of heterozygosity. This information is important for predicting the fitness and persistence of populations because, within *O. mykiss*, it is known that inbreeding interacts with demographic factors

by reducing fecundity, juvenile survival, and lifespan (Kincaid 1983; Leary *et al.* 1985; Su *et al.* 1998). By not having an N_e above 500, however, this population is unlikely to undergo increases in population genetic parameters, such as additive genetic variation and heterozygosity, that have been positively associated with long-term evolutionary potential (Lande & Barrowclough 1987; Lynch 1996).

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