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Census vs. effective population size in chinook salmon: large- and small-scale environmental perturbation effects

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Abstract

Population viability has often been assessed by census of reproducing adults. Recently this method has been called into question and estimation of the effective population size (N_e) proposed as a complementary method to determine population health. We examined genetic diversity in five populations of chinook salmon (*Oncorhynchus tshawytscha*) from the upper Fraser River watershed (British Columbia, Canada) at 11 microsatellite loci over 20 years using DNA extracted from archived scale samples. We tested for changes in genetic diversity, calculated the ratio of the number of alleles to the range in allele size to give the statistic *M*, calculated N_e from the temporal change in allele frequency, used the maximum likelihood method to calculate effective population size (N_{eM}), calculated the harmonic mean of population size, and compared these statistics to annual census estimates. Over the last two decades population size has increased in all five populations of chinook examined; however, N_e calculated for each population was low (81–691) and decreasing over the time interval measured. Values of N_{eM} were low, but substantially higher than N_e calculated using the temporal method. The calculated values for *M* were generally low ($M < 0.70$), indicating recent population reductions for all five populations. Large-scale historic barriers to migration and development activities do not appear to account for the low values of N_e; however, available spawning area is positively correlated with N_e. Both N_e and *M* estimates indicate that these populations are potentially susceptible to inbreeding effects and may lack the ability to respond adaptively to stochastic events. Our findings question the practice of relying exclusively on census estimates for interpreting population health and show the importance of determining genetic diversity within populations.

Keywords: census population size, chinook salmon, effective population size, genetic diversity, microsatellite

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Introduction

Animal populations have been assessed traditionally by direct census counts for conservation and management purposes. Estimates of total population size are logistically problematic for aquatic animals; however, indirect census estimates have been used widely in anadromous Pacific salmon from the west coast of North America to determine catch quotas for commercial, aboriginal and recreational fisheries. Although Pacific salmon spend one or more years at sea before migrating back to freshwater to spawn, they show a remarkable ability to return to their natal spawning areas to breed, with generally very low levels of straying (Quinn 1993). Unfortunately, the combination of their natal homing behaviour and anthropogenic impacts has led to declines and losses of individual river populations, or 'stocks', over much of the 20th century. Specifically, blockage of migratory routes by dams, destruction of spawning habitat and over harvesting have led to significant declines in numbers of returning fish, and the extirpation of some populations (Nehlsen *et al*. 1991; Waples 1994; Slaney *et al*. 1996).

Pacific salmon stocks in the upper Fraser River show high variability in numbers, but have been increasing from very low levels since the 1980s (R. Bailey, Fisheries and

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Oceans, Canada, pers. comm.). Although there are currently no dams on the mainstem of the Fraser River, there have been major intentional and unintentional changes in water flow over the last 100 years. Specifically, a major water diversion project on the Nechako River has reduced flows markedly in one of the Fraser River's major tributaries. In addition to such large environmental perturbations, the upper Fraser River salmon have also been subject to increases in fishing pressure and habitat loss. It is not clear whether the large historic perturbations or the ongoing low-level impacts (fishing and habitat loss) have had any impact on the genetic diversity of salmon in the upper Fraser watershed.

The impact of the historic low numbers in the upper Fraser salmon stocks on population viability is not clear, but may be profound. It is expected that small population size will lead to a loss of genetic variation through genetic drift, while periodic population bottlenecks will accelerate the erosion of genetic diversity. Such a loss of genetic diversity is, in turn, expected to impact population fitness and viability negatively, although that relationship has mixed empirical support (Britten 1996; David 1998; Heath *et al*. 2002). The rate of loss in genetic diversity, however, is dependent on the effective population size, N_e, rather than the actual number of animals in a population, N (Kalinowski & Waples 2002). N_e is the size of an ideal population that would be affected by genetic drift at the same rate as the actual population. Reduced population size and population bottlenecks lead to reduced N_e (Franklin 1980). N_e is therefore a fundamental parameter for conservation and management when assessing population fitness.

Chinook salmon (*Oncorhynchus tshawytscha*) populations of the upper Fraser River have experienced both large- and small-scale environmental perturbations. Although only a limited number of stocks were impacted by diversion of the headwaters of the Nechako River, all stocks have been impacted to varying degrees by habitat loss and overfishing. To examine the relative impacts of large-scale perturbations vs. localized impacts, we selected for detailed temporal genetic analysis five chinook salmon populations that have been impacted variously by large- and small-scale environmental perturbations. Two populations (Nechako River and Stuart River) were impacted directly by the Nechako Diversion, while three other populations (Willow River, Bowron River and Dome Creek) were chosen from tributaries above the confluence with the Nechako River, and hence were not affected by the large-scale perturbation. To evaluate differences in variation in genetic diversity and N_e, we extracted DNA from scale samples archived over approximately 20 years, and assessed allelic variation at 11 polymorphic microsatellite loci. These data were used to estimate genetic diversity (unbiased heterozygosity and mean number of alleles), estimate population bottleneck history using the ratio of the number of alleles to the range in allele size (the statistic '*M*'; Garza & Williamson 2001), and to calculate effective population size (N_e) from the harmonic mean of census size, the temporal change in allele frequency (Waples 1990a) and the maximum-likelihood estimation method of Beerli & Felsenstein (1999).

Materials and methods

Study areas

Five chinook salmon populations (Nechako River, Stuart River, Willow River, Bowron River and Dome Creek; Fig. 1) were chosen for inclusion in this study based on their history of large-scale environmental perturbation, the availability of archived scale samples and historic data and geographical proximity. The five stocks represent populations that have been subject to different levels of human impact, and have shown variation in census population size (N). Within the last 20 years the populations increased from moderate numbers (Bowron), increased from very low numbers (Willow), were relatively stable over time (Nechako), showed considerable annual variation (Stuart) or remained small (Dome). The current size of the Bowron, Nechako and Stuart populations are similar. Although the Willow River is approximately the same length as the Bowron River (Table 1) the population of chinook spawning in this system is much smaller, and close to that of Dome Creek. Dome Creek is the smallest system of the five rivers included in this study (Table 1).

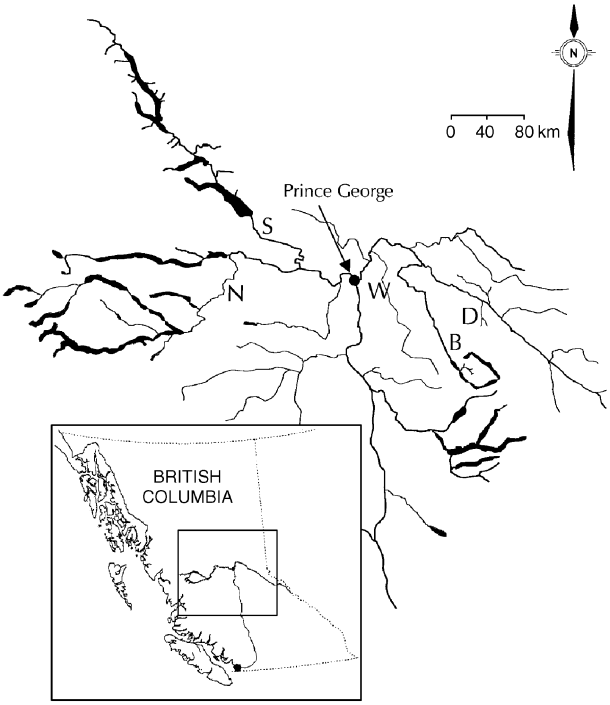

Fig. 1 Map of the upper Fraser River watershed showing the locations of the five rivers where fish samples were collected and used in this study.

Table 1 Habitat characteristics for five upper Fraser River tributaries and a summary of development activities

	River length (km)	Mean discharge m^3/s	Spawning habitat (km)	Cumulative development activity
Nechako	240*	103.6¶	59**	High (MacDonald *et al*. 1997a)
Stuart	145†	129	27††	Low (MacDonald *et al*. 1997a)
Willow	223‡	36.5	1.6‡‡	High (MacDonald *et al*. 1997b)
Bowron	238	63.4	14§§	High (MacDonald *et al*. 1997b)
Dome	41§	11.4	6¶¶	Low (MacDonald *et al*. 1997b)

*Length of Nechako River from confluence with the Fraser River to the Kenney Dam.
†Length of Stuart River from confluence with the Nechako River to Stuart Lake.
‡Upstream migration barrier at km 35 due to a 150-m section with large boulders and restricted channel width (Murray *et al*. 1981).
§Upstream migration barrier at km 9 due to a small splash dam constructed ~80 years ago (R. Argue, pers. comm.).
¶Flow measured above the confluence with the Stuart River at Vanderhoof (NFCP 1989–94).
**Chinook spawn in a section 154 km long; most spawning occurs in a section 20 km long below Cheslatta Falls (383%) and in a section 39 km long upstream of the town of Vanderhoof (343%; NFCP 1989–94).
††A 27-km section of the Stuart River covers 97% of identified spawning areas (NFCP 1989–94).
‡‡Murray *et al*. (1981) estimate 1.6 km of channel exist with substrate for spawning within an 8-km section below Highway 16.
§§Murray *et al*. (1981) estimate 14 km of channel with substrate for spawning within a 32.5-km section of the upper Bowron River.
¶¶Enhanced juvenile chinook have been released above the migration barrier since 1986 (R. Argue, pers. comm.).

The chinook salmon returning to each of the rivers have been enumerated to generate census estimates for up to 50 years. Until the late 1970s, the spawner counts were ground-based and conducted by stream-side observations. After this time, aerial surveys were used to estimate numbers of spawners on up to five flights for each watershed. The reliability of the aerial counts is better than ground-based visual surveys, but it is still variable from year to year depending on water level, clarity and flying conditions. The absolute numbers of returning chinook must be interpreted with caution; however, they do represent reliable comparative data. Variation among years for the estimated numbers of chinook probably reflects true variation in fish numbers.

Several ongoing and historic events are expected to affect the upper Fraser chinook populations. The Nechako and Stuart Rivers were affected by a major water diversion project on the Nechako River. In 1952 the Kenney Dam was completed and blocked all flows to the headwaters of the Nechako River. The reservoir behind Kenney Dam filled for 4 years, during which time the upper Nechako River flows were approximately 10% of historic levels. Following reservoir filling the flows in the Nechako River were similar to historic values, but have declined in subsequent decades to allow for power generation. Other human activities are also extensive through most of the watersheds. Roads and railway rights-of-way are considerable for the Willow, Bowron and Dome systems, but less for the mainstem of the Nechako and Stuart Rivers. Forest harvesting has been extensive through the Bowron and Willow watersheds. Agriculture impacts are extensive through the Nechako watershed. The Stuart River is the least impacted of the systems examined.

The physical characteristics of the five rivers are given in Table 1. The length of the Nechako River is listed as 240 km: the distance from the confluence with the Fraser to the Kenney Dam. The Stuart River includes only river length from Stuart Lake to the confluence with the Nechako, and although it is a shorter distance than the Nechako River water discharge is similar. The Willow and Bowron Rivers are adjacent watersheds and are similar in many respects; however, spawning of chinook salmon occurs in the headwaters of the Bowron River, whereas spawning is limited to the lower 35 km of the Willow due to an upstream migration barrier. Dome Creek is the smallest watershed, although the total length is similar to the accessible length of the Willow River. Due to the physical dimensions, natural barriers and human impacts on each of the watersheds examined, the total length of river that chinook are found to spawn varies from 1.6 km in Willow River to 59 km in the Nechako River (Table 1).

Genetic analyses

Scales were collected from carcasses of adult chinook salmon spawners and archived at the Pacific Biological Station by Fisheries and Oceans Canada. Scales from each of the rivers were chosen from two or three dates over a 20-year period (1978–98). For each population, we identified years for which we could collect at least 30 scale samples from fish sampled over a period of approximately 2 weeks. For the 1998 Bowron River fish scale samples were not collected, but a limited number of opercular punch samples (n = 20) were taken from spawning chinook and preserved in 95% ethanol.

DNA was extracted from scales (or operculum punches for Bowron 1998) using a modified proteinase K digestion method. Briefly, two to four unwashed scales (or one opercular punch) were digested in 200 μL proteinase K buffer

Table 2 Microsatellite loci used for analysis of chinook historic population genetic structure. Primer sequences and repeat size were taken from the original published sources; the number of alleles and allele size range were calculated using data from all populations and years combined. Annealing temperatures were optimized for chinook salmon

Locus	No. of alleles	Allele size range	Repeat	Annealin temp (°C)	Source
Ots1	4	176–192	2	56–52*	Banks *et al*. (1999)
Ots3	14	82–120	2	50	Banks *et al*. (1999)
Ots4	13	140–172	2	56–52*	Banks *et al*. (1999)
Ots104	28	168–296	4	54	Nelson & Beacham (1999)
Ots107	35	160–328	4	58	Nelson & Beacham (1999)
Omy207	5	74–86	2	52	Olsen *et al*. (1996)
Omy325	18	84–128	2	62–56†	Olsen *et al*. (1996)
Oneμ3	9	144–176	2	56–52*	Scribner *et al*. (1996)
Ssa85	29	112–224	2	62–56†	O'Reilly *et al*. 1996
Ssa197	45	134–290	2	56–52*	O'Reilly *et al*. 1996
Sfo8	29	206–300	2	50	Angers *et al*. (1995)

*Includes a 'touchdown' step with four cycles of –1 °C until final annealing temp. of 52 °C.
†Includes a 'touchdown' step with five cycles of –1 °C until final annealing temp. of 56 °C.

[10 mM Tris (pH 8.0); 10 mM ethylene diamine tetraacetic acid (EDTA); 0.5% sodium dodecylsulphate (SDS)] with 25 μg proteinase K overnight at 37 °C with gentle rocking. The digestion was extracted twice with phenol:chloroform:isoamyl alcohol (24:24:1), and alcohol/salt precipitated. The pellet was washed with 70% ethanol, dried and redissolved in 100 μL water.

Variation at nine dinucleotide and two tetranucleotide repeat microsatellite loci was assessed (Table 2). The optimized polymerase chain reaction (PCR) conditions determined for chinook salmon were as follows: a 'hot-start' and a 1-min denature cycle (94 °C), followed by 35 cycles of a 1-min denature step (94 °C), a 1-min annealing step (variable temperature, Table 2) and a 1.5-min extension cycle (72 °C). For some primer sets we used a touchdown protocol to increase the clarity of the PCR product (Table 2; Don *et al*. 1991). Reactions included 1.0 μL of the extracted DNA, 0.5 μL of each primer (100 ng/mL), 2.5 μL of the 10× reaction buffer (200 mM Tris-HCl (pH 8.4), 500 mM KCl; Gibco-BRL), 0.5 μL dNTPs (10 mM each dNTP), 1.5 μL of 25 mM $MgCl_2$, 1.0 U *Taq* DNA polymerase (Gibco-BRL) and ddH_2O to make up a total 25 μL reaction volume. The amount of scale-extracted DNA was varied if amplification was not successful with 1.0 μL as recommended by Nielsen *et al*. (1999); template DNA was increased or decreased until successful amplification was achieved, or the sample was ruled too degraded for use. The forward primer for all PCR was dye-labelled and the resulting dye-labelled amplified fragments were run on an automated sequencer with appropriate size standards (Visible Genetics, Toronto, ON, Canada). All microsatellite fragment sizes were determined to the nearest 0.3 base pairs (bp), and rounded to the nearest whole repeat number.

Statistical analysis

Genetic diversity was estimated using two statistics; unbiased heterozygosity (Nei 1978) and mean allele number. We calculated the ratio of the number of alleles to the range in allele size, '*M*', following Garza & Williamson (2001), for each population at each sample time. The ratio *M* reflects past reductions in population size, or repeated bottlenecks. The expected behaviour of *M* under a number of conditions has been described and values below $M = 0.68$ are strong indications of recent population reductions (Garza & Williamson 2001).

Effective population size was estimated using three approaches. First, the harmonic mean was used to estimate effective population size based on fluctuations in census population size (N_h). We calculated N_h from census estimates over the time interval for which we had scales. Second, the maximum-likelihood estimation method (MIGRATE, version 1.7.1) of Beerli & Felsenstein (1999) was used to determine θ, which can be used to calculate effective population size (N_{eM}) from the relationship between θ, N_e and mutation rate ($\theta = 4N_e\mu$). There were 10 short chains (500 used trees of 10 000 sampled) and three long chains (5000 used trees of 100 000 sampled) used for the analysis. We used the mutation rate of 5×10^{-4} as suggested by Heath *et al*. (2001). N_{eM} was calculated for Nechako 1988, Stuart 1988, Willow 1992, Bowron 1988 and Dome 1991 sample dates. The dates were chosen as the method estimates migration between populations and these dates were within one generation. Third, we calculated effective population size from the temporal change in allele frequencies (N_e). Allele frequencies were calculated for all observed alleles for each sampling year within a population. Standardized allele variance, $\hat{F}$, was estimated for each locus following Waples's (1990b) formula:

$$\hat{F}_j = \frac{1}{L-1}\sum_{i=1}^{L}\frac{(X_{i1}-X_{i2})^2}{(X_{i1}+X_{i2})/2}$$

where L is the number of alleles at the jth locus and X_{i1} and X_{i2} are the allele frequencies for the ith allele at the first and second temporal sampling, respectively. We then calculated a weighted mean standardized allele variance across all loci using:

$$\hat{F} = \frac{\sum[(L_j-1)\hat{F}_j]}{\sum(L_j-1)}$$

where L_j is the number of alleles at locus j. The effective population size was then estimated using:

$$N_e = \left[\frac{b}{2(\hat{F}-1/S+1/N)}\right]\bar{y}$$

where b is an empirically derived parameter that reflects mean generation time and length of time between samples (Waples 1990b; Tajima 1992), S is the harmonic mean of the sample sizes in the two sample years for each locus, N is the estimated mean number of spawning fish in the first sample year, and $\bar{y}$ is the mean generation time. We chose a generation time of 5 years and either a 10-year interval (for a value of b = 2.01) or a 20-year interval (for a value of b = 2.75; see Tajima 1992). The actual mean generation times were 4.83, 4.79, 4.97, 4.68 and 4.67 for Nechako, Stuart, Willow, Bowron and Dome populations, respectively (Fig. 2). The calculation of N_e is relatively insensitive to variation in generation time, and distribution of spawning age within semelparous salmon with overlapping generations, especially given the relatively large sampling intervals (Waples 1990a). Inaccuracies in the estimation of N when the population size is greater than 1000 spawning fish will have little effect on our estimate of N_e. Our data set includes a number of alleles with low frequency which has been shown to lead to an upward bias in estimates of N_e (Waples 1990a). We calculated N_e on a revised data set where all alleles (within a river system over all sampled years) that did not have a frequency of three or higher were pooled. There was little or no difference between our estimates of N_e pooling low-frequency alleles and that of N_e using the data set containing rare alleles. This is in agreement with Waples (1989) and data presented in this paper are for the original data set. The 95% confidence interval associated with N_e was calculated using $\hat{F}$ values modified by:

$$\left[\frac{n\hat{F}}{\chi^2_{0.025(n)}}, \frac{n\hat{F}}{\chi^2_{0.975(n)}}\right]$$

where n is the total number of independent alleles among the 11 microsatellite loci [i.e. $\Sigma(L_j-1)$].

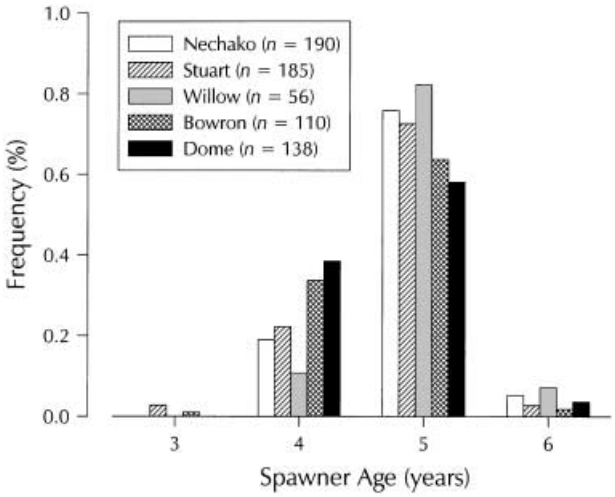

Fig. 2 Age class distribution for spawning chinook salmon from the five populations examined. Data are from ages determined from the scales used in the present study.

Results

There is a generally increasing trend in the census counts and harmonic mean (N_h) for population size (Fig. 3). The increase has been moderate in two of the stocks (Willow and Dome), substantial in two of the stocks (Stuart and Bowron) and has not reached historic levels in one stock (Nechako). Population size, however, shows considerable yearly variation with differences of several thousand spawners between years for some of the larger populations (Fig. 3).

The 11 microsatellite loci showed high levels of variation among the chinook populations examined with a total of four to 45 alleles at each locus (Table 2) and from two to 31 alleles observed within individual stocks (Table 3). Repeatability of allelic scores was determined to be greater than 96% on a subset of samples that were re-amplified. Observed heterozygosity ranged from 0 to 1.0 among loci, years and populations (Table 3). A portion of the data showed significant (after Bonferroni correction) departures from Hardy–Weinberg equilibrium (nine of 154 tests). Although most were due to heterozygote deficiency, two of nine significant departures were due to heterozygote excess.

Genetic diversity showed varying patterns of change among the five populations: the Bowron was characterized by decreasing genetic diversity, the Willow was relatively constant and the Dome showed a consistent increase over time (Fig. 4). The Nechako and Stuart populations showed a more complex pattern of change; however, mean heterozygosity and allele number agreed on the general shape of the change (Fig. 4).

The calculated values for M were generally low ($M < 0.70$; Fig. 4), indicating recent population reductions for all the five stocks. Although values were generally low, there was considerable change over time. M also shows patterns of

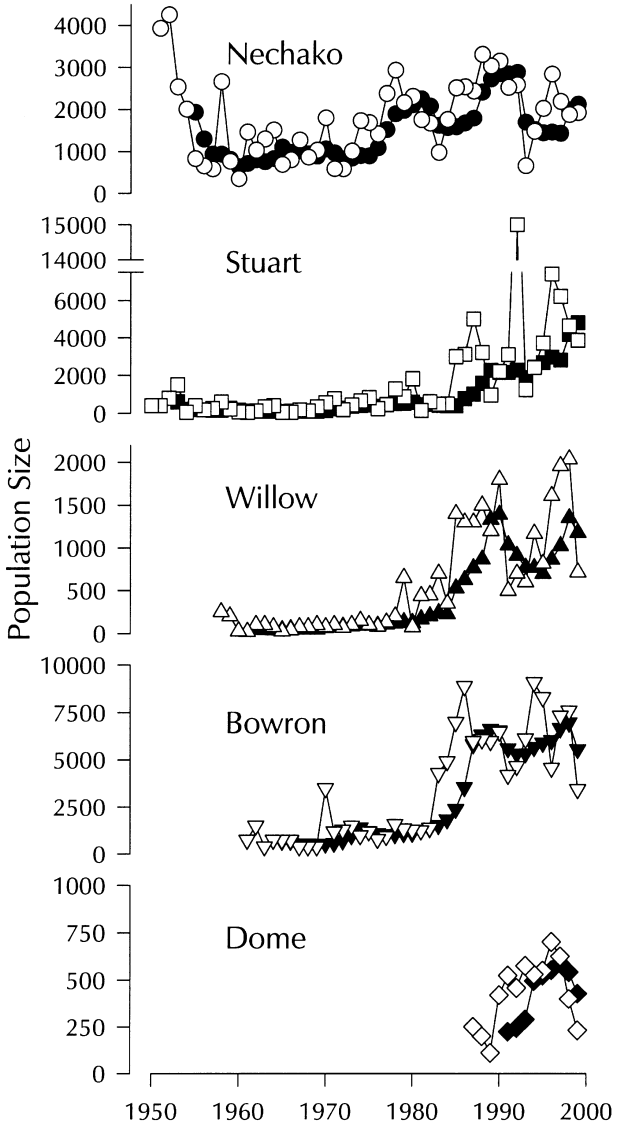

Fig. 3 Observed population size (open symbols) and harmonic mean calculated as a 5-year running average (approximately one generation) on the census data (closed symbols) for five upper Fraser River chinook stocks. Harmonic mean was calculated from data for the year of census and 4 years prior to the census date.

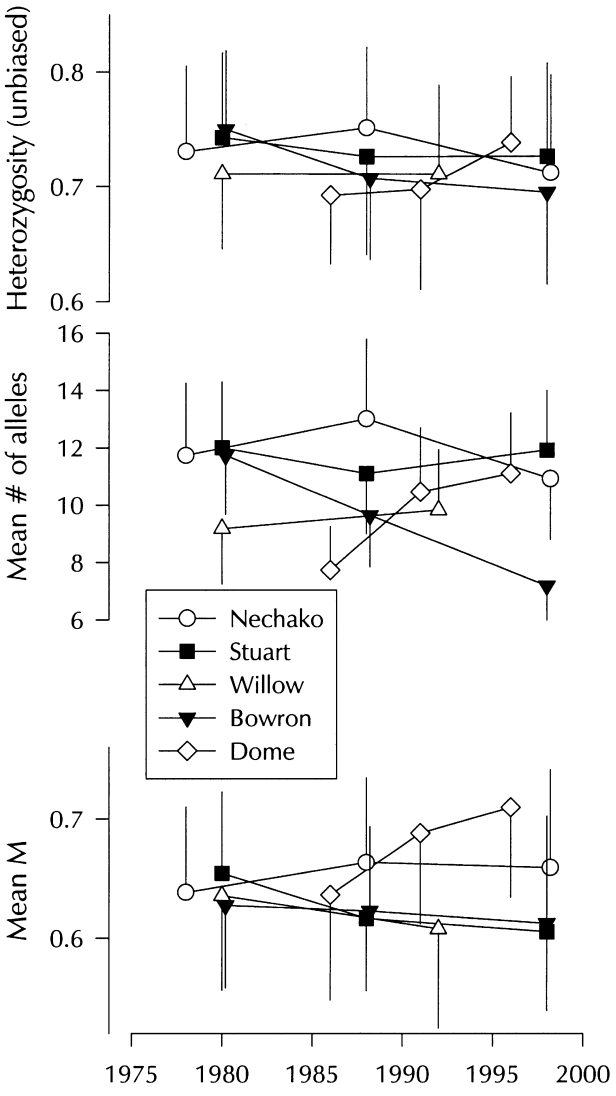

Fig. 4 Mean heterozygosity (unbiased), number of alleles and the ratio of the number of alleles to the range in allele size (*M*) for five populations of upper Fraser chinook salmon.

change over time and among populations similar to those of mean heterozygosity and number of alleles. A noteable exception was for Dome Creek, where heterozygosity and number of alleles were generally lower than the other stocks, yet *M* was higher and the 1996 Dome sample had the highest value of *M*.

We found no relationship between N_e and N or N_h. Differences in the ratios of effective population size to census size are shown for each population in Fig. 5. The Stuart and Willow populations showed the greatest range in census size over the interval examined, and have the lowest value of the ratio of N_h/N. The lower variation among years in the Nechako population is reflected in the similarity between harmonic mean and census values, with values of N_h/N of approximately 0.9 (Table 4). The Bowron and Dome populations showed considerable variation in population size from the earlier dates examined, but show less variation at the later dates as N_h approached N and the ratio N_h/N became greater than 0.9 (Table 4). Estimates of N_e range from 81 to 691 for the five stocks. N_e for the Nechako stock is highest, followed by Stuart, while N_e for the remaining stocks were similar over the longest time interval (Table 4). The 95% confidence intervals calculated for N_e were relatively narrow for all estimates. N_e shows a decline over time for the four populations where multiple calculations of N_e were possible, and the ratio of N_e/N was also lower for more recent sample dates. Values of N_e/N ranged from

Table 3 Number of fish sampled (*n*), allele numbers (*A*), sample sizes (*N*), observed and expected heterozygosity (H_O and H_E) at 11 microsatellite loci for the five chinook salmon populations at two or three sample dates

	Nechako			Stuart			Willow		Bowron			Dome		
Locus	1978	1988	1998	1980	1988	1998	1980	1992	1980	1988	1998	1986	1991	1996
n	49	60	48	55	44	43	27	30	36	34	18	29	58	59
Ots1														
N	42	55	47	30	36	29	26	27	32	27	12	28	40	58
A	2	2	2	2	2	3	2	2	2	2	3	2	2	2
H_O	0.48	0.56	0.60	0.43	0.53	0.52	0.62	0.70	0.50	0.44	0.42	0.43	0.45	0.47
H_E	0.48	0.50	0.48	0.50	0.50	0.46	0.49	0.51	0.51	0.49	0.54	0.45	0.47	0.47
Ots3														
N	46	56	46	51	41	37	24	29	35	30	18	29	56	52
A	6	7	7	8	10	6	4	3	6	5	5	2	3	5
H_O	0.61	0.66	0.67	0.74	**0.63***	0.57	0.63	0.59	**0.77***	0.53	0.67	0.41	0.71	0.63
H_E	0.68	0.73	0.76	0.78	0.79	0.68	0.64	0.56	0.71	0.51	0.71	0.49	0.51	0.64
Ots4														
N	48	54	47	52	33	35	24	29	30	33	18	29	53	56
A	9	9	8	9	9	10	5	7	10	8	5	6	7	7
H_O	0.73	0.74	0.70	0.65	0.64	0.77	0.67	0.76	0.77	0.7	0.78	0.62	0.81	0.73
H_E	0.78	0.81	0.75	0.69	0.75	0.83	0.65	0.69	0.80	0.79	0.64	0.77	0.80	0.83
Ots104														
N	31	58	42	49	33	33	24	22	33	34	11	28	56	58
A	23	20	21	23	22	22	19	18	22	21	13	16	19	18
H_O	0.93	0.91	0.93	0.96	0.94	0.91	0.95	0.91	0.97	0.91	1.0	1.0	0.96	0.86
H_E	0.96	0.94	0.95	0.94	0.96	0.95	0.95	0.95	0.95	0.94	0.93	0.93	0.93	0.93
Ots107														
N	40	59	46	33	28	34	27	29	36	23	15	27	57	59
A	17	20	15	18	17	19	18	19	18	14	11	14	17	18
H_O	0.93	0.88	0.72	0.90	0.89	0.97	0.77	0.79	**0.78***	0.89	0.80	0.78	0.96	0.81
H_E	0.91	0.93	0.82	0.92	0.93	0.93	0.94	0.94	0.92	0.91	0.88	0.90	0.92	0.90
Omy207														
N	47	44	38	52	42	41	26	28	36	33	18	29	58	41
A	3	3	3	3	3	4	3	3	3	2	1	3	2	4
H_O	0.23	0.30	0.08	0.13	0.05	0.07	0.19	0.11	0.25	0.15	0	0.55	0.07	0.34
H_E	0.30	0.41	0.08	0.13	0.05	0.07	0.28	0.10	0.27	0.19	0	0.43	0.07	0.41
Omy325														
N	49	55	48	54	33	37	27	30	35	34	18	29	58	58
A	9	12	11	11	11	13	10	7	14	10	5	9	13	16
H_O	0.71	0.84	**0.81***	0.81	0.82	0.92	0.70	0.87	0.83	0.80	0.67	0.86	0.90	0.93
H_E	0.81	0.87	0.87	0.85	0.85	0.89	0.86	0.74	0.89	0.81	0.74	0.83	0.87	0.87
Oneμ3														
N	30	56	43	49	44	40	27	28	35	31	16	24	53	56
A	2	3	3	5	2	4	3	4	6	5	5	2	3	5
H_O	0.43	0.38	0.33	**0.73***	0.57	0.60	**0.92***	0.71	0.46	0.64	0.63	0.58	0.38	0.52
H_E	0.35	0.31	0.37	0.66	0.45	0.56	0.53	0.62	0.49	0.57	0.60	0.45	0.40	0.56
Ssa85														
N	46	54	40	47	34	38	23	27	31	26	17	27	56	58
A	15	19	15	16	16	16	10	10	13	11	9	10	14	11
H_O	0.70	0.85	0.90	0.77	**0.77***	0.66	0.65	0.89	0.87	0.88	0.76	0.67	0.84	0.67
H_E	0.90	0.88	0.91	0.90	0.92	0.85	0.75	0.85	0.86	0.85	0.87	0.70	0.87	0.71
Ssa197														
N	43	51	38	43	35	37	21	28	32	29	18	29	57	59
A	25	31	22	24	19	19	17	20	19	13	11	10	22	23
H_O	0.79	0.82	0.79	0.77	0.80	0.73	0.86	0.86	0.75	0.69	0.61	0.69	0.77	0.8
H_E	0.95	0.96	0.94	0.91	0.92	0.92	0.92	0.95	0.93	0.84	0.86	0.81	0.92	0.89

Table 3 *Continued*

	Nechako			Stuart			Willow		Bowron			Dome		
Locus	1978	1988	1998	1980	1988	1998	1980	1992	1980	1988	1998	1986	1991	1996
Sfo8														
N	28	44	25	35	25	25	20	26	26	28	17	28	57	59
A	18	17	13	13	11	15	10	15	16	15	11	11	13	13
H_O	0.71	0.75	0.80	0.77	0.60	**0.68***	0.75	0.77	0.73	0.89	0.76	0.75	0.81	**0.69***
H_E	0.94	0.91	0.91	0.88	0.87	0.83	0.83	0.89	0.92	0.89	0.86	0.86	0.90	0.90

*Significant departures from Hardy–Weinberg equilibrium (after Bonferroni correction) are shown in bold type.

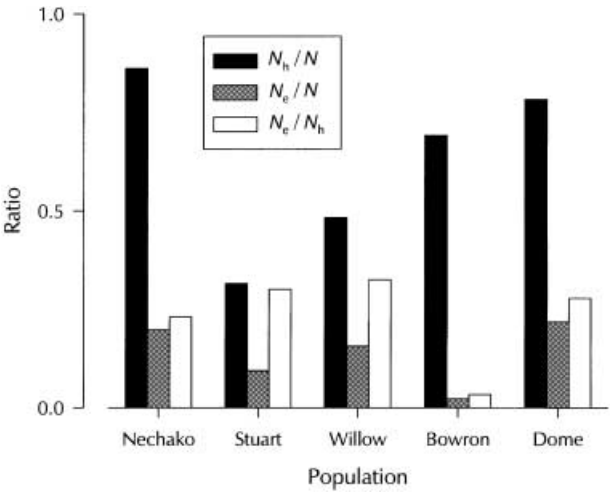

Fig. 5 Ratios of harmonic mean of census population size to mean population size (N_h/N), effective population size (calculated from the temporal change in allele frequency, Waples 1990a) to census size (N_e/N), and effective population size to the harmonic mean of population size (N_e/N_h).

0.021 to 0.566. The ratio of N_e/N_h was also lower for more recent sample dates. Values of N_e/N_h ranged from 0.022 to 0.765. Effective population size calculated from MIGRATE (N_{eM}) were similar for the Nechako, Willow and Bowron populations, but markedly lower for Stuart and Dome populations (Table 4).

Discussion

In general we found little concordance between census population size and the genetic-based population parameters (i.e. genetic diversity, N_e, N_{eM}; Fig. 5). This is despite considerable variation in both census population size and genetic parameters among the study populations over the time frame of the study. Population sizes of all stocks examined in this study have shown a gradual increase, particularly the Bowron River population. The observed increase in population size should lead to lower susceptibility to inbreeding effects and stochastic events (Franklin 1980). At face value, management efforts for these populations have been successful. Genetic recovery from population

Table 4 Average population size (N), harmonic mean of population size (N_h), effective population size using the temporal variation in allele frequency (N_e) and effective population size using the maximum likelihood estimate from MIGRATE (N_{eM}) for five stocks of upper Fraser River chinook. N_e is calculated over three time intervals for four of the stocks; N and N_h are calculated for the same interval as N_e

Stock	Years	N	N range	N_h	N_h/N	N_e	N_e 95% CI	N_e/N	N_e/N_h	N_{eM}
Nechako	1978–88	2206	975–3300	1992	0.903	691.0	333–3131	0.313	0.347	
Nechako	1978–98	2216	654–3300	1907	0.861	437.9	271–785	0.197	0.230	983.9
Nechako	1988–98	2325	654–3300	1900	0.817	301.1	179–569	0.130	0.158	
Stuart	1980–88	1995	157–5010	654	0.328	306.0	169–682	0.153	0.468	
Stuart	1980–98	3417	157–15000	1077	0.315	322.8	191–614	0.094	0.300	575.6
Stuart	1988–98	4564	970–15000	2639	0.578	264.3	146–559	0.060	0.100	
Willow	1980–92	901	75–1500	435	0.483	140.8	85–253	0.156	0.324	1088
Bowron	1980–88	4576	1260–8896	2728	0.596	267.5	123–1343	0.058	0.098	
Bowron	1980–98	5560	1260–9104	3838	0.690	126.0	78–217	0.023	0.033	940.0
Bowron	1988–98	6414	4200–9104	6058	0.945	132.7	72–295	0.021	0.022	
Dome	1986–91	301	110–523	223	0.741	170.5	100–331	0.566	0.765	
Dome	1986–96	432	110–702	338	0.782	93.6	64–137	0.222	0.277	641.3
Dome	1991–96	545	400–702	531	0.974	81.0	57–113	0.149	0.153	

bottlenecks or sustained low effective population size, however, may not mirror census population size recovery, i.e. healthy census values may mask small N_e (Nunney & Elam 1994; Frankham 1995a). For this reason it is important to evaluate genetic-based parameters such as genetic diversity and N_e as measures of population health (Tringali & Bert 1998).

Genetic diversity

We found highly variable relationships between genetic diversity and census measurements. The Bowron River chinook show a decline in heterozygosity and allele number over the study period (Fig. 4), which does not reflect the dramatic increase in population size in the last 10 years (Fig. 3). Although the decline in heterozygosity between 1988 and 1998 could be an artefact of the smaller sample size taken in 1998, the trend is evident between the 1980 and 1988 sample dates. A similar lack of concordance can be seen for Nechako, Stuart and Willow populations across all dates examined. The Dome is the only stock within this study to show an increase in census numbers associated with a consistent increase in genetic diversity. Our estimations of genetic diversity are probably robust given the large number of individuals and loci used, as well as the agreement between mean number of alleles and heterozygosity.

Our calculated values for the ratio of the number of alleles to the range in allele size, M, were well below the theoretical threshold value for populations that experienced recent reductions in population size (Fig. 3; Garza & Williamson 2001). It could be argued that Pacific salmon in general would be expected to have low values of M as large fluctuations in population size are common for most stocks. Data from a captive population of chinook salmon derived originally from a large coastal population generated a value of $M > 0.9$ (200 fish, seven microsatellite loci; D.D. Heath, unpubl. data). Thus, not all chinook salmon populations are characterized by low M. Our values of M indicate that all of the stocks included in this study have experienced recent bottlenecks.

Effective population size

The harmonic mean of the census estimates reflects reduced effective population size due to fluctuating population size (Franklin 1980). In the Nechako, Bowron and Dome populations, the harmonic mean was close to mean census size and the ratio of N_h/N was high, approximately 0.9 (Table 4, Fig. 5). The Stuart and, to a lesser extent the Willow, showed more variation in census size, consequently the ratio of N_h/N was less than 0.5. Fluctuations in population size have been demonstrated to reduce heterozygosity within a population (Motro & Thomson 1982) and potentially produce very small values of N_e compared to the census population size (Vucetich *et al*. 1997). Greater decreases in heterozygosity occur following declines to very small population size (bottlenecks) and with a long cycle period (Motro & Thomson 1982). Census estimates reveal years with extreme population bottlenecks for three of the populations examined; Stuart 140 fish in 1981, Willow 75 fish in 1980 and Dome 120 fish in 1989 (B. Huber and R. Argue, Fisheries & Oceans Canada, pers. comm.). Such fluctuation in population size may thus be an important factor in the low values of N_e estimated for the Stuart, Willow and Dome populations (Fig. 5). In the Nechako population no such extreme fluctuations were seen in the census data, and this river has among the highest values of N_e and N_{eM}. The Bowron, however, has exibited a relatively constant increase in population size, yet has very low levels of N_e. The relatively high N_h/N ratio, but low N_e/N ratio, indicate that the effect is not driven by population size variability.

Our estimates of N_e from the temporal method are at, or below, the minimum value of 500 suggested by Waples (1990b) for Pacific salmon and by Franklin (1980) for other species. Low values of N_e, however, are not without precedent in the literature. A number of studies have shown relatively low N_e for populations of salmonids considered at conservation risk, but also for populations not currently of management concern. Winter run chinook salmon from the Sacramento River listed as endangered had N_e from 21.9 to 401 calculated from the variance in male and female spawners (Hedrick *et al*. 1995) and 88.5 using the temporal method (Bartley *et al*. 1992). Three populations of steelhead trout from the Skeena Drainage in British Columbia that were not considered to be of management concern had values of N_e ranging from 92 to 560 (temporal method; Heath *et al*. 2001). Similarly, Jorde & Ryman (1996) found populations of brown trout from four lakes in Sweden to have N_e from 52 to 480 using the temporal method. Using mtDNA from one of these lakes, Laikre *et al*. (1998) estimated N_e using the temporal method for females to be 58 (while nuclear allele N_e estimate was 97). Simon *et al*. (1986) used estimates of variance in family survival to measure the effective number of breeders in coho salmon (*O. kisutch*) and found that N_e varied between 64 and 282 over a 25-year interval. The consequence of such low N_e may be profound. Waples (1990b) showed that low-frequency alleles are subject to rapid extinction in Pacific salmon where N_e is less than 500. Small N_e has been theorized to lead to inbreeding depression and a lack of response to stochastic events. Thus, populations with small N_e are at a much greater risk for extinction (Newman & Pilson 1997), but also may give little warning of impending extinction (Frankham 1995b).

Estimates of effective population size calculated by MIGRATE (N_{eM}) (Beerli & Felsenstein 1999) were considerably

higher than those from the temporal method (Waples 1990a). The differences in N_e calculated by the two methods are due probably to the estimate of mutation rate used; however, the relative differences between populations is probably valid. In contrast to the findings using the temporal method to calculate N_e, Willow had the highest and Stuart the lowest values of effective population size (Table 4). This discrepancy is due probably to violations of at least two assumptions associated with the calculation of N_{eM} (discrete generations and constant population size) using the method of Beerli & Felsenstein (1999). Thus, there is little agreement between the census population size, N_e, and N_{eM} calculated using MIGRATE.

Why is N_e *so low?*

N_e can be depressed due to a variety of factors, including fluctuating population size, small initial population size, unequal sex ratio and variance in family size (Franklin 1980). The overall low effective population size observed in this study are due, in part, to fluctuating census size as N_h/N was generally much less than 1 (Fig. 5). Small initial population size or population bottlenecks can also lead to reduced N_e (Franklin 1980; Garza & Williamson 2001), and some of our study populations have clearly experienced both (Fig. 3). Our temporal method estimates of N_e were very much lower than N_h, hence other factors are also impacting these populations.

Effective population size also depends on sex ratio and variance in family size: two parameters that are difficult to determine for wild populations (Waples 1990a). The ratio of N_e/N_h estimates the cumulative effect of departures in sex ratio and family size from ideal conditions (Kalinowski & Waples 2002). The low values of this ratio suggest that these two parameters may be influencing effective population size strongly. There are limited data on the sex ratios for the five populations of chinook we examined. Female to male ratios ranged from 0.86 for Dome Creek to 1.67 for Stuart River (Murray *et al*. 1981; NFCP 1994; R. Argue, pers. comm.). Interestingly, Heath *et al*. (2001) found a significant female bias in three of nine populations of steelhead, which were also characterized by very low N_e. Our sex-ratio data for all populations except Dome Creek are based on carcass recovery data and may represent a biased estimate; it is therefore difficult to determine whether a bias in sex ratio is affecting N_e in any of these populations. Simon *et al*. (1986) found that the variance in reproductive success among families in an enhanced population of coho salmon was much higher than expected and consequently N_e was much lower than the number of breeders. In naturally spawning Atlantic salmon (*Salmo salar*), Garant *et al*. (2001) found a high degree of variance in individual reproductive success; the number of offspring was correlated positively with the number of mates. Conversely, equalizing reproductive success of all individuals can cause N_e to be greater than the census size (Kalinowski & Waples 2002). A small public development project has enhanced chinook on Dome Creek since 1985. This project has utilized only broodstock from adults returning to the Dome system. The high ratio of N_e/N_h in the Dome population (Table 4, Fig. 5) is consistent with improved reproductive success of all individuals. The level of hatchery supplemenation, however, is generally low (approximately 12% of spawners) and juveniles are released at an early life stage back into Dome Creek (R. Argue, personal communication). The effect of this project on N_e therefore is not clear.

Perhaps the most striking finding of our study is the trend for a decline in N_e, despite clear evidence for increasing population size (N, Table 4). Generally, N_e is less than N (Nunney 1993; Frankham 1995a), but the expectation is that N_e should increase with increasing N. To our knowledge this is the first report of measurements of N_e that show no increase, even though population size has increased. This finding questions the benefit of relying exclusively on census estimates for interpreting population health. In isolated populations, genetic drift causes a loss of variation with each generation. The ratio of N_e/N is an important parameter to understand genetic drift in natural populations. Nunney (1993) showed that the ratio of N_e/N should be close to 0.5 and that it is unusual for N_e/N to be outside the range of 0.25–1.0. Many of our estimates of this ratio are less than 0.25 (Table 4, Fig. 5). Frankham (1995a) calculated N_e/N-values for 102 species from published sources, and found an average of 0.11. From Frankham's (1995a) analysis, the main factors responsible for low N_e/N estimates were fluctuation in population size, variance in family size, and to a lesser extent unequal sex-ratio. Vucetich *et al*. (1997) derived theoretically N_e/N for 44 populations to obtain an average of 0.21. Our values of N_e/N are within the range of estimates calculated by Frankham (1995a) and derived theoretically by Vucetich *et al*. (1997), although two of the populations we studied (Stuart and Bowron; Table 4) are well below these averages.

Large-scale vs. small-scale factors

A major construction project that impacted population size for two of the five stocks of chinook examined in this study was diversion of the Nechako River headwaters following the completion of Kenney Dam in 1952. This event should have impacted N_e and M differentially for these affected stocks. In terms of census population size, construction of the dam impacted the Nechako chinook and to a lesser extent the Stuart stock (Fig. 3). The decline in chinook salmon from the Nechako and Stuart, however, levelled off by the late 1960s and they have been increasing generally since that time. Flows in the Nechako are now regulated to minimize temperature increases during the autumn for migration and provide sufficient discharge to cover suitable habitat

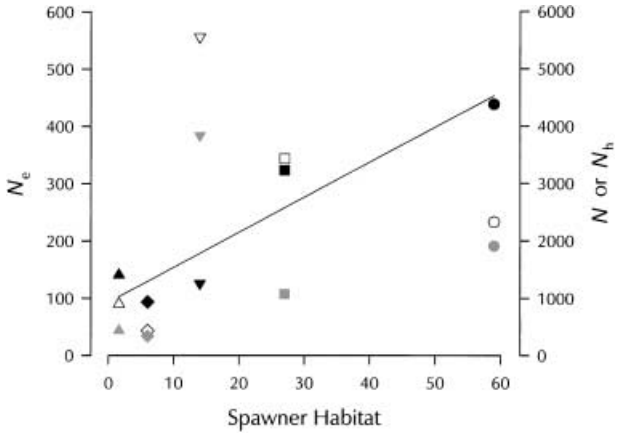

Fig. 6 Effective population size (N_e: closed symbols), mean census population size (N: open symbols) and harmonic mean of census size (N_h: shaded symbols) vs. river km of available spawner habitat. Each data point was calculated over the widest time interval from Table 4. The solid line is the significant linear regression of N_e on habitat ($P < 0.05$). Symbol shapes designate each river system as in Fig. 2.

for chinook spawners (NFCP 1989–94). Despite declines in numbers in the mid 1950s, N_e is greatest for the Nechako stock. Our results therefore do not link large-scale impacts on these chinook stocks to low N_e.

A number of development activities are likely to impact N_e and population size of chinook salmon within each of these watersheds. Forest harvesting, construction of road and railway rights-of-way, high water temperature and sedimentation are a concern for all the watersheds to varying degrees (Table 1; MacDonald *et al*. 1997a, 1997b). A cumulative development activity index for each watershed was assigned by MacDonald *et al*. (1997a, 1997b) that included factors likely to affect reproductive success of chinook within these watersheds, yet there is no relationship between the index score and N_e. Highly impacted watersheds (Nechako, Bowron and Willow) have N_e values greater than low impacted watersheds (Stuart and Dome). As a consequence, we have little evidence for these factors to be affecting genetic diversity, N_e or M directly. Spawning habitat area, however, is correlated significantly with N_e ($R^2 = 0.88$; $P < 0.05$; Fig. 6). Available spawning habitat was assessed by length of each river system over which chinook have been observed to spawn (Table 1) and was regressed on N_e for data from the widest range in sample dates. There was no correlation between spawning habitat and N ($P > 0.5$) or N_h ($P > 0.3$) (Fig. 6). The correlation between spawning habitat and N_e argues for the importance of maintaining or creating additional suitable habitat for spawning within each of these river systems. A similar finding was also reported by Jorde & Ryman (1996) for brown trout from four small lakes in Sweden. They found that N_e corresponded to the number of inflowing streams and available spawning habitat. Access to appropriate spawning sites may be a limiting factor for all five populations examined. If spawning habitat is limiting, mature fish may die without spawning or later spawning fish may reuse redd sites, removing eggs placed previously and influencing the reproductive success of early spawners. Loss of eggs from early spawners has been linked directly to spawner abundance in pink salmon (*O. gorbuscha*; Fukushima *et al*. 1998). As a consequence the number of individuals contributing to N_e may be very much less than N or even N_h. Interestingly, Ardren & Kapuscinski (2003) found increased N_e/N ratios at low N, which they identified as genetic compensation. A reduction in variance in reproductive success was responsible for this compensation effect. The lack of an increase in N_e despite the increase in census size in the present study, therefore, may be a direct consequence of limited habitat for spawning and reproductive failure in a subset of the returning salmon. Fish that do not reproduce successfully will be counted as part of N and used in calculations of N_h, but will not contribute to the next generation or N_e.

Control over factors that lead to a reduction in N_e (fluctuations in population size, variance in family size and unequal sex-ratio) may not be possible for chinook stocks from the upper Fraser River watershed. Through continuing current management efforts, we may be able to ensure populations continue to increase. Arresting the decline in N_e, however, is perhaps even more pressing for the long-term conservation of these stocks (Tringali & Bert 1998). Although large-scale anthropogenic impacts receive considerable attention, our study indicates that they may have relatively minor relevance to long-term population genetic health. Restoration and maintenance of habitat essential for successful spawning and survival of juveniles is probably the best method to limit or prevent any further declines in the N_e of these upper Fraser River chinook stocks. Our data also highlight the potential danger in basing management decisions solely on estimates of census population size for Pacific salmon stocks. We recommend that management decisions be based not only on knowledge of population size, but on multiple estimates of effective population size as well.

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This paper represents part of an ongoing effort to conserve and effectively manage salmon populations in British Columbia. Mark Shrimpton is examining how environmental disturbances and alterations to the aquatic ecosystem affect habitat use, physiological performance and population structure in fish. Daniel Heath is working on microgeographic population genetics, historic genetic changes, and the relationship between genetic diversity and performance in aquatic organisms.
