

Iteroparity in complex mating systems of steelhead *Oncorhynchus mykiss* (Walbaum)

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This study investigated diverse reproductive types in complex mating systems of steelhead *Oncorhynchus mykiss*. Postspawned steelhead (kelts) were sampled during attempted downstream migration over Lower Granite Dam on the Snake River, U.S.A. Multilocus microsatellite genotypes (14 loci) were used to assign unknown origin, kelt individuals to upstream populations of origin. Results indicated that iteroparity is a life-history trait that remains in several tributaries of the Snake River basin despite strong selection against downstream adult passage because of hydroelectric dams. The largest populations of steelhead in the Snake River, however, were only weakly represented (Clearwater River = 7.5% and Salmon River = 9.4%, respectively) in the kelt steelhead mixture relative to the Grande Ronde River (18.2%), Imnaha River (17.4%), Pahsimeroi Hatchery (25.2%) and Asotin Creek (22.2%). A lack of correlation between population escapement size and kelt proportions ($P > 0.05$) suggests that iteroparity was not uniformly expressed across populations, but was significantly negatively correlated with body size ($P < 0.05$). Iteroparity may be a valuable source of genetic variability and a conservation priority, especially in years with poor recruitment or in recently bottlenecked populations.

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Key words: iteroparity; mating systems; *Oncorhynchus mykiss*; semelparity; sex ratio.

INTRODUCTION

Variable mating systems are common in nature with species exhibiting a wide range of reproductive strategies. Most animals demonstrate iteroparous reproductive behaviour, but semelparity is observed in some groups such as snails

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Littorina spp. (Hughes & Roberts, 1980) and fishes [e.g. shad *Alosa sapidissima* (Wilson) and Pacific salmon *Oncorhynchus* sp.]. Fishes in the subfamily Salmoninae exhibit both iteroparity (*Salmo*, *Salvelinus*) as well as semelparity (most species of *Oncorhynchus*). Within the genus *Oncorhynchus*, the more derived species (Stearley & Smith, 1993; Oakley & Phillips, 1999) are exclusively semelparous [*Oncorhynchus nerka* (Walbaum), *Oncorhynchus tshawytscha* (Walbaum), *Oncorhynchus gorbuscha* (Walbaum), *Oncorhynchus keta* (Walbaum) and *Oncorhynchus kisutch* (Walbaum)], whereas the two ancestral species [*Oncorhynchus mykiss* (Walbaum) and *Oncorhynchus clarki* (Richardson)] retain iteroparity. Species with a semelparous strategy seek to maximize fitness by placing all energy and gametes into a single spawning season (e.g. individuals attempt multiple fertilizations and have high fecundity). In contrast, iteroparity may be a method of spreading genetic contribution over multiple generations, with less energy expenditure during each spawning season (Fleming & Reynolds, 2004). The only Pacific salmon that have relatively high potential for iteroparous reproduction are *O. mykiss* and *O. clarki*, although Unwin *et al.* (1999) describes unusual occurrences of iteroparity in the typically semelparous species, *O. tshawytscha*. Recent studies of Atlantic salmon *Salmo salar* L. and the anadromous form of *O. mykiss*, steelhead, show that most anadromous repeat spawners are female (Burgner *et al.*, 1992; Wertheimer & Evans, 2005), suggesting selection for anadromous males with a semelparous reproductive strategy. Iteroparity in the male gender of *O. mykiss* occurs through residence or precocity in fresh water, a life-history strategy infrequently observed in females.

Complex mating systems may have evolved to act as a balance for stochastic events (Taborsky, 2001). *Oncorhynchus mykiss* is considered to have one of the most diverse life histories in Salmonidae (Behnke, 1992) including resident, estuarine and anadromous ecotypes, wide ranges in age and timing of juvenile and adult migrations, and reproductive strategies including precocity, semelparity and iteroparity. This complex array of life-history variation is possibly a compensating or bet-hedging tactic for life in stochastic environments (Fleming & Reynolds, 2004). Different age at maturation and overlapping generations also provide a buffer for recovery potential in the event of catastrophic failure of any single brood year. If life-history variation buffers demographic risk, then disruption of complex mating systems that have evolved over geological time might threaten the long-term viability of populations or species.

Historically, iteroparity was a naturally occurring reproductive strategy in *O. mykiss* (Busby *et al.*, 1996), with the potential to provide an important source of genetic variation to steelhead populations over multiple generations. Prior to dam construction in the north-west U.S.A., iteroparity in steelhead was once estimated to be as high as 17% in some rivers in the lower Columbia River basin (Busby *et al.*, 1996) with some individuals spawning up to four consecutive times (Leider *et al.*, 1990). Evolutionarily, iteroparous steelhead (*i.e.* kelts) were probably an important source of genetic variability that reduced the extent of genetic drift within populations, especially in years with poor recruitment or recently bottlenecked populations.

In the Snake River, U.S.A., iteroparity may be limited by postspawning mortality as kelts attempt to navigate eight dams during seaward migration. A key component to the iteroparous reproductive strategy is the migration of postspawners

adults to the open ocean for nourishment to replenish energy levels necessary for additional spawning migrations (Jonsson *et al.*, 1997). In the Snake River, the sex ratio of kelt steelhead is predominantly female (77.0%; Evans *et al.*, 2004). This postspawning behaviour is in contrast with semelparity, in which females defend nest sites rather than attempt downstream migration (Crespi & Teo, 2002). Construction of several hydropower dams in the Columbia River basin during the 20th century has greatly restricted downstream adult steelhead migration (Busby *et al.*, 1996). While modern dams incorporate adult fish ladders and juvenile bypass facilities to assist upstream and downstream migration, there is little provision for downstream migrating adult kelt steelhead, nearly eliminating individuals attempting this reproductive strategy. Telemetry studies indicate that kelt steelhead suffer up to 96% mortality during downstream migration (Wertheimer & Evans, 2005).

In this study, postspawned kelt steelhead were sampled during attempted downstream migrations over the Lower Granite Dam, the furthest upstream of the eight hydropower facilities on the Snake River. Multilocus microsatellite genotypes were used to assign unknown origin, kelt steelhead individuals to upstream population of origin. This information was examined to determine the geographic distribution of potential repeat spawning in populations in the Snake River, despite several generations of strong selection against downstream adult passage at hydroelectric dams.

METHODS

BASELINE SAMPLES

All the Snake River sub-basins upstream of Lower Granite Dam were represented in the baseline reference (Fig. 1) if they were accessible to anadromous steelhead. Although many tributaries in the Snake River basin with steelhead populations were not sampled, such a scale of resolution was beyond the scope of this study. There were 20 populations included in the baseline reference for assigning mixtures of kelt steelhead to population of origin as shown in Fig. 1. Baseline populations were collected and genotyped as reported previously by P.M. (unpubl. data) and Narum *et al.* (2006). A subset of the genotypic data associated with this study were provided from an ongoing study that expands on the results of the baseline samples (M. Paquin, NOAA Fisheries, unpubl. data).

KELT STEELHEAD SAMPLES

Tissue samples of kelt steelhead were collected at the Lower Granite Dam juvenile bypass facility, located at river km 107.5 on the Snake River. Adult steelhead were removed from the Lower Granite bypass facility and transferred to a nearby 190 l sampling tank containing circulated river water. Fish were then anaesthetized, measured for fork length (L_F), a non-lethal fin clip taken and checked for marks such as adipose or other fin clips. Specimens were then scanned with ultrasound to determine reproductive state (prespawning or postspawning) and sex (Evans *et al.*, 2004). Kelt steelhead were collected from the bypass facility for 11 weeks, from 31 March to 15 June 2002, and were not observed in the facility during other times of the year. Sampling occurred throughout the kelt steelhead outmigration period, with sampling effort that corresponded to daily numbers of fish collected in the trap. Sampling was conducted for 2 days during week 1, 6 days per week during weeks 2 to 8, 5 days per week from

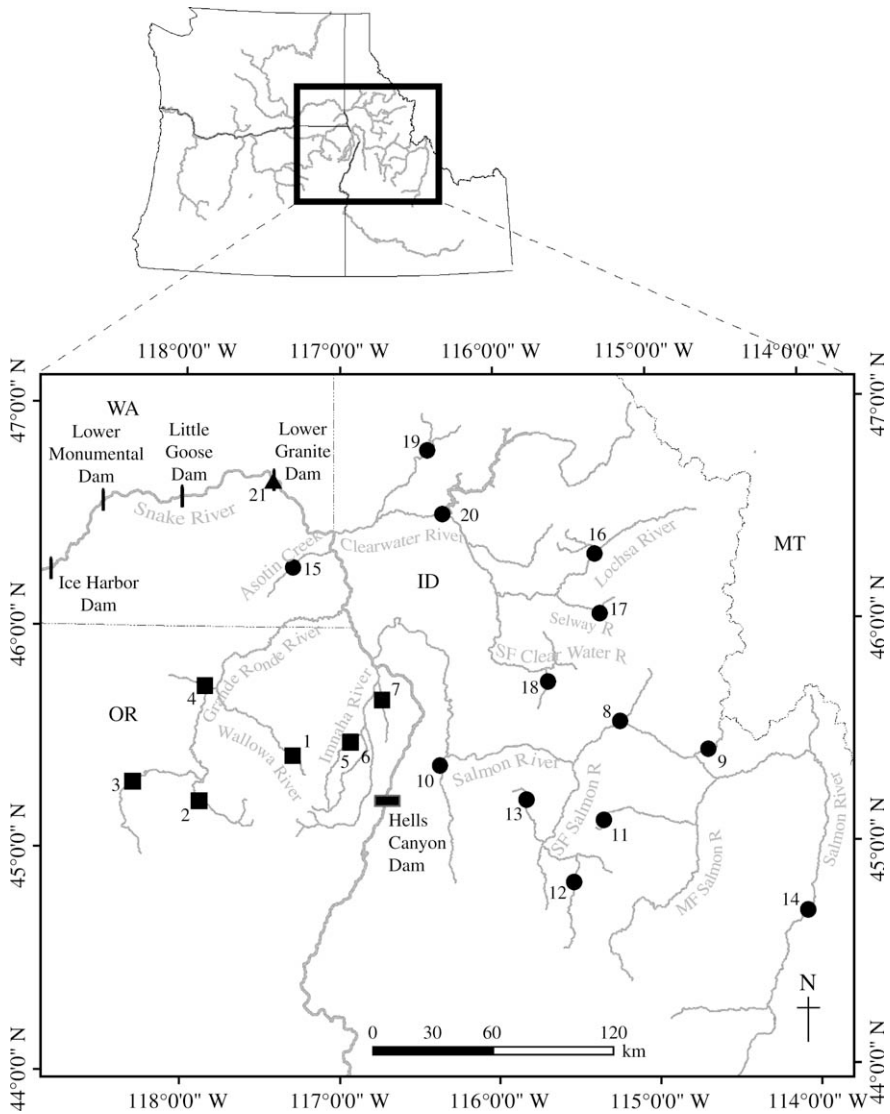


FIG. 1. Map of the Snake River basin, Pacific north-west U.S.A., including baseline reference populations of *Oncorhynchus mykiss*, kelt collection site at Lower Granite Dam, and location of three other lower Snake River hydroelectric dams (■, adult collection; ●, juvenile collection; ▲, kelt mixture sample). Collections numbered: (1) Wallowa Hatchery, (2) Catherine Creek Weir, (3) upper Grande Ronde Weir, (4) Lookingglass Creek, (5) Little Sheep Creek Weir, (6) Little Sheep Creek hatchery stock, (7) Lightning Creek Weir, (8) Bargamin Creek, (9) Horse River, (10) Rapid River, (11) Big Creek, (12) Johnson Creek, (13) Secesh River, (14) Pahsimeroi Hatchery, (15) Asotin Creek, (16) Fish Creek, (17) Gedney Creek, (18) Ten Mile Creek, (19) Potlatch Creek, (20) Dworshak Hatchery and (21) Lower Granite Dam kelt mixture.

weeks 9 to 10 and 3 days in week 11. A total of 2617 kelt steelhead were encountered at the Lower Granite Dam facility across the run and tissue samples were collected from each fish. Of these tissues, 361 samples were randomly selected (<http://www.randomizer.org/form.htm>) across the collection period for genotyping.

LABORATORY ANALYSIS

Fin clips were digested and DNA extracted using standard protocols from Qiagen® DNeasy™ in conjunction with a Qiagen® 3000 robot (Qiagen Inc., Valencia, CA, U.S.A.). Genomic DNA was quantified and arrayed into 96 well plates for genotyping.

The polymerase chain reaction (PCR) was used to amplify 14 microsatellite loci: *Ocl1* (Condrey & Bentzen, 1998), *Ogo4* (Olsen *et al.*, 1998), *Oke4* (Buchholz *et al.*, 1999), *Oki23MMBL* (Smith *et al.*, 1998), *Omy7* (K. Gharbi, pers. comm.), *Omy1001* (Spies *et al.*, 2005), *Omy1011* (Spies *et al.*, 2005), *Oneu14* (Scribner *et al.*, 1996), *Ots3* (Banks *et al.*, 1999), *Ots4* (Banks *et al.*, 1999), *Ots100* (Nelson & Beacham, 1999), *Ssa289* (McConnell *et al.*, 1995), *Ssa407* (Cairney *et al.*, 2000) and *Ssa408* (Cairney *et al.*, 2000). Eleven loci were dinucleotide repeats (*Ocl1*, *Ogo4*, *Omy7*, *Omy1001*, *Oneu14*, *Ots3*, *Ots4*, *Ots100*; *Oke4*, *Ssa289* and *Ssa407*) and three were tetranucleotide repeats (*Oki23*, *Omy1011* and *Ssa408*). Amplifications were performed with *c.* 25 ng of genomic DNA and Applied Biosystems (Foster City, CA, U.S.A.) reaction chemistry in 7 µl total volume. Cycling conditions (9700 thermal-cyclers; Applied Biosystems) included an initial denaturation of 1 min at 92° C, followed by 30 cycles of 30 s at 92° C (denature), 30 s at 50–60° C (anneal) and 30 s at 72° C (extension). A final extension was carried out for 40 min at 72° C. Annealing temperatures were adjusted to optimize PCR conditions for each locus (*Ocl1* = 58° C, *Ogo4* = 56° C, *Oke4* = 52° C, *Oki23MMBL* = 56° C, *Omy7* = 58° C, *Omy1001* = 58° C, *Omy1011* = 58° C, *Oneu14* = 58° C, *Ots3* = 58° C, *Ots4* = 58° C, *Ots100* = 58° C, *Ssa289* = 58° C, *Ssa407* = 58° C and *Ssa408* = 58° C). Forward primers were fluorescently labelled (Applied Biosystems), and PCR products were genotyped using manufacturer's protocols with an Applied Biosystems model 3730 genetic analyser. Alleles were scored with GeneMapper software from Applied Biosystems.

STATISTICAL ANALYSIS

Fisher's exact tests were used to evaluate each locus and population for departures from Hardy–Weinberg equilibrium with a Markov Chain Monte-Carlo (MCMC) algorithm as implemented in GENEPOP v. 3.4 (Raymond & Rousset, 1995). Significant deviations from Hardy–Weinberg equilibrium may indicate a violation of one of the assumptions of the null model, including admixed populations in a sample collection. Corrections were made against type I error in exact tests with the sequential Bonferroni method (Rice, 1989). Tests for linkage disequilibrium between all pairs of loci were also performed with the MCMC method in GENEPOP.

Pair-wise genetic differentiation among baseline collections was estimated from allele frequencies (F_{ST} ; Weir & Cockerham, 1984) with GENEPOP. Exact tests were also completed with GENEPOP to compare allele frequencies between all pairs of populations. Significance levels were adjusted for multiple tests with the sequential Bonferroni method (Rice, 1989).

To infer the degree of relatedness between sample collections, pair-wise genetic distances (Cavalli-Sforza & Edwards, 1967) were estimated between all populations with GENDIST in PHYLIP v. 3.5 (Felsenstein, 1993). Genetic chord distances were then used to construct a neighbour-joining dendrogram of sample populations with NEIGHBOR (PHYLIP v. 3.5). Bootstrap replicates of 1000 iterations were attained with SEQBOOT and a consensus tree was formed with CONSENSE in PHYLIP v.3.5.

To determine the appropriate number and composition of baseline reporting groups, the number of distinct populations in the baseline was estimated with the programme STRUCTURE v. 2.1 (Pritchard *et al.*, 2000). All baseline individuals were included without *a priori* population identity. The number of distinct populations (k) was determined by averaging three iterations of k ranging from one to eight, at a 'burnin' of 100 000 iterations, and a simulation with 500 000 iterations. The average k value with the greatest probability defined the number of reporting groups, and population composition within each distinct group provided information for clustering populations into broader reporting groups for mixture analyses.

Three types of mixture simulations were examined with the programme GMA (Genetic Mixture Analysis; Kalinowski, 2003) to evaluate the power of the baseline to analyse mixture samples. First, simulations of 20 mixtures, each with 100% composition of the 20 baseline populations, were completed independently. In each simulation, 400 multilocus genotypes were drawn from the baseline (sampling with replacement assuming random mating and independent assortment of loci) and stock composition of the mixture was estimated from the average of 1000 replicates, given 100% as the parametric value. The second type of simulations included three different mixtures of five populations in the baseline, each with 20% composition. Again, 400 multilocus genotypes were drawn from the baseline as described above and stock composition of the mixture was estimated from the average of 1000 replicates, this time with 20% as the parametric value. The third type of simulated mixture sample included all baseline individuals to determine the individual assignment accuracy to both population and reporting group. Finally, the observed genotypes from individuals in the kelt steelhead mixture were analysed in GMA and both population and reporting group of origin were determined based on highest assignment probability (Rannala & Mountain, 1997).

Average L_F and median collection date were estimated from individuals that were assigned to each population and reporting group. An ANOVA and pair-wise comparisons were used to test for significant differences in average L_F among reporting groups. The Bonferroni method was used to correct the critical value for multiple tests in pair-wise comparisons ($\alpha = 0.05/15 = 0.0033$). The 95% CI for median collection date were estimated with a non-parametric distribution in the programme 'R' (<http://www.r-project.org>). The programme 'R' was also used to determine correlations (Pearson's test) between reporting group L_F and kelt steelhead proportions, and between reporting group proportions of escapement and kelt steelhead. Escapement proportions were determined from radio tagged steelhead passing Lower Granite Dam during upstream spawning migrations (C. Peery, unpubl. data).

RESULTS

BASELINE ANALYSES

Tests for deviations from Hardy–Weinberg equilibrium in baseline populations revealed that only one of 280 tests (20 populations at 14 loci) was significant (Bonferroni adjusted critical value = $0.05/280$ tests = 0.0002). Tests of Hardy–Weinberg equilibrium in the kelt steelhead mixture, however, indicated that two of 14 loci (*Omy7* and *One14*) were statistically significant (Bonferroni adjusted critical value = $0.05/14$ tests = 0.0036) due to heterozygote deficiencies. These heterozygote deficiencies were probably due to a Wahlund effect in the collection of kelt steelhead that was an admixture of multiple distinct populations.

Pair-wise F_{ST} tests of 20 baseline populations revealed significant genetic structure between 188 of 190 pair-wise comparisons (Bonferroni adjusted $\alpha = 0.005 = 0.05/190$ tests = 0.0003). The two non-significant tests were both among Catherine Creek, to Wallowa Hatchery ($P = 0.777$) and upper Grande Ronde River ($P = 0.012$). Average F_{ST} values ranged from 0.007 to 0.031 within sub-basins to 0.025 to 0.043 among sub-basins (Table I). A neighbour-joining dendrogram (Fig. 2) provides additional evidence supporting the phylogenetic relationship of sites within sub-basins. One exception was the Pahsimeroi Hatchery collection that clustered with a site from the Grande Ronde River (Lookingglass Creek) due to mixed ancestry of these stocks from hatchery transplants (Keifer *et al.*, 1992). Further, Pahsimeroi Hatchery is not

TABLE I. Summary of pair-wise F_{ST} (minimum, maximum and mean) of *Oncorhynchus mykiss* within and among sub-basins of the Snake River, U.S.A.

	Within sub-basins			Among sub-basins		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Grande Ronde River	-0.0019	0.0177	0.0077	0.0035	0.0553	0.0247
Imnaha River	0.0099	0.0155	0.0134	0.0057	0.0703	0.0295
Salmon River	0.0108	0.0558	0.0282	0.0103	0.0824	0.0370
Pahsimeroi Hatchery	0.0073	0.0073	0.0073	0.0066	0.0630	0.0276
Clearwater River	0.0120	0.0444	0.0305	0.0176	0.0824	0.0427
Asotin Creek	na	na	na	0.0035	0.0533	0.0216

na, not applicable since only one site from Asotin Creek.

included in the Salmon River cluster since broodstock are of mixed origin that were taken throughout the Snake River (Busby *et al.*, 1996). Analysis with STRUCTURE software suggested nearly equal probability of either five to six distinct population clusters within the baseline. Since six reporting groups

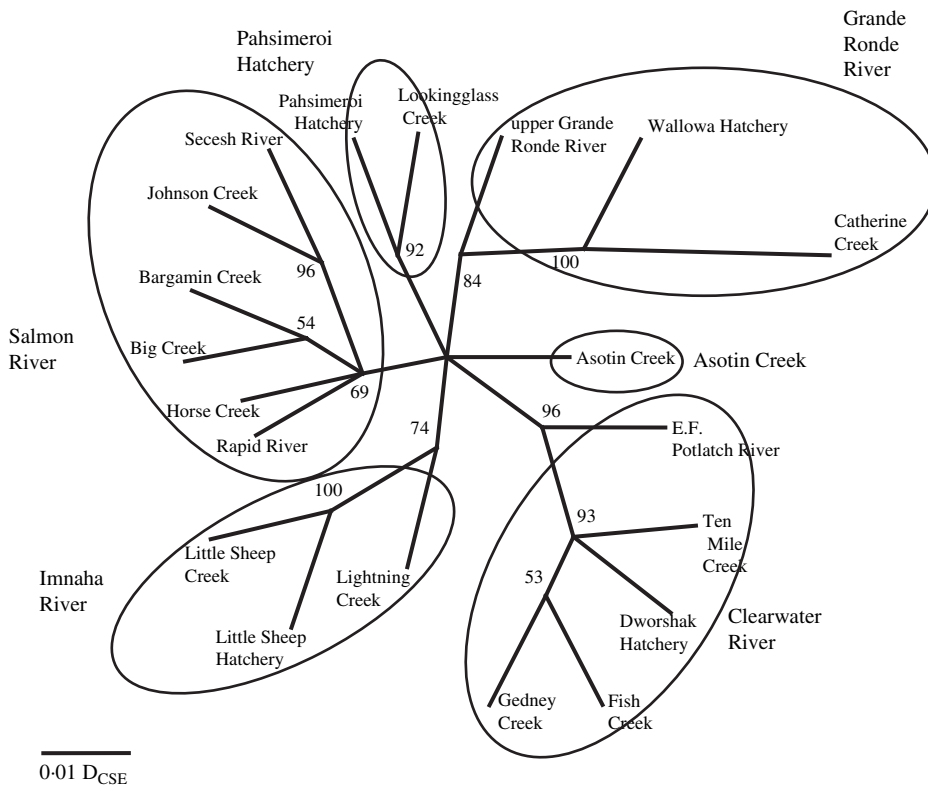


FIG. 2. Neighbour-joining dendrogram of all 20 populations of steelhead included in the baseline (see Fig. 1). Numbers at nodes represent bootstrap percentages from 1000 iterations. Ovals indicate six reporting groups for mixture analyses as defined by genetic clusters.

were most concordant with the clusters of population aggregates in the neighbour-joining dendrogram, six ovals corresponding to reporting groups are shown in Fig. 2. The baseline populations contained in each oval were considered reporting groups for mixture analyses (Asotin Creek, Grande Ronde River, Imnaha River, Clearwater River, Salmon River and Pahsimeroi Hatchery).

MIXTURE ANALYSES

Results from 100% simulations had a range of 98.3–99.9% correct assignment for each of the 20 baseline populations. Accuracy was slightly higher with a narrower error range when the results were defined by reporting groups (99.1–99.9%). Three mixture simulations with 20% population composition were within 0.41% of population composition and 0.22% of reporting group composition (Table II). Finally, a mixture simulation with all individual

TABLE II. Simulations of three hypothetical mixtures ($n = 400$) of *Oncorhynchus mykiss* with expected and estimated proportions

	Mixture 1		Mixture 2		Mixture 3	
	Expected	Estimated	Expected	Estimated	Expected	Estimated
Reporting group						
Clearwater River	0.20	0.2010	0.20	0.2015	0.20	0.2019
Pahsimeroi Hatchery	0.20	0.1989	0.20	0.2008	0.20	0.1992
Salmon River	0.20	0.1997	0.20	0.1995	0.20	0.1988
Imnaha River	0.20	0.1982	0.20	0.1979	0.20	0.2001
Grande Ronde River	0.20	0.2022	0.20	0.2003	0.20	0.2000
Population						
Wallowa Hatchery	0.20	0.1972	0.00	0.0024	0.00	0.0012
Catherine Creek	0.00	0.0037	0.20	0.1963	0.00	0.0018
Upper Grande Ronde River	0.00	0.0014	0.00	0.0016	0.20	0.1971
Lookingglass Creek	0.20	0.1977	0.00	0.0012	0.00	0.0010
Little Sheep Creek (weir)	0.00	0.0005	0.00	0.0008	0.20	0.1980
Lightning Creek (weir)	0.20	0.1970	0.00	0.0012	0.00	0.0009
Little Sheep Hatchery	0.00	0.0007	0.20	0.1959	0.00	0.0011
Bargamin Creek	0.00	0.0006	0.00	0.0007	0.00	0.0006
Horse River	0.00	0.0006	0.20	0.1981	0.00	0.0005
Rapid River	0.00	0.0003	0.00	0.0002	0.20	0.1972
Big Creek	0.00	0.0003	0.00	0.0003	0.00	0.0003
Johnson Creek	0.20	0.1978	0.00	0.0002	0.00	0.0002
Secesh River	0.00	0.0001	0.00	0.0000	0.00	0.0000
Pahsimeroi Hatchery	0.00	0.0012	0.20	0.1996	0.20	0.1982
Asotin Creek	0.00	0.0016	0.00	0.0017	0.00	0.0017
Fish Creek	0.00	0.0001	0.00	0.0003	0.00	0.0002
Gedney Creek	0.00	0.0002	0.00	0.0003	0.00	0.0003
Ten Mile Creek	0.00	0.0001	0.00	0.0001	0.20	0.1993
Potlatch Creek	0.20	0.1987	0.00	0.0003	0.00	0.0002
Dworshak Hatchery	0.00	0.0002	0.20	0.1986	0.00	0.0001

baseline samples provided estimates that were similar to the composition of each population (mean of 5.8% difference from expected value of 100%). Of the 20 populations in the mixture, estimates for 15 of these were within 10% of the expected composition in the mixture and none were outside 14%. The five populations with estimates outside 10% included three populations that were under estimated, Horse Creek (88.2%), Gedney Creek (89.0%) and Ten Mile Creek (86.9%) and two that were over estimated, Rapid River (110.6%) and Fish Creek (112.2%). When estimates were distributed among the six reporting groups, all were within 3% of actual composition with the exception of the Imnaha River group that was over represented (119.1%).

Kelt steelhead samples assigned widely throughout the Snake River basin. Individual kelt steelhead were assigned to 17 of 20 populations and to every sub-basin (*i.e.* reporting group) with composition ranging between 7.5 and 25.2% (Table III). The three populations with no kelt steelhead assignments were Secesh River from the Salmon River sub-basin and Gedney Creek and Ten Mile Creek from the Clearwater sub-basin. The least represented sub-basins in the kelt steelhead mixture were the Clearwater River (7.5%) and the Salmon River (10.0%), and the most highly represented reporting group was Pahsimeroi Hatchery (25.2%). Other than the high mixture composition attributed to nearby Asotin Creek (22.2%), population distance from Lower Granite Dam did not influence kelt steelhead composition (Table III). Individuals that were assigned to reporting groups near Lower Granite Dam, however, had earlier median collection dates at the dam than more distant reporting groups (Table III). Individuals that assigned to Clearwater River and Salmon River reporting groups had larger L_F on average than other reporting groups (Table III), consistent with larger 'B-run' steelhead that spend an extra year in the ocean and are known to occupy those drainages. Mean L_F among reporting groups were significantly different in an ANOVA ($F_{5,355}$, $P < 0.001$). Pair-wise tests indicated that the Clearwater River had significantly larger mean L_F than all other groups, and the Salmon River had significantly larger mean L_F than the Imnaha River ($P < 0.001$) and Pahsimeroi Hatchery ($P < 0.01$). Mean L_F of reporting units was significantly negatively correlated with kelt

TABLE III. Median collection date with 95% CI and mean \pm S.E. fork length (L_F) for kelt *Oncorhynchus mykiss* reporting groups

Reporting Group	Percentage of kelt <i>O. mykiss</i>	Median collection date	95% CI		Mean \pm S.E. L_F (mm)
			Lower	Upper	
Clearwater River	7.5	15/04/2002	11/04/2002	20/04/2002	731 \pm 21
Asotin Creek	22.2	23/04/2002	20/04/2002	16/05/2002	637 \pm 7
Grande Ronde River	18.2	10/05/2002	20/04/2002	18/05/2002	645 \pm 10
Pahsimeroi Hatchery	25.2	16/05/2002	05/05/2002	18/05/2002	628 \pm 6
Imnaha River	17.5	17/05/2002	16/05/2002	20/05/2002	620 \pm 7
Salmon River	9.4	16/05/2002	20/04/2002	21/05/2002	672 \pm 15

steelhead proportions ($r = -0.83$, $P < 0.05$), and kelt steelhead proportions were not significantly correlated with escapement proportions by reporting groups ($r = -0.58$, $P > 0.05$).

All hatcheries in the baseline, even those from sub-basins with low numbers of kelt steelhead, were evident in the mixture sample (range of 2.5–7.5% composition). Within sub-basins, mixture composition of hatchery and tributaries differed, but not consistently. In the Imnaha and Grande Ronde Rivers, hatcheries had lower numbers of kelt steelhead than the average composition of natural populations in tributaries of those sub-basins (5.0 and 2.4% less than tributary populations, respectively). Conversely, hatcheries in the Clearwater and Salmon Rivers represented a larger percentage in the mixture than the average of natural populations in tributaries of each sub-basin (5.9 and 6.2% more than natural populations, respectively).

DISCUSSION

Despite strong selection against iteroparity in the Snake River basin because of lengthy downstream migration distance and impediments of the hydro-system (up to 96% mortality; Wertheimer & Evans, 2005), the large number of kelt steelhead encountered at Lower Granite Dam is an indication that this reproductive strategy has not been extirpated. Resident rainbow trout (*O. mykiss*) are commonly iteroparous (Behnke, 1992), and high gene flow with steelhead in some populations (Zimmerman & Reeves, 2000; Olsen *et al.*, 2005) may act to maintain this trait in anadromous *O. mykiss*. Results from the current study suggest that iteroparous behaviour (downstream migration) is less common in some Snake River populations than others. Populations in the Clearwater and Salmon Rivers had much lower incidence of kelt steelhead observed at Lower Granite Dam than populations in the Imnaha River, Grande Ronde River and Asotin Creek. This is in contrast to estimates of steelhead run sizes from tagging studies that indicate the Clearwater and Salmon Rivers support the largest populations of steelhead above Lower Granite Dam (C. Peery, unpubl. data). The lack of correlation between population size and kelt proportions ($P > 0.05$) suggests that the trait of iteroparity is not uniformly expressed across populations, but is more abundant in some drainages than others in the Snake River basin.

The small proportion of Salmon River and Clearwater River origin kelt steelhead in the mixture suggests that either these kelt steelhead were not collected during sampling or iteroparous activity is minimal in fish from these sub-basins. Since samples were collected throughout the entire period of known kelt steelhead 'outmigration', sampling bias is unlikely. Another possibility is that kelt steelhead from these two sub-basins 'outmigrate' in completely different months than the sampling period, but this is also unlikely since kelt steelhead have not been observed in the bypass trap at other times of the year (Evans *et al.*, 2004). The results indicate that iteroparous behaviour is limited in the Salmon and Clearwater Rivers, possibly due to selection against this reproductive strategy along with limited gene flow between resident and anadromous components of the population. The Imnaha and Grande Ronde Rivers should have been subject to similar levels of selection, but stocking of hatchery fish with mixed broodstock (Keifer *et al.*, 1992) or high gene flow with resident

populations may have provided a source to maintain this reproductive trait. Additionally, larger steelhead ('B-run') are more common in the Clearwater and Salmon Rivers (Busby *et al.*, 1996; Keefer *et al.*, 2002), and larger body and egg size select for semelparous reproduction rather than iteroparity (Crespi & Teo, 2002). Larger individuals require more nutrients than smaller fish to re-establish body condition after long distance spawning migrations, leading to higher postspawn mortality rates. Further, the larger egg size of individuals with large body size often relates to higher juvenile survival and selection for semelparous reproduction.

Tributary latitude (Withler, 1966) and distance from the ocean (Meehan & Bjornn, 1991) have each been suggested to have some influence on the rate of iteroparity in steelhead populations. Withler (1966) observed that populations in British Columbia and further north have lower rates of iteroparity than those in California Oregon and Washington. Subsequent studies in Alaska (Lohr & Bryant, 1999), however, report relatively high levels of repeat spawning (39.6% of winter run and 21% of summer run) that are contrary to the trend observed by Withler (1966). Additional studies of steelhead from high latitude countries on other continents (Kamchatka Peninsula of Russia; Savvaitova *et al.*, 1999; Argentina; Pascual *et al.*, 2001), with predominant iteroparous populations, also suggest that latitude is not a major influence on iteroparity. Tributary distance from the ocean, however, probably influences iteroparity rate as migration has high energetic costs (Roff, 1991; Hogasen, 1998; Hendry & Berg, 1999). Busby *et al.* (1996) reviewed rates of repeat spawning of North American steelhead and found almost all coastal populations had higher levels of iteroparity than inland populations. One exception to this observation was the population of inland steelhead in the Rogue River, Oregon. In the present study, there was no consistent trend of population composition of kelt steelhead related to distance from the ocean. Other than the high mixture composition attributed to nearby Asotin Creek (*c.* 30 river km), population distance from Lower Granite Dam did not appear to consistently influence kelt steelhead composition. For example, composition of kelt steelhead of lower river populations in the Clearwater River sub-basin were low (*i.e.* only 0.8% composition from East Fork Potlatch River). Inconsistencies in kelt steelhead population composition related to distance may be because Snake River populations above Lower Granite Dam are sufficiently inland that all have high energetic costs due to migration distance relative to coastal populations. Kelt steelhead collected at Lower Granite Dam, however, reflect *c.* 17% of adult escapement (Evans *et al.*, 2004), suggesting that a significant proportion of adult steelhead retain the energy to attempt downstream migration. The effect of the hydro-system on mortality during downstream migration may be a severe additive source of mortality and selection against iteroparity for these inland steelhead populations.

Previous studies that have quantified repeat spawning with scale analysis have shown that steelhead of the west coast of North America range in iteroparity rate from 2 to 26% (Withler, 1966; Howell *et al.*, 1985; Busby *et al.*, 1996). Results of these studies indicate that populations of coastal, ocean-maturing, steelhead typically have higher rates of iteroparity than inland, stream-maturing, steelhead populations. While this trend may be expected

given the proximity of coastal rivers to the ocean, inland steelhead from the Rogue River in Oregon had the second highest proportion of repeat spawners of sites included in the study (17%; Busby *et al.*, 1996). Few evaluations of iteroparity of inland steelhead have occurred, and only one study (Long & Griffin, 1937; average repeat spawning rate of 3.4%) occurred prior to the construction of the hydro-system in the Columbia River basin (Bonneville Dam was built in 1938). An additional study (Whitt, 1954) of repeat spawning in the Snake River prior to construction of four lower Snake River dams, however, found that repeat spawning of 'B-run' steelhead in the Clearwater River was *c.* 2%. These studies suggest that repeat spawning may have only constituted a small proportion of the historical anadromous run in the Snake River basin, but overall estimates of steelhead iteroparity rates prior to human disturbance are vague. Therefore, direct comparisons of iteroparity rate before and after human disturbance are not possible. It is apparent from other populations of *O. mykiss*, however, that relatively high levels of iteroparity are possible in this species (*e.g.* Rogue River; Busby *et al.*, 1996; Alaska; Lohr & Bryant, 1999; Kamchatka; Savvaitova *et al.*, 1999).

CONSERVATION IMPLICATIONS

As females are often the limiting resource in salmonid mating systems (Fleming, 1998), special concern is warranted to conserve life histories dominated by females. Operational sex ratios (Emlen & Oring, 1977) of salmonids are generally skewed heavily towards males (Hendry *et al.*, 1995; Quinn *et al.*, 1996; Fleming & Reynolds, 2004; Kuligowski *et al.*, 2005) and conservation of iteroparous females may provide improved balance to sex ratios of populations. Genetic drift will be reduced in populations with multiple reproductive strategies (Fleming & Reynolds, 2004) that include precocial males, resident 'sneakers' and both iteroparous and semelparous anadromous adults.

Snake River steelhead are at a considerable disadvantage during downstream migration because summer spill is negligible at four lower Snake River dams (Fig. 1) and their preferred passage route over dam spillways (Wertheimer & Evans, 2005) may not be an option in low flow years. This is in contrast to mainstem Columbia River hydropower dams that continue to spill water throughout much of the summer and provide a more direct and favourable migration route for kelt steelhead (Evans *et al.*, 2004; Wertheimer & Evans, 2005). To improve downstream migration of kelt steelhead through the Snake River hydro-system, options under consideration include increased summer spill, permanent artificial side channels, removable spillway weirs and dam breaching.

Kelt reconditioning is also an option that is likely to improve survival of downstream migrating kelt steelhead and improve operational sex ratios. Reconditioning was initially attempted and successful with iteroparous Atlantic salmon (Gray *et al.*, 1987; Crim *et al.*, 1992; Johnston *et al.*, 1992), brown trout *Salmo trutta* L. (Poole *et al.*, 1994) and Arctic charr *Salvelinus alpinus* (L.) (Boyer & Van Toever, 1993). Subsequent efforts with *O. mykiss* have also been effective at improving survival of kelt steelhead (Hatch *et al.*, 2004). This is potentially a conservation option for disrupted mating systems of steelhead that are heavily skewed towards males. Continuing research (*e.g.* reproductive

success studies of reconditioned kelt steelhead) will assist in determining whether this could be a potentially important recovery technique for steelhead populations.

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