Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon

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Executive Summary

This report presents results of studies testing for genetically based differences in performance (growth, migration, and survival) between hatchery and wild populations of steelhead and Chinook salmon (Project Number 90-052). The report is organized into 10 chapters with a general study introduction preceding the first chapter. A growing body of data shows that domestication and a resulting loss of fitness for natural rearing occur in hatchery populations of anadromous salmonids; however, the magnitude of domestication will vary among species and hatchery programs. Better information on domestication is needed to accurately predict the consequences when hatchery and wild fish interbreed. The intent of hatchery supplementation is to increase natural production through introduction of hatchery fish into natural production areas. The goal of this study was to provide managers with information on the genetic risks of hatchery supplementation to wild populations of Columbia River Basin summer steelhead and spring Chinook salmon.

We employed a common garden experimental design, where artificially spawned progeny of hatchery (H) and wild (W) fish were reared together from an early age (eyed-embryo or button-up fry developmental stage). Communal rearing ensured that performance differences among progeny groups (HH, HW, and WW) were from genetic differences, or from maternal effects such as egg size or quality, rather than from environmental differences. Progeny of hatchery females spawned with wild males were included to separate genetic from maternal effects. Because HH and HW progeny were maternal half siblings, performance differences between them would indicate genetic effects. Genetic marks or DNA parentage analysis were used to identify progeny group for individuals sampled after the groups were mixed. Fitness for natural rearing was evaluated by releasing progeny groups in natural streams and comparing their performance through juvenile outmigration. Domestication is also expected to increase fitness for hatchery rearing. Fitness in the hatchery program was tested by rearing progeny groups in the hatchery and comparing their performance at least through seaward migration after release from the hatchery and sometimes to adult return.

Chapter 1 describes comparisons between progeny of hatchery steelhead from Dworshak National Fish Hatchery and wild steelhead from Fish Creek, a Lochsa River tributary. Comparisons were made for the 1992 and 1993 year-classes for HH and WW progeny only (no HW cross). The hatchery population originated 5-6 generations earlier with wild steelhead from the North Fork Clearwater River. Natural rearing occurred in Brushy Fork Creek, also a Lochsa River tributary. Survival of 1992 year-class juveniles to smolt release from Dworshak Hatchery was only 18% due to a disease outbreak. Under these low survival conditions, survival was 2.8 times higher for HH than for WW fish. All 1993 year-class fish had died halfway through the scheduled 10 month hatchery rearing period, again due to disease. Survival to when 1% of the initial fry were still alive was 4.2 times higher for HH than for WW fish indicating that WW fish succumbed to the epizootic sooner than did HH fish. Emigrants from the Brushy Fork study reach were sampled for three years and fish residing in the study reach were sampled for six years following fry release. Most emigrants were one or two years old and too small to be smolts (mean fork length at age-2 = 93 mm). Survival in Brushy Fork was unambiguously lower for HH than for WW fish of the 1992 year-class. Survival of the 1993year-class was higher for HH than for WW fish during the first two months in Brushy Fork but was lower for HH than for WW fish thereafter, and net survival from release to ages 3 and older was also lower for HH than for WW fish if our emigrant samples were representative (periods of inoperative emigrant traps prevented certainty about this). A thunderstorm-induced power outage interrupted flow to the incubation trays at the hatchery and compromised a major tenet of the common garden design for the 1992 year-class, possibly contributing to the inconsistency in relative survival in Brushy Fork between year-classes. The storm caused the incubation environment to differ between the progeny groups as a result of reduced oxygen levels and higher densities for HH alevins. This difference was illustrated by a 55% loss for HH fish during the event, about twice that for WW fish. Differences between progeny groups were also found for growth at the hatchery (HH

substantially greater than WW), detection rate at lower Snake and Columbia River dams for seaward migrating juveniles of the 1992 year-class after release from the hatchery (HH again much greater than WW), and growth in Brushy Fork (HH<WW for the 1992 year-class; HH>WW for the 1993 year-class). Many HH and WW males and females residualized and matured in Brushy Fork. Ovaries weight at ages 4 and 5was greater for HH than for WW females of both year-classes; testes weight at age-3 was less for HH than for WW males of the 1993 year-class. Results were generally consistent with the expectations of domestication: performance better for HH than for WW fish in the hatchery but worse for HH than for WW fish in the stream.

Chapters 2 and 3 present comparisons between hatchery steelhead from Dworshak and wild steelhead from the Selway River. Comparisons were made for the 1994 year-class (HH and WW crosses) and the 1995 year-class (HH, HW, and WW crosses). Chapter 2 describes tests of fitness for hatchery rearing. Fish were reared at Dworshak Hatchery, where smolts were produced in the standard one year time period, and also at nearby Clearwater Hatchery where lower rations, lower winter temperatures, and two years of rearing more closely mimicked the natural life history (natural smolt age = 2-4 years). At both hatcheries, progeny groups were reared together in the same raceways (mixed treatment) and also in separate replicate raceways (separate treatment) to test for competition. Smolts were acclimated at and released from a satellite hatchery facility on Crooked River, a South Fork Clearwater River tributary. We found little or no difference among crosses in survival in the hatchery but higher rates of growth in the hatchery and seaward migration after release for HH than for WW fish (HW intermediate). Faster growth for HH fish resulted in greater size at release which contributed to their higher migration rate, but other as yet uncharacterized traits also affected migration since the migration difference among crosses was apparent even within size classes. Growth of WW fish was slower in the mixed than in the separate treatment indicating that WW fish were competitively inferior to HH fish in the hatchery environment. Precocious males sometimes composed a large percentage of the fish in raceways at Clearwater Hatchery (the maximum was 30% for WW fish of the 1995 year-class in the separate treatment). Incidence of precocious males was higher for WW than for HH fish (HW intermediate) in the separate treatment but not in the mixed treatment. Incidence of HH precocious males was similar between treatments. Apparently, the presence of HH fish suppressed early maturation by WW males. A direct effect beyond the suppression of WW growth by HH fish was involved because the effect persisted within size categories. The difference in growth rate between HH and WW fish was consistently, although not significantly, less at Clearwater Hatchery (two-year rearing) than at Dworshak Hatchery (one-year rearing), perhaps suggesting a slight benefit to WW fish of the more natural rearing environment at Clearwater. Greater seaward migration for HH than for WW fish was primarily due to greater residualization for WW than for HH fish in Crooked River rather than to immediate differential mortality. Based on sampling and tagging of 1994 year-class residuals in Crooked River, condition factor of both HH and WW residuals in late summer was considerably lower than it had been in the hatchery the previous March, and the residuals produced almost no seaward migrating smolts the following spring. Adult return rate was higher for HH than for WW fish of the 1995 year-class (HW intermediate), consistent with the difference in seaward migration; only three adults (all WW) returned from the 1994 year-class. Results of hatchery rearing tests were generally consistent with domestication of the hatchery population: performance better for HH than for WW fish in the hatchery programs.

Chapter 3 describes natural rearing tests for steelhead of the 1994 and 1995 year-classes. Rearing occurred in Twentymile Creek, a South Fork Clearwater River tributary. Emigrants from and fish residing in Twentymile Creek were sampled for five years following fry release. Over 90% of the emigrants were one or two years of age and too small to be smolts (mean fork length at age-2 = 103 mm). Per fry released, the HH cross produced 0.64-0.83 times as many emigrants and 0.63-0.68 times as many age-4 residuals as the WW cross (HW intermediate). Survival from age-1 to age-4 was lower for HH than for WW residuals (i.e., non-migrants) of the 1995 year-class (HW intermediate) and survival from age-2 to age-4 may have been lower for HH than for WW residuals of the 1994 year-class (P=0.06).

Collectively, these results indicate lower survival for HH than for WW fish. Size was often greater for HH than for WW fish indicating faster growth for the former (HW size usually intermediate). Dispersal of fry from release sites and emigration of one- and two-year olds from the study stream were greater for WW than for HH fish (HW intermediate). Greater dispersal for WW than for HH fish was probably not caused by competitive displacement of small by larger fish. Incidence of flowing milt was higher for HH than for WW fish at age-2. Peak incidence of flowing milt for older residuals was similar among crosses (about 50%), but the peak occurred at greater size and age for WW than for HH residuals (HW intermediate). Natural rearing results were generally consistent with domestication of the hatchery population: performance worse for HH than for WW fish.

Chapter 4 describes calibration and testing of a steelhead incubation model that predicts time between fertilization and maximum alevin wet weight (MAWW) from incubation temperature. MAWW corresponds to the button-up fry stage of development. Model accuracy was tested by incubating embryos from Dworshak hatchery steelhead at warm (mean=11.6^{o}C) or cold (mean=7.3^{o}C) temperatures and measuring the time between fertilization and MAWW for each temperature. Model projections of time to MAWW were within 1% of measured time to MAWW. Mean egg weight ranged from 0.101-0.136 g among tested females (mean across females = 0.116 g). For each temperature, time to MAWW was positively related to egg size (i.e., to mean egg weight per female), but the increase in time to MAWW with increasing egg size was greater for embryos reared at the warm than at the cold temperature. We developed equations accounting for the effect of egg size on time to MAWW for each temperature, and also for the mean of those temperatures (9.3^{o}C).

Chapter 5 presents tests of whether varying incubation temperatures to match development between embryos from different spawning dates affected survival or growth of steelhead fry released in a stream (North Fork Palouse River) and in hatchery raceways (at Dworshak Hatchery). Dworshak hatchery steelhead were artificially spawned on two dates separated by a four week interval. Progeny from the early date (ExE, from early males and early females) were incubated in chilled (7°C) water, and those from the late date (LxL) were incubated in ambient (12°C) water, until developmental stage matched. A third group, created by fertilizing eggs from late females with cryopreserved milt from early males (ExL), was included to control for any genetic differences between early and late returning adults. Survival in the stream to 3 and 15 months after release was similar among crosses. Survival in the hatchery to near the end of the standard one year rearing period was similar among crosses for one of two year-classes but different for the other; however, it was difficult to ascribe the differences (ExL>ExE; LxL intermediate but closer to ExE) to incubation temperature differences. We conclude that there was little if any effect of incubation temperature on survival. Length of juveniles of one year-class differed among crosses in the stream and in the hatchery. Length of the other year-class differed among crosses in one pond at the hatchery but not in the other pond or in the stream. When length differed the pattern was always the same: ExE>LxL; ExL intermediate but closer to LxL. We speculate that incubation temperature may have affected growth of juveniles, and in particular that a longer period of incubation in chilled water may have caused fast juvenile growth relative to a shorter incubation period in ambient water.

Chapter 6 describes tests of whether differences in developmental stage of fry at release affected subsequent survival and growth of steelhead in a stream (North Fork Palouse River) and in hatchery raceways (at Dworshak Hatchery). Differences in development were created by artificially spawning Dworshak hatchery steelhead and incubating their progeny at three different temperatures (means=10.9, 11.3, and 11.7^{o}C). Time between fertilization and maximum alevin wet weight (MAWW) was predicted from incubation temperature using a model. MAWW is equivalent to the button-up fry stage of development. Developmental stages at release were “underdeveloped” (97.7% of model-predicted time to MAWW, mean alevin weight=0.177 g, mean alevin proportion yolk [yolk weight/total body weight]=0.087), “intermediate” (102.5%, 0.179 g, 0.044), and “overdeveloped” (107.9%, 0.156 g, 0.030).

Neither survival nor growth in the hatchery to near the end of the standard one year rearing period differed among groups. In the stream, frequency of overdeveloped fish relative to the other two groups decreased from release in May to September, probably indicating lower survival for the overdeveloped fish during that interval since our other studies showed emigration of steelhead sub-yearlings to be negligible. Length in September was less for overdeveloped than for intermediate fish and was in between for underdeveloped fish, suggesting that growth between May and September was less for overdeveloped fish than for intermediate fish. Relative frequency of the fry development groups changed from September to one year later; however, the change may have reflected differences in emigration rate during the interval rather than differential survival. Our results show a cost to survival and growth in a stream, but not in a hatchery, from overdevelopment characterized by loss of weight and yolk reserves relative to fry closer to MAWW at release. We didn't find any cost from underdevelopment; however, our underdeveloped fry were closer to MAWW than the overdeveloped fry, and they may have been no farther from MAWW than the intermediate fry based on percentage of model-predicted time to MAWW at release.

Chapter 7 presents tests of whether differences in size of unfed fry at release affected survival and growth of juvenile steelhead in hatchery ponds (at Dworshak Hatchery) and streams (Silver and Twentymile creeks, both South Fork Clearwater River tributaries). Differences in fry size were produced by selecting and spawning females that differed in the mean size of their eggs. Experiments were initiated in 1996 and 1999 with Dworshak hatchery steelhead. Fry size groups were small (mean fork length=26.7 mm, mean weight=0.149 g) and large (28.1 mm, 0.197 g) in 1996 and small (27.5 mm, 0.159 g), medium (28.2 mm, 0.190 g), and large (28.9 mm, 0.201 g) in 1999. Survival in the hatchery to near the end of the standard one year rearing period and in streams to late summer, three months after release, was higher for the large than for the small group in 1996 but was similar among groups in 1999. Survival in streams to age-1 appeared to show the same pattern (large>small in 1996; no difference in 1999), but differences among fry size groups in emigration rate may also have been involved. The inconsistency between years may have resulted because some 1996 female parents of the small fry size group had exceptionally small eggs and were a year younger than the other 1996 females and all 1999 females. Growth in the hatchery was similar among groups in both years whereas growth in streams was faster for the large than for the small group in both years and intermediate for the medium group in 1999. Growth in streams appeared to be limited by food availability. Initially large fry probably out-competed smaller fry for limited food; however, we found no evidence that dispersal from release sites or emigration from streams was caused by competitive displacement of small by larger fish.

Chapter 8 describes comparisons between progeny of hatchery spring Chinook salmon from Warm Springs National Fish Hatchery and wild spring Chinook salmon from the Warm Springs River. The hatchery population was initiated in 1978-1981 with Warm Springs River salmon. Hatchery managers included wild fish in the hatchery broodstock most years and avoided artificial selection to minimize genetic divergence from the wild founder population. We compared performance among HH, HW, and WW progeny of the 1992 and 1996 year-classes. Both year-classes were reared at Warm Springs Hatchery; only the 1996 year-class was reared in a natural stream (Little White Salmon River). The progeny groups differed on several measures including survival to outmigration in the stream (WW>HH>HW) and juvenile growth in the hatchery (1992 year-class; WW>HW>HH); however, results may have been confounded. The genetic marks we used (genotypes at the *sSOD*-1* allozyme locus) were found to differentially effect survival in a companion study (HH mark favored over WW mark; HW mark intermediate). Furthermore, HW survival in the current study was neither intermediate, as would be expected from additive genetic effects, nor similar to that of HH fish as would be expected from maternal effects since HW and HH fish were maternal half-siblings. Finally, the unexpected performance of HW fish precluded ruling out maternal differences between hatchery and wild mothers as the cause of differences between HH and WW fish. The key finding that survival of HH fish in the stream was 0.91 that for WW fish, indicating a small loss of fitness for natural rearing in the hatchery population, is valid

only if three conditions hold: (1) any selection on the genetic marks was in the same direction as in the companion study, (2) lower survival in the stream for HW than for WW or HH fish resulted because some HW families were genetically atypical, not from problems with either of the other two groups, and (3) lower survival for HH than for WW fish was not due to maternal effects. Although all three conditions had support, none of it was conclusive. Study results provided only suggestions, not definitive answers for the primary question of whether the hatchery population had diverged genetically from its wild founder population in fitness-related traits.

Chapter 9 describes a study that replicated comparisons between progeny of hatchery spring Chinook salmon from Warm Springs National Fish Hatchery and wild spring Chinook salmon from the Warm Springs River. The problem of non-neutral genetic marks was avoided by using DNA parentage analysis to identify progeny group (HH, HW, or WW). Comparisons were made for the 2000 year-class reared at Warm Springs Hatchery and in the Metolius River. A goal of the hatchery program was to minimize genetic divergence from the wild founder population. One technique used to achieve this goal was fall and spring volitional releases that allowed juveniles to express natural migration behavior. Hatchery rearing of our experimental fish included volitional releases in two of three raceways. About 40% of the fish in these two raceways left the hatchery in the fall. The fall migrants comprised 2-3 times more WW than HH fish (HW intermediate), a clear difference in migration behavior. We simulated a fall volitional release in the third raceway by siphoning water into an adjacent raceway. Fish going through the siphon composed only 14% of the fish in the original raceway and were similar among crosses (0.33 HH, 0.31 HW, 0.36 WW). Survival of hatchery reared juveniles to adult return was 35% higher for HH than for WW fish (27% higher for HW than for WW fish). Growth of juveniles in the hatchery was similar among crosses. Mean length of age-4 returning adults was greater for HH (693 mm) and HW fish (691) than for WW fish (678). Performance in the stream did not differ statistically between HH and WW fish; however some comparisons were not definitive due to high emigration rates shortly after fry release. About 23% of the released fry emigrated from the study reach during the first 39 days of the 16 month outmigration period, many of them within three days of release. These early emigrants were 38% WW, 30% HW, and 32% HH fish (significant difference between WW and HW) and composed 74% of total outmigrants. Early emigration precluded knowing anything about the performance of the population components that emigrated. It also precluded comparing survival among crosses between the unfed fry and summer parr life stages. Nevertheless, some comparisons were possible. Survival from summer parr sampling to fall-spring outmigration was similar among crosses. Migration timing after the first 39 day period was also similar among crosses, as was size of migrants and of summer parr. Performance differences among hatchery-reared crosses were consistent with domestication (higher survival to adult for HH than for WW fish in the hatchery program) and suggested that selection against fall emigration from the hatchery was a mechanism of domestication.

Chapter 10 presents tests of the relative importance of maternal versus genetic effects on performance of spring Chinook salmon. Pure-strain and reciprocal crosses were made with fish from Carson National Fish Hatchery (C) and Warm Springs National Fish Hatchery (W): CC, WW, CW=Carson female x Warm Springs male, and WC=Warm Springs female x Carson male. The crosses were mixed as button-up fry, reared at Warm Springs Hatchery to the smolt stage, and then transferred to a seawater facility where they were reared for three months. Growth and final size at the hatchery and in seawater were always least for WW fish compared to fish from the other crosses, and were significantly less for WW fish than for fish from at least one of the other crosses. Growth and final size at the hatchery and in seawater were always statistically similar among CC, CW, and WC fish. Survival at the hatchery and for the entire three month seawater rearing period were similar among crosses; however, survival during the first month of seawater rearing was significantly higher for WW fish than for fish from the other crosses. In general, fish with at least one Carson parent (CC, CW, and WC) performed similarly to each other but differently from pure-strain Warm Springs fish (WW). One explanation is that genetic effects were non-additive, with Carson genetics being dominant over Warm Springs genetics.

Alternatively, there could have been an interaction between maternal and genetic effects such that if the female parent was from Warm Springs then the genetic difference between populations was expressed, but if the female was from Carson then the genetic difference between populations was not expressed.

In addition to these chapters, we also published three papers on ancillary studies conducted in support of our main experiments with hatchery and wild fish:

Reisenbichler, R. R., M. C. Hayes, S. P. Rubin, L.A. Wetzel, and B. M. Baker. 2006. *PEPA** genotype affects return rate for hatchery steelhead. Transactions of the American Fisheries Society 135:28-39.

This paper describes tests for the neutrality of the genetic marks used to differentially mark progeny of hatchery and wild steelhead (chapters 1-3). The marks were genotypes at the cytosol non-specific dipeptidase locus (*PEPA-1**). The two most common alleles at this locus in Clearwater River steelhead were **100* and **110*. The marks for HH, HW, and WW progeny were **110/*110*, **100/*110*, and **100/*100*, respectively. The mark neutrality study tested for differential growth and survival among fish that differed in *PEPA-1** genotype but were similar in other respects. Heterozygous (**100/*110*) Dworshak hatchery fish were spawned. Each mating produced all three genotypes in a 1:2:1 ratio (confirmed by sampling alevins) following the rules of Mendelian inheritance. Half of the progeny from each mating were reared at Dworshak Hatchery for one year and then released to migrate to the ocean and return to the hatchery as adults. The other half were released in a natural stream (North Fork Palouse River) as unfed fry and monitored for three years. Two year-classes (1992 and 1993) were evaluated. We found no differences in size or survival among *PEPA-1** genotypes for either the naturally reared fish or the hatchery-reared yearlings just before smolt release. For females, survival to returning adult was also similar among genotypes; however, hatchery-reared males with the **110/*110* genotype returned at a higher rate than did males with the **100/*100* genotype; survival of heterozygous males was intermediate. These results indicate that selection occurs at the *PEPA-1** locus or at one or more loci tightly linked to it.

Discussion of the implications of these findings for interpretation of our performance comparisons between hatchery and wild progeny is included in the relevant report chapters (1-3). Briefly, because *PEPA-1** genotype did not affect growth or survival of naturally reared fish or hatchery-reared juveniles in the mark neutrality study, it was unlikely to have caused the differences we observed among HH, HW, and WW progeny for those life stages and rearing environments. *PEPA-1** genotype was also unlikely to have caused the large differences among HH, HW, and WW progeny in seaward migration after release from the hatchery (chapters 1 and 2) because those migration differences were due in large part to growth differences in the hatchery prior to release. Finally, independent data, presented in the Chapter 2 discussion section, showed that juvenile growth patterns in the early years of the Dworshak Hatchery program were similar to WW growth patterns in our study, suggesting that genetic change from generations of selection against slow grow at Dworshak Hatchery, rather than *PEPA-1** genotype, was responsible for the growth differences we observed among HH, HW, and WW progeny. The performance comparison most likely to have been affected by the *PEPA-1** genetic marks was adult return rate of hatchery-reared juveniles (Chapter 2).

Hayes, M. C., R. R. Reisenbichler, S. P. Rubin, and L.A. Wetzel. 2011. Differential survival among *sSOD-1** genotypes in Chinook salmon. Transactions of the American Fisheries Society 140:1305-1316.

This paper presents tests for the neutrality of the genetic marks used to differentially mark progeny of hatchery and wild Chinook salmon (Chapter 8). The marks were genotypes at the superoxide dismutase locus (*sSOD-1**). The marks for HH, HW, and WW Chinook salmon progeny were **-100/*-100*, **-100/*-260*, and **-260/*-260*, respectively. The Chinook salmon mark neutrality study was

conducted similarly to the mark neutrality study for steelhead. Heterozygous (**-100/*-260*) Chinook salmon were spawned, and growth and survival of their progeny were evaluated under hatchery rearing to returning adult return and under natural rearing to summer parr or juvenile outmigration. Warm Springs hatchery fish were spawned in 1992, 1993, 1996, and 1997, and their progeny were reared at the hatchery but not in a natural stream. Carson hatchery fish were spawned in 1993 and their progeny reared at the hatchery and in Buck and Rattlesnake creeks (White Salmon River drainage). Fish from Little White Salmon National Fish Hatchery were spawned in 1999 and their progeny reared in the Little White Salmon River but not at the hatchery. Survival to returning adult at Warm Springs Hatchery was significantly higher for the **-100/*-100* genotype than for **-260/*-260* (**-260/*-260* survival 0.76 that for **-100/*-100*; **-100/*-260* survival 0.88 that for **-100/*-100*). Adult return rate to Carson Hatchery showed a similar but non-significant pattern (**-260/*-260* survival 0.77 that for **-100/*-100*; **-100/*-260* survival 0.80 that for **-100/*-100*). Size was similar among genotypes for juveniles and adults at Warm Springs Hatchery. Size differed significantly among genotypes for juveniles (**-100/*-100* greater than **-100/*-260*) and age-4 adult males (**-100/*-100* greater than **-100/*-260* or **-260/*-260*) at Carson Hatchery. Survival in Buck and Rattlesnake Creeks to the summer parr stage was significantly higher for **-100/*-100* than for **-260/*-260* (**-260/*-260* survival 0.76 that for **-100/*-100*; **-100/*-260* survival 0.88 that for **-100/*-100*). Survival in the Little White Salmon River to juvenile outmigration was similar among genotypes. Size was similar among genotypes for all natural rearing comparisons. In summary, survival and size differences among *sSOD-1** genotypes commonly occurred in both rearing environments (juveniles in the stream; juveniles and returning adults at the hatchery), and those differences always showed better performance for **-100/*-100* than for **-260/*-260* (**-100/*-260* usually intermediate). These results indicate selection at the *sSOD-1** locus or at loci tightly linked to it.

Performance differences among genetic marks were more pervasive for Chinook salmon (*sSOD-1** genotypes) than for steelhead (*PEPA-1** genotypes). Many of the performance comparisons between hatchery and wild Chinook salmon progeny described in Chapter 8 may have been confounded by non-neutrality of the genetic marks. A possible exception was the finding that survival in the stream was lower for HH than for WW fish. This finding may have been valid since the mark neutrality study showed survival in the stream to be higher, not lower, for fish with the HH mark (**-100/*-100*) than for fish with the WW mark (**-260/*-260*). Note that performance comparisons between hatchery and wild Chinook salmon progeny described in Chapter 9 were not confounded because the progeny were not genetically marked. Instead, DNA parentage analysis was used to identify progeny group (HH, HW, or WW).

Hayes, M. C., S. P. Rubin, J. E. Hensleigh, R. R. Reisenbichler, and L. A. Wetzel. 2005. Performance of juvenile steelhead trout (*Oncorhynchus mykiss*) produced from untreated and cryopreserved milt. Aquaculture 249:291-302.

This paper describes tests for performance differences between steelhead produced from untreated milt (UM) and cryopreserved milt (CM). Two year-classes, 1996 and 1997, were tested. Differences between UM and CM fish of the 1996 year-class were found for alevin weight, final size of juveniles reared sympatrically (UM and CM fish in the same tanks) at low ration levels for 7 months, and mean cortisol level of juveniles exposed to chronic (48-hour) stress. No performance differences were found between UM and CM fish of the 1997 year-class. Length of 1996 year-class alevins was influenced by a significant milt-by-family interaction, suggesting a greater treatment effect (UM versus CM) for some families than for others. Results indicate that milt cryopreservation can affect post-fertilization performance under some circumstances.

Part of the material contained in Chapter 2 was presented in a previously published paper:

Reisenbichler, R. R., S. P. Rubin, L.A. Wetzel, and S. R. Phelps. 2004. Natural selection after release from a hatchery leads to domestication in steelhead, *Oncorhynchus mykiss*. Pages 371-383 In K.M. Leber, H.L. Blankenship, S. Kitada, and T. Svåsand [editors] Stock Enhancement and Sea Ranching: developments, pitfalls, and opportunities. 2nd edition. Blackwell Science Ltd, Oxford.

This paper uses results of our comparison between hatchery and wild steelhead progeny for survival and growth in the hatchery and seaward migration after release to illustrate how domestication can occur even though mortality of juveniles in the hatchery is much lower than mortality in the natural environment after release. We found little or no difference among crosses in survival in the hatchery but much higher rates of growth in the hatchery and seaward migration after release for HH than for WW fish (HW intermediate). Seaward migration rate was positively correlated with size at release; therefore, fish that grew fast (i.e., performed well) in the hatchery had a selective advantage after release. Slow growing WW fish were at a selective disadvantage. Generations in the hatchery program would select for faster growth in WW fish descendants, resulting in growth rates similar to those observed for HH fish. Even after growth differences were accounted for (i.e., when similarly sized fish were compared), post-release migration differences were apparent between HH and WW fish, suggesting that domestication selection also operated on traits other than growth.

Introduction

A growing body of data shows that domestication and a resulting loss of fitness for natural rearing occur in hatchery populations of anadromous salmonids (Reisenbichler and McIntyre 1977; Reisenbichler and Rubin 1999; Berejikian and Ford 2004; Araki et al. 2008). The magnitude and consequences of domestication, however, will vary among streams and species, and better information on domestication is required to accurately predict the consequences when hatchery fish interbreed with wild fish (Reisenbichler et al. 2003; McClure et al. 2003; Fraser 2008). This issue has important management implications in part because substantial interbreeding is the *intent* of supplemention programs which are commonly proposed or implemented to increase natural production.

Risk from hatchery supplementation of naturally spawning salmon and steelhead populations was a poorly understood issue in the Pacific Northwest when this study was conceived. Many biologists dismissed the possibility of domestication and felt that simply avoiding out-of-basin transfers into a hatchery was sufficient to avoid significant genetic risks from supplementation. One consequence of this thinking was that hatchery steelhead from Dworshak National Fish Hatchery on the North Fork Clearwater River were being considered for use in supplementing the wild populations in both the Lochsa and Selway Rivers (Steve Yundt, personal communication; all three populations originated within the Clearwater River, Idaho). This situation suggested an important need to evaluate whether genetic differences in performance existed between Dworshak Hatchery fish and wild Lochsa or Selway fish, and whether domestication was largely responsible for any such differences.

Some biologists held that domestication in anadromous salmonids could be eliminated by starting the hatchery population with local wild fish, avoiding selective breeding in the hatchery and by routinely introducing wild fish into the hatchery broodstock. This progressive strategy was implemented for spring Chinook salmon at Warm Springs National Fish Hatchery, Oregon even though domestication had not been demonstrated previously for Chinook salmon. We tested whether the program at Warm Springs Hatchery has been effective at avoiding domestication in Warm Springs Chinook salmon.

This study tested for genetic differences in growth, migration, and survival between hatchery and wild steelhead in Idaho and spring Chinook salmon in Oregon. We employed a common garden experimental design, rearing artificially spawned progeny of hatchery and wild fish together in natural streams and in hatcheries. Naturally reared progeny were tracked through juvenile outmigration. Hatchery-reared progeny were followed to returning adult. The steelhead were from the Clearwater River: Dworshak National Fish Hatchery and the Lochsa and Selway Rivers. The salmon were from the Deschutes River: Warm Springs National Fish Hatchery and the Warm Springs River.

The results are presented in separate chapters corresponding to different objectives and different study sites. Our work with steelhead is covered by chapters 1-7*;* our work with Chinook salmon is covered by chapters 8-10. We conducted a number of ancillary studies to support our main experiments with hatchery and wild fish. Some of the ancillary studies are covered by chapters in this report (chapters 4-7 and 10) whereas others were previously published in journals. One previously published paper presents part of the material contained in Chapter 2 of this report. Abstracts of the previous publications are appended to this report, and results summaries are included in the Executive Summary section.

Rearing sites and other details of our main experiments changed over time for various reasons. Steelhead experiments were conducted with four year-classes. In 1992 and 1993, wild adults were collected from a Lochsa River tributary (Fish Creek), natural rearing also occurred in a Lochsa tributary (Brushy Fork Creek), and hatchery rearing took place at Dworshak Hatchery (Chapter 1). In 1994 and 1995, wild adults were collected from the Selway River at Selway Falls, natural rearing occurred in a South Fork Clearwater River tributary (Twentymile Creek) (Chapter 3), and the majority of hatchery

rearing took place at Clearwater Hatchery located on the North Fork Clearwater River near (across the river from) Dworshak Hatchery. We changed sites for the 1994 and 1995 year-classes to combine efforts with regional managers who were evaluating introduction of Selway River steelhead to the South Fork Clearwater River. Wild steelhead had been extirpated from the South Fork in the mid-1900's by Harpster Dam which has since been removed. Our experimental fish were reared at Clearwater Hatchery for two years to more closely mimic the 2-4 year freshwater rearing period of naturally produced steelhead in the region, and smolts were acclimated at and released from a satellite hatchery facility on Crooked River (South Fork Clearwater River drainage). A small portion of the 1995 year-class fish were reared at Dworshak Hatchery for the standard one year period which allowed us to compare relative performance of hatchery and wild fish between the two hatchery programs (two-year rearing at Clearwater Hatchery; one-year rearing at Dworshak). At both Clearwater and Dworshak hatcheries, hatchery and wild fish were reared in sympatry (in the same raceways) and allopatry (in separate raceways) to test for competition between hatchery and wild fish. Results of hatchery rearing for the 1994 and 1995 year-classes are reported in Chapter 2. Rearing sites, source populations, and year-classes associated with each report chapter are given in italics in the Table of Contents and on chapter title pages.

Chinook salmon experiments were conducted with the 1992, 1996, and 2000 year-classes. Low returns of salmon in the intervening years did not allow enough wild fish for our study. Hatchery rearing of all three year-classes took place at Warm Springs Hatchery. No suitable natural rearing site was available for the 1992 year-class; natural rearing of the 1996 year-class occurred in the Little White Salmon River in south-central Washington. One of our ancillary studies (Hayes et al. 2011) showed that the genetic marks we used for the 1992 and 1996 year-classes (*sSOD-1* genotypes) were not selectively neutral and therefore confounded our results. These results are nevertheless reported in Chapter 8 with careful discussion of limitations in interpretation due to the non-neutral marks. We adopted an inherently neutral genetic mark, DNA-based parentage analysis, for use with the 2000 year-class. This technique was in its infancy in 1992 but had matured enough to be feasible by 2000. The Metolius River, a Deschutes River tributary, became available to us as a natural rearing site for the 2000 year-class because of efforts to reintroduce spring Chinook salmon. The salmon were eliminated in the 1960's by dam construction and the creation of two large reservoirs (Simtustus and Billy Chinook) that presented navigation difficulties for downstream-migrating juveniles. Solutions to this problem were under investigation by 2000. Results for the 2000 year-class are reported in Chapter 9 in extended abstract format. Full results have been submitted to a journal that does not accept material previously published in agency reports with wide circulation.

The common garden design of our experiments required that hatchery and wild progeny be mixed together at an early age (unfed fry). We genetically marked the progeny groups (or used parentage analysis) so that population of origin could be identified for individuals sampled after mixing. Differences in spawning time between hatchery and wild fish posed the challenge of giving hatchery and wild fish an "equal start" in our experiments. We manipulated incubation temperatures to slow development of early progeny or speed up development of late progeny, thereby matching development by the eyed-embryo or button-up fry developmental stage. Most of our ancillary studies tested whether performance (growth, migration, or survival) was differentially affected by the genetic marks (Reisenbichler et al. 2006; Hayes et al. 2011) or by incubation temperature manipulations (chapters 4-7 herein). A finding of performance differences would indicate that the common garden assumption of equal treatment of progeny groups was invalid.

Our main experiments sometimes included hybrid progeny produced by crossing wild males with hatchery females. We initially thought that spawning time would be enough later for wild steelhead than for hatchery steelhead to require cryopreservation of wild milt for use in wild-by-hatchery crosses made the following year. We therefore tested whether performance differed between fish conceived with

thawed compared to fresh milt (Hayes et al. 2005). As it turned out, spawning time overlapped sufficiently to make milt cryopreservation unnecessary.

An original goal of our work with spring Chinook salmon was to compare the performance of Warm Springs wild and hatchery fish to that of fish from Carson National Fish Hatchery, a well-established hatchery stock that had been cultured for many generations without any introgression from wild fish. Confounding from maternal effects was a concern for this study particularly because Carson females tended to carry lower levels of bacterial kidney disease (BKD) than Warm Springs females (Susan Guttenberger, U. S. Fish and Wildlife Service, personal communication, 2012), and BKD is readily transmitted from mother to offspring (Pascho et al. 1991). We therefore tested the relative importance of maternal versus genetic effects on performance of fish from Warm Springs and Carson hatcheries (Chapter 10). The plan to include Carson fish in our experiments was ultimately dropped in favor of restricting the comparison to wild, hatchery, and wild-hatchery hybrids from Warm Springs.

Natural rearing sites for ancillary studies with steelhead were the North Fork Palouse River (Palouse River Drainage, Idaho), and Silver and Twentymile creeks (South Fork Clearwater River drainage). Natural rearing sites for ancillary studies with Chinook salmon were the Little White Salmon River, and Buck and Rattlesnake creeks (White Salmon River drainage, Washington).

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References

Araki, H., B. A. Berejikian, M. J. Ford, and M. S. Blouin. 2008. Fitness of hatchery-reared salmonids in the wild. Evolutionary Applications **1**:342-355.

Berejikian, B.A., and M.J. Ford 2004. Review of the relative fitness of hatchery and natural salmon. US Dept. Com., NOAA Tech. memo. No. NMFS-NWFSC-61.

Fraser, D.J. 2008. How well can captive breeding programs conserve biodiversity? A review of salmonids. Evol. Appl. 2: 1-52.

Hayes, M. C., R. R. Reisenbichler, S. P. Rubin, L.A. Wetzel, and A. R. Marshall. 2011. Differential survival among *sSOD-1** genotypes in Chinook salmon. Transactions of the American Fisheries Society 140:1305-1316.

Hayes, M. C., S. P. Rubin, J. E. Hensleigh, R. R. Reisenbichler, and L. A. Wetzel. 2005. Performance of juvenile steelhead trout (Oncorhynchus mykiss) produced from untreated and cryopreserved milt. Aquaculture **249**:291-302.

McClure, M.M., E.E. Holmes, B.L. Sanderson, and C.E. Jordan. 2003. A large-scale multispecies status assessment: anadromous salmonids in the Columbia River Basin. Ecol. Appl. 13: 964-989.

Pascho, R. J., D.G. Elliott, and J.M. Streufert. 1991. Brood stock segregation of spring Chinook salmon *Oncorhynchus tshawytscha* by use of the enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody technique (FAT) affects the prevalence and levels of *Renibacterium salmoninarum* infection in progeny. Diseases of Aquatic Organisms 12(1), 25-40.

Reisenbichler, R. R., M. C. Hayes, S. P. Rubin, L.A. Wetzel, and B. M. Baker. 2006. *PEPA** genotype affects return rate for hatchery steelhead. Transactions of the American Fisheries Society 135:28-39.

Reisenbichler, R.R., and J.D. McIntyre. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, Salmo gairdneri. Journal of the Fisheries Research Board of Canada 34: 123 128.

Reisenbichler, R.R., and S.P. Rubin. 1999. Genetic changes from artificial propagation of Pacific salmon affect the productivity and viability of supplemented populations. ICES Journal of Marine Science 56: 459-466.

Reisenbichler, R.R., F.M. Utter, and C.C. Krueger. 2003. Genetic concepts and uncertainties in restoring fish populations and species. Pages 149-183 *in* R.C. Wissmar and P.A. Bisson [editors]. Strategies for restoring river ecosystems – sources of variability and uncertainty in natural and managed systems. American Fisheries Society, Bethesda, MD.

Chapter 1: Differences in survival and growth in hatchery and stream environments, and in maturation of residuls in a stream, between progeny of hatchery and wild steelhead *(Study sites: Brushy Fork Creek and Dworshak Hatchery; Stocks:Dworshak hatchery and Fish Creek wild; Year classes: 1992 and 1993)*

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Abstract

Freshwater survival in hatchery and natural rearing environments was compared between progeny of hatchery (H) and wild (W) steelhead *Oncorhynchus mykiss* from the Clearwater River drainage in Idaho. Adults from Dworshak National Fish Hatchery and wild adults from Fish Creek fish were artificially spawned, and their progeny were genetically marked at the *PEPA* allozyme locus and released together as unfed fry in production facilities at the hatchery and in Brushy Fork Creek, also in the Clearwater River drainage, in a common garden design. Survival was higher for H than for W progeny at the hatchery but lower for H than for W progeny in Brushy Fork, indicating reduced fitness of the hatchery population for natural rearing and suggesting domestication as the cause. Survival at the hatchery was lower than is typical due to disease outbreaks. Survival of the first year-class of experimental fish to smolt release was only 18%. Survival of H fish was 3.8 times that of W fish under these poor survival conditions. All fish from the second year-class died halfway through the scheduled 10 month rearing period. Survival of H fish was 5.2 times that of W fish to when 1% of the initial fry were still alive indicating that W fish succumbed to the epizootic sooner than did H fish. Emigrants from the Brushy Fork study reach were sampled for three years and fish residing in the study reach were sampled for six years following fry release. Most emigrants were one or two years old and too small to be smolts (mean fork length at age-2 = 93 mm). Survival in Brushy Fork was lower for H than for W fish of the first year-class. Survival of the second year-class was higher for H than for W fish during the first two months in the stream but was lower for H than for W fish thereafter, and net survival from release to ages 3 and older was also lower for H than for W fish if our emigrant samples were representative (periods of inoperative emigrant traps prevented certainty about this). Differences between progeny groups were also found for growth (H>W) and condition (H>W) in the hatchery and downstream migration success of hatchery-reared fish after release (H>W), and for growth (H<W for one year-class; H>W for the other), condition (H>W), downstream dispersal (H>W for one year-class; H=W for the other), and maturation of residuals (ovaries weight was greater for H than for W females at ages 4 and 5; testes weight was less for H than for W age-3 males of one year-class) in Brushy Fork. A thunderstorm-induced power outage interrupted flow to the incubation trays at the hatchery and compromised a major tenet of the common garden design for the second year-class, possibly contributing to the inconsistency in relative survivals in Brushy Fork between year-classes. The storm caused the incubation environment to differ between the stocks as a result of reduced oxygen levels and substantially higher densities for H alevins. This difference was illustrated by a 55% loss for H fish during the event, about twice that for W fish.

Introduction

This study was conceived and initiated in the early 1990's when managers were considering using steelhead *Oncorhynchus mykiss* from Dworshak National Fish Hatchery (Figure 1) to supplement wild steelhead in selected rivers within the Clearwater River drainage in Idaho. Previous studies with steelhead in Oregon and Washington (Reisenbichler and McIntyre 1977; Leider et al. 1990) suggested that reduced (genetic) fitness for natural rearing, including reduced freshwater survival and reduced production of smolts and returning adults, should be expected for the hatchery (sea-ranched) population

so that interbreeding of hatchery and wild fish would reduce the fitness of the wild population; however, no supporting data were available for steelhead in Idaho. Our main objective was to provide such data by testing for genetic differences in fitness for natural rearing between Dworshak Hatchery steelhead and wild steelhead from the Clearwater River. Our primary measure of fitness was freshwater survival.

An additional objective was to test for domestication of the hatchery stock (natural selection for fish adapted to the hatchery program; Reisenbichler and Rubin 1999). Domestication is expected to increase fitness for hatchery rearing but decrease fitness for natural rearing. We therefore hypothesized that if the Dworshak Hatchery stock was domesticated, survival would be better for hatchery than for wild progeny reared at Dworshak Hatchery whereas the opposite would be true under natural rearing. The extent to which domestication occurs is still a contentious subject (Brannon et al. 2004a) despite evidence of its prevalence from several studies (Reisenbichler and Rubin 1999; Araki et al. 2008).

We tested for differences in survival and growth between progeny of Dworshak Hatchery steelhead and of wild steelhead returning to Fish Creek (Figure 1). Progeny of hatchery and wild fish were genetically marked, mixed as unfed fry, and reared together at Dworshak Hatchery and in Brushy Fork Creek (Figure 1); thus any survival or growth differences were from genetic differences, or maternal differences such as vertically transmitted disease, egg size, or egg quality, rather than from environmental differences. Performance in the hatchery was monitored by sampling fish at the end of the 10 month rearing period and assessing downstream movement after release as fish passed dams lower in the river system. Performance in the study stream was monitored by sampling fish in the study reach or emigrating downstream. Because steelhead can express resident as well as anadromous life histories (Zimmerman and Reeves 2000), we also assessed maturity of older stream-reared fish.

Source populations

Dworshak Hatchery was built to maintain the North Fork Clearwater River steelhead population after Dworshak Dam blocked access to spawning grounds (Figure 1). The number of wild adults returning to the North Fork Clearwater River during 1967-1971, when the dam was being constructed, ranged from 2,413-7,657 (mean=4,448) (Miller 1987). The hatchery stock was initiated with wild adults trapped at the base of the dam in 1969-1972. Scale analysis indicated that the freshwater age composition of wild adults used for hatchery broodstock (all four years combined) was 12% age-1 (we incremented fish ages [e.g., from age-0 to age-1] on January 1), 76% age-2, and 12% age-3, and ocean age composition was 3% 1-ocean, 57% 2-ocean, 39% 3-ocean, and 1% 4-ocean (note that age at maturity is one year more than the sum of fresh water and ocean ages because returning adults spend an additional year in fresh water before spawning) (Pettit 1976). Mean fork length of hatchery broodstock during 1969-1971 was 82 cm (Wold 1970; Pettit 1976).

The hatchery stock has been maintained to the present with ancestors of the original North Fork stock; no introductions from other hatcheries have occurred. The ocean age composition of adult returns to the hatchery averaged 7% 1-ocean, 88% 2-ocean, and 5% 3-ocean for release years 1980-1999 (Burge et al. 2004). The hatchery used both one- and two-year rearing strategies for the first 12 year-classes (1969-1980), when an average of 23% of the juveniles were reared for two years prior to release (range 0-100%). After 1980 all juveniles were reared in the hatchery for only one year. Thus most fish matured at age-4 (one year in the hatchery, two years in the ocean, and one more year in fresh water before spawning). With a generation time of four years, the hatchery population had been cultured for 5-6 generations when adults were collected in 1992 and 1993 to initiate our study.

Embryos at Dworshak Hatchery are incubated in vertical-tray incubators (Piper et al. 1982). Fry are reared indoors in rectangular tanks; fingerlings are moved outside into Burrows ponds (Piper et al. 1982) in late summer and reared there until release the following spring (Roseberg et al. 1992). Water is pumped to the ponds from the North Fork Clearwater River below Dworshak Dam. During the winter,

ponds containing smaller fingerlings (from later egg takes) are supplied with heated and sometimes ion supplemented reuse water to promote growth and smoltification. Losses of juveniles to disease primarily result from infectious hematopoietic necrosis virus (IHNV), which first appeared in 1981, and the protozoan parasite *Ichthyophthirius multifilis* (Ich) (Howell et al. 1985).

A weir has been operated in Fish Creek about 1 km upstream from its mouth since 1992. Annual escapement during 1992-1999 averaged 91 (range 29-267; Byrne 2001) and was 105 in 1992 and 267 in 1993 when adults were collected for our study. The length frequency of wild adults captured during 1992-1999 (Byrne 2001) suggests that most spent at least two years in the ocean. Scale analysis indicated that the ocean age composition of adults returning in 2003 and 2004 averaged 18% 1-ocean, 81% 2-ocean, and 1% 3-ocean (Byrne 2005). Mean fork length of adults captured during 1992-1999 was 78 cm (Byrne 2001). Median arrival date in years when the weir remained intact throughout the spawning run (1993, 1994, 1998, and 1999) ranged from 29 April to 7 May (Byrne 2001). Length of smolts leaving Fish Creek (Byrne 2001), and length at age, from scale analysis, of juveniles captured in Fish Creek (Byrne 2005) indicate that most juveniles (>95%) smolt at ages 2 or 3. Thus ocean age composition and size are generally similar between Fish Creek wild adults and the North Fork Clearwater River wild adults used to start the Dworshak Hatchery stock; however, mean smolt age may be older for Fish Creek juveniles than for the original North Fork wild juveniles.

The influence of hatchery fish in Fish Creek has been slight. Only two releases of hatchery steelhead – Dworshak stock in both cases – have occurred in Fish Creek: 80,000 fry on 11 June 1979 and 20,000 fingerlings (mean weight = 1.8 g) on 24 September 1980 (Dworshak National Fish Hatchery cumulative distribution data, U.S. Fish and Wildlife Service, Idaho Fishery Resource Office). Hatchery strays composed 0.7% of the total adult steelhead captured at the Fish Creek weir in 1992-1994 (U.S. Geological Survey, unpublished data).

Study stream

The natural rearing portion of the study was conducted in Brushy Fork Creek (Figure 1), henceforth called Brushy Fork, above a waterfall impassable to anadromous fish. Mean wetted width between the lower trap site and the upper bridge, a distance of 8.4 km, was 5.4 m in July 1994, and elevation was 1707 m at the bottom and 1853 m at the top (mean gradient=1.7%). Where the stream flowed through Elk Meadows (from the middle bridge upstream most of the way to the upper bridge), it had a low gradient, meander bends, alternating pools and riffles, and sand, gravel, and cobble substrate. Downstream from Elk Meadows the gradient was steeper, run and pocket water habitats were more prevalent, and substrate was coarser. The reach between the waterfall and the lower trap was 3.9 km long and had an even steeper mean gradient (4.5%) than above that trap. Mean monthly water temperature measured at the lower bridge during 1992-1996 ranged from 0°C during December-February to 11.7^{o}C in August (mean annual temperature=3.5^{o}C). The only resident fish we encountered above the waterfall were westslope cutthroat trout *O. clarki lewisi*. Identification of this subspecies was confirmed by analysis of several diagnostic allozyme loci (Leary et al. 1987) for a sample of 60 adults collected in 1993.

Methods

Experimental crosses, embryo incubation, and fry release

Adults were collected in 1992 and 1993. Hatchery adults were collected during 24 March-8 April 1992 and 23 March-14 April 1993 when they entered Dworshak Hatchery. They were recognized as of hatchery fish because the adipose fin was removed from all juveniles before release from the hatchery. Wild adults, identified by an intact adipose fin, were captured at the Fish Creek weir during 27 March-9 May (median=2 April) 1992 and 27 March-20 May (median=12 April) 1993.

Hatchery and wild adults were screened for genotype at the cytosol non-specific dipeptidase locus (*PEPA**; Shaklee et al. 1990). A small piece of fin tissue was excised from each adult, frozen on dry ice, and analyzed electrophoretically following Aebersold et al. (1987). Alleles were designated **100* and **111*, reflecting their relative electrophoretic mobilities. Hatchery males and females homozygous for the **111* allele were mated with each other to create a hatchery progeny group (H) marked **111*/**111*. Wild progeny (W) were marked either **100*/**100* or **100*/**111* by ensuring that at least one of the wild parents involved in each mating was homozygous for the **100* allele (Table 1). We did not make crosses between hatchery and wild adults. The genetic marks allowed us to distinguish between the progeny groups after they were mixed as fry.

Hatchery adults were individually tagged and released in holding ponds after being sampled for fin tissue. Wild adults were held individually in PVC tubes (Lindsay et al. 1992) near the Fish Creek weir site until their *PEPA** genotypes were known, then those with the desired genotypes were tagged and transferred to holding ponds at Idaho Fish and Game's Powell satellite facility (1992) (Figure 1) or Dworshak Hatchery (1993). All hatchery adults were spawned on one day each year, and wild adults were spawned over a several week period as they became ripe (Table 1). Some wild males contributed milt on more than one spawning date. On each date, gametes were stripped and held on ice until fertilization (<8 hours). We usually divided each female's eggs into two equivalent portions, and each portion was fertilized with milt from a different male; exceptions are noted in Table 1. Eggs from the wild female spawned on 21 May 1993 were fertilized with milt cryopreserved on earlier spawning dates (Wheeler and Thorgaard 1991). Most males sired two families (i.e., half of the eggs from each of two females), but the range was one to six (Table 2). Hatchery and wild parents were similar in size and presumably in ocean-age composition except that in 1992 only two of seven hatchery males appeared to be 1-ocean compared to four of seven wild males (Figure 2). Samples of $\geq$30 unfertilized eggs were collected from each female, preserved in 10% buffered formalin, and weighed collectively >90 days later to determine mean egg weight per female.

Fertilized eggs were placed in vertical-tray incubators (Piper et al. 1982). In 1992, each tray contained a single full-sib family. In 1993, each tray was fitted with a divider so that the two half-sib families from each female could be accommodated. Water temperatures were manipulated during incubation to synchronize development among embryos from different spawning dates. Embryos conceived on different dates were incubated in separate stacks, and each stack received a different schedule of ambient (~12°C) and chilled (~7°C) water (Table 3). A model that predicts developmental stage from incubation duration and temperature (Rubin et al. 2012c) was used to synchronize embryo development.

When embryos reached the eyed stage, dead embryos and unfertilized eggs were removed and hand counted, and the number of live embryos was determined by volumetric displacement (1992) (Piper et al. 1982) or with an electronic counter (1993) (Jensorter^{TM}). Mortalities occurring after eye-up were removed.

On 30 June 1992, the predicted date of button-up that year, fry were counted into two groups, one for release in Brushy Fork and the other for ponding at Dworshak Hatchery. The number of fry in each family was counted electronically (Applebee and Tipping 1991) or by hand, and total weight of fry was measured to determine mean fry weight for each family. Five percent of the fry from each family were allocated to a nursery tank at the hatchery (number of fry released in the nursery tank are given in Table 4) and the other 95% to Brushy Fork (number of fry released in Brushy Fork given in Table 5). Fry were transferred to the nursery tank on 30 June. Fry for outplanting were held overnight in troughs at the hatchery (stocks mixed in the troughs), and on 1 July they were transported to Brushy Fork and

approximately equal numbers were released along three 2-km long segments, each centered on a bridge in the study reach (Figure 1).

Predicted date of button-up for the 1993 year-class was 3 July, but a week earlier our experimental fish sustained heavy mortality because a power outage (caused by lightening) interrupted the flow of water to the incubation stacks. The backup generators failed to start automatically, and manual startup was delayed by complications from a power surge. After flow was restored, mortalities were removed and live alevins counted to assess the extent of the losses. The two half-sib families (same mother) in each tray were mixed during this process. Further, one whole tray was inadvertently mixed with a second, and part of a third tray was mixed with a fourth (all four trays contained hatchery stock). Thus, all maternal families except these four remained in separate trays. Large differences in density between H and W alevins during the power outage seemed to result in large differences in mortality between these groups (see results section below) and a violation of the assumption of equal conditions for the two groups. Recognizing this problem, we continued the experiment with this year-class to see whether the results would be comparable to those from the uncompromised, 1992 year-class.

On 2 July 1993, total weight of 100 fry from each tray was measured, and on 3 July fry were counted into release groups as in 1992 except that half of each tray was allocated to Dworshak Hatchery and half to Brushy Fork (number of fry ponded at the hatchery and released in Brushy Fork are given in Tables 4 and 5, respectively). The total number of fry available was about the same as in 1992 but fewer were released in Brushy Fork than in 1992. Fry were ponded at Dworshak Hatchery during counting and released in Brushy Fork the next day as in 1992 except that releases occurred only in segments associated with the lower and middle bridges.

Juveniles at the hatchery

1992 year-class.—On 24 November, experimental fish in the nursery tank were given a right ventral clip and moved outside to a Burrows pond. A total of 2,926 fish (mean weight=7.2 g) were clipped and moved; survival from when button-up fry were released in the nursery tank was 91%. To attain standard rearing density, the experimental fish were placed in a Burrows pond that already contained about 30,000 ad-clipped (no ventral clip) production fish that were larger (mean weight=13.8 g) than the experimental fish. On 8-9 April 1993, about 75% of the fish in the Burrows pond were sorted according to fin clip. All experimental fish encountered were measured, weighed, and tagged with a passive integrated transponder (PIT) tag (Prentice et al. 1990); their left ventral fins were removed, frozen and analyzed later for *PEPA** genotype; and the fish were then returned to the pond. Fish were released from the hatchery on 3 May 1993. Combined survival of experimental and production fish between when experimental fish were moved outside to the Burrows pond and release was relatively low (about 40%) due to an outbreak of Ich.

The PIT tags were individually coded (one code per tagged fish) and could be detected when fish moved through juvenile bypass facilities at Lower Snake and Columbia River dams. Records of PIT-tagged fish detected at the dams were obtained from the PIT Tag Information System (Martinson et al. 1997). Detection rate for PIT-tagged fish was computed as the number of fish detected at least once at any dam divided by the number of tagged fish released.

1993 year-class.—Fish were moved outside into a Burrows pond on 29 October. There were enough of them to attain standard density with our experimental fish alone. Survival between button-up and 29 October was 94%. On 16 December, fish in the pond received coded wire tags and had their adipose and right ventral fins clipped. Survival in the pond up to marking was 73%. Beginning in early December outbreaks of IHNV and Ich caused heavy mortality, and by mid-January all of the fish in the pond had died.

On 6-7 January, the remaining live fish (*n*=356) were sampled for size and *PEPA** genotype as for the previous year-class. Additionally, 32 fish that had been dead for less than 24 hours were similarly sampled on 7 January.

Juveniles in the stream

Downstream migrants were trapped at two sites in Brushy fork (Figure 1). A v-shaped weir (apex pointing downstream) that spanned the entire stream (Blankenship and Tivel 1980) was installed at the lower site. The weir was covered with 3.2 mm mesh screening and was designed to prevent downstream passage except through a pipe leading from the weir apex downstream into a trap box. The pipe outflow was above the water surface in the trap box under most flow conditions, preventing fish from swimming back upstream. Either an inclined screen trap (Kray-Meekin type) with a rectangular 0.6 x 0.9 m mouth or a screw trap (Thedinga et al. 1994) with a 1.5 m diameter cone was operated at the upper site. Traps were fished during 1992-1996; dates of operation for each trap type are given in Table 6. The reason for having more than one trap type was that the inclined screen or screw trap could be operated earlier in the spring than we were able to install the screen weir, and the screen weir functioned better than the other traps in summer and fall when flows were low. In 1994 and 1995 trap malfunctions caused extended gaps in coverage (Table 6). Traps were checked $\geq$2 days per week during operation. Efficiency was not estimated for any of the traps, although the weir trap likely caught all downstream migrants. All trapped fish were sampled for size, tissue, and aging structures (scales or otoliths; see below) except in 1993, when only 50 were sampled (and the remainder counted) on days when more than 50 were trapped.

Fish residing upstream from the barrier falls (henceforth termed residents) were captured by backpack electrofishing each summer from 1992-1999; dates are given in Table 7. Electrofishing always proceeded upstream with the intent of thoroughly covering all habitats. All captured fish were sampled. Fish were collected near each bridge in 1992-1993, and at six sites whose lengths averaged 193 m (range 130-241) in 1994-1995 (Figures 4 and 5). In 1995, we placed blocknets at upper and lower boundaries and conducted 2- or 3-pass removal at sites 2, 4, and 5 (numbered downstream to upstream; only 2 passes were made at site 4 because pass 2 catch was <25% of pass 1 catch). We made one upstream pass at the other three sites and used no blocknet at the upper boundaries. From 1996-1999, our goals were to intensively sample the steelhead remaining in the stream and to remove as many of them as possible to minimize interbreeding with westslope cutthroat trout, thus all residents captured during these years were euthanized. In 1996, the entire stream from 1200 m below the weir to 400 m above the upper bridge was electrofished. A second pass was made between the weir and the middle bridge. Collections were grouped into five sub-reaches (Barrier falls-weir, weir-lower bridge, lower-middle bridge, middle-upper bridge, above upper bridge; Figures 4 and 5). From 1997-1999, various portions of all but the uppermost sub-reach were electrofished (Figures 4 and 5) by making one pass with two backpack electrofishers operated side-by-side; electrofishing in previous years had been conducted with a single backpack unit.

Captured fish (migrants and residents) lacking orange throat slashes and other markings characteristic of westslope cutthroat trout were measured and weighed, white muscle or fin tissue was collected and frozen for electrophoretic analysis, and starting in 1994 otoliths or scales were taken for aging (Table 8). Tissue was analyzed for genotype at *GPI-A** (a locus of glucose-6-phosphate isomerase; Shaklee et al. 1990) to determine species (the ***100* allele is diagnostic of steelhead and the ***92* allele of westslope cutthroat; Leary et al. 1987) and at *PEPA** to determine the experimental group for the steelhead. *GPI-A** and *PEPA** genotypes were resolved for 99.4% of the samples. Weight frequency analysis completely separated the two year-classes of steelhead collected in 1993. Steelhead collected in 1994-1999 were aged from otoliths or scales (Peven et al. 1994). Accuracy of aging otoliths could not be evaluated with Brushy Fork samples; however, accuracy of the same personnel was 100% for known-age steelhead collected from Twentymile Creek and ranging in age from 1 to 4 (*n*=184; USGS unpublished data; Twentymile Creek collections described in Rubin et al. 2012b). Sub-samples of Brushy Fork

residents were aged from both scales and otoliths (Table 8). Agreement between the two methods was 95% in 1995 (n=61) and 90% in 1996 (n=60).

Migrants and residents collected in 1995 and aged from otoliths (Table 8) were also sexed. Residents collected in 1996-1997 were examined in the field for flowing milt and then frozen. Those not flowing milt were later thawed and sexed. Gonads were weighed for a sub-sample of females, males with flowing milt, and males without. Gonadosomatic index (GSI) was computed as 100 times thawed gonad weight divided by fresh (in the field) whole body weight. Residents collected in 1998-1999 were examined for flowing milt and sexed in the field; GSI was not measured.

For sites of known length (1994-1999 collections), we computed a density index equal to the number of fish caught on the first pass (total catch where only one pass was made) per m stream length (Figure 4). Where more than one pass was made, density index was partitioned into separate indices for each year-class and stock according to the proportion of each year-class and stock in the total catch (all passes combined). First pass efficiency (proportion of total fish captured) undoubtedly varied among sites, years, and gear types (one versus two electrofishers); however, we expect that density index provides an index of abundance that is roughly consistent over these strata. Population estimates were generated for multiple-pass sites (Van Deventer and Platts 1985). The percentage of the total estimate caught on the first pass was 54, 52, and 77 for 1995 sites 2, 4, and 5, respectively, and 15 and 38 for 1996 sub-reaches 2 and 3.

Analyses

Survival of hatchery relative to wild progeny ($RS_{H/W}$) was computed as:

$(P_{H2}*P_{W1})/(P_{H1}*P_{W2})$;

P_{H1} = proportion of H fish at release, P_{H2} = proportion of H fish at time 2 (i.e., juvenile sampling), P_{W1} = proportion of W fish at release, and P_{W2} = proportion of W fish at time 2. Relative survival was termed "apparent" (and abbreviated $ARS_{H/W}$) for Brushy Fork juveniles because estimates may have reflected emigration as well as mortality. For each year-class, age-specific and total (all ages combined) estimates of ARS were made for migrants by pooling over capture dates, and age-specific estimates were made for residents by pooling over capture sites. Relative survival was tested with *G*-tests for goodness of fit (Sokal and Rohlf 1995); expected frequencies were the proportion of each stock from the original number of fry released. Differences in the relative frequency of the two stocks among different ages of residents or between migrants and residents were tested with contingency table *G*-tests of independence. Exact *P*-values of goodness of fit and independence tests were obtained from StatXact-3 (Cytel Software Corporation 1997).

Differences in length were tested with one-way AOV. For Brushy Fork juveniles, analyses were conducted separately by year-class, age, and sub-population (migrants or residents), with data again pooled over dates for migrants and sites for residents. Condition (weight at length) was tested with analysis of covariance (AOCV) on $\log_{10}$ transformed weight and length. If slopes of the weight-length relation were similar between stocks (P>0.05), intercepts were compared between stocks using the "common slope" model (i.e., with the stock*length interaction term removed). Weight at a common length (the geometric mean of lengths pooled over stocks) was evaluated using relations from the common slope AOCV if slopes were similar, or from the model including the stock*length interaction term if not. Condition factor (k) was computed as:
k = 100,000*W/L3;
W = weight at common length (g); L = common length (mm). This method avoided the problem of correlations between k and length that occur when the exponent of the weight-length relation differs from 3 (Cone 1989).

Sex ratio and the proportion of males with flowing milt were compared between stocks with *G*-tests of independence. GSI was arcsine-square root transformed and compared between stocks with AOV. Gonad weight was $\log_{10}$ transformed and compared between stocks with AOCV; $\log_{10}$ transformed length was the covariate. Testes weight and GSI of males were tested in 2-way analyses with factors stock, milt status (flowing or not), and their interaction. We used logistic models (SAS 2000) to test whether relative frequency of the stocks was affected by multiple factors (sex, age, capture date, etc.).

Results

Egg size; embryo survival; fry size and developmental stage

In 1992, egg size was greater for W than for H females. Percent eye-up was lower for W than for H embryos; survival from eye-up to button-up was uniformly high for both groups (Table 9). Fry weight (Table 9) and developmental stage (according to incubation temperature model; Table 3) at release were similar between stocks.

In 1993, egg size was similar between stocks; percent eye-up was again lower for W than for H embryos (Table 9). Mean eye-up for the two wild families from cryopreserved milt was only 25%, but even with these families excluded eye-up for W embryos was only 86.1%, still significantly lower than eye-up for H embryos. Embryo density between fertilization and eye-up was lower for W than for H embryos because fecundity was lower for wild females, and the density difference between stocks increased after eye-up because of low percent eye-up for W embryos (Table 9).

Survival of W embryos from eye-up to button-up in 1993 was 55%, twice that of H embryos (Table 9). Survival between eye-up and the power outage was 97% for both stocks, and live alevin counts made right after the outage were similar to those made a week later at button-up; thus almost all of the mortality between eye-up and button-up occurred during the outage and was probably from lack of oxygen. Survival over the outage was inversely related to density of alevins in incubation trays (Figure 3). Apparently, lower density of W alevins during the outage resulted in higher per capita oxygen availability and higher survival. Fry weight and development at release were similar between stocks as in 1992 (Tables 3 and 6).

Juveniles at the hatchery

1992 year-class.—Survival of H fish from button-up to juvenile sampling was >3x that of W fish, and length and condition of juveniles and detection rate after release were also substantially greater for H than for W fish (Table 4). If 75% of the experimental fish were sampled (about 75% of the total fish in the raceway were sorted to separate experimental from production fish), then our estimate of relative survival to juvenile sampling translates into true survival estimates of 25% for H, 7% for W, and 18% for both groups combined.

Almost all detections of PIT-tagged fish at dams were for fish $\geq$120 mm in length at tagging, and a greater proportion of H than of W fish were above this threshold (Figure 6); thus the difference in detection rate between stocks was related to length differences. Eighty-three percent of the H detections were at the uppermost Snake River dam (Lower Granite), 10% at the next Snake River dam encountered (Little Goose), and the rest at McNary Dam on the Columbia River. There were only two W detections, both at Little Goose. Travel time to the dams was inversely related to length at tagging (Figure 7). The last detection in 1993 was on 1 June, and there were no detections in later years of downstream migrants or adult returns.

1993 year-class.—Similar to 1992, survival of H fish to juvenile sampling was >5x that of W fish, length was greater for H than for W juveniles, and condition also appeared greater for H juveniles but could not be tested due to a violation of the equal slopes assumption of the AOCV (Table 4). Only 1% of the initial fry were still alive when juveniles were sampled, and no juveniles of either stock survived to release.

Juveniles in the stream

Migrants.—No age-0 migrants were captured from either year-class even though traps were operated from before fry release in July until late October. Over 3,300 1992 cohort yearlings were trapped from mid June through August, 1993 (Figure 8), of which about 20% were sampled. The trajectory of catch versus time and the timing of age-1 migrants from our other experiments (Rubin et al. 2012b; 2012f) suggest that most of the run was sampled even though trapping did not start until mid June. $ARS_{H/W}$ estimated from samples pooled over capture dates was significantly <1 (Table 5); however, H fish may have been underrepresented in this estimate because frequency of H relative to W fish increased from early to late in the run, but sampling efficiency (proportion of the catch that was sampled) decreased from early to late in the run (Figure 9). The increase in the relative frequency of H fish over time was significant (P=0.028; logistic regression) and mean capture date was three days later for H than for W fish (P=0.027; 1-way AOV). $ARS_{H/W}$ was re-estimated by computing a weighted mean (and associated variance; Scheaffer et al. 1986) of the relative frequency of H fish where the weights were total catch in the four time periods shown in Figure 9. $ARS_{H/W}$ by this method was still <1 but not significantly so (P=0.13; binomial test [Ott 1984]; Table 5).

Few migrants of either year-class were caught after 1993, and $ARS_{H/W}$ was not significantly different from 1 for 1992 year-class migrants aged 2-4 or for 1993 year-class migrants aged 1-3 (Table 5). The traps did not operate through the entire spring or fall migration seasons in 1994 or 1995 (Table 6). In 1996 the screw trap was deployed in early April, when winter low flow conditions were still in effect, and fished through late June, well past the peak of high spring flows, a period that should have included most smolts migrating to the ocean during spring 1996(Byrne 2001).

After age-1, emigration rate (proportion of the population that emigrated) appeared to decrease with age. Although screw trap efficiency was not measured in Brushy Fork, it was never less than 20% for similarly sized and aged emigrants in a different but similarly sized stream (Rubin et al. 2012b). Even if efficiency was only 10% in Brushy Fork, the 10 age-3 and 1 age-4 migrant trapped in 1996 would have represented only 100 age-3 and 10 age-4 total migrants, but there were roughly 760 age-3 and 520 age-4 residents above the screw trap in 1996 based on mean density index (0.04 fish/m for age-3 and 0.03 for age-4; Figures 4 and 5), mean efficiency of 1-pass electrofishing determined from multiple pass sites (0.39), and distance from the middle bridge to the upstream limit of resident sampling in 1996 (6.9 km). In contrast, there were at least 3,300 age-1 migrants in 1993 and perhaps a comparable number of age-1 residents that year based on a rough estimate of 2,300 age-2 residents in 1994 (mean density index = 0.17 fish/m; mean efficiency of 1-pass electrofishing in 1995 = 0.61; distance from the weir to the upper bridge = 8.4 km). Also, the ratio of 1992 to 1993 year-class migrants was less than the ratio of 1992 to 1993 year-class residents each year from 1994-1996, indicating that emigration rate was lower for older, 1992 year-class fish than for younger, 1993 year-class fish each year (Table 10). A steady decline in emigration rate through age-5 was observed in a similar study (Rubin et al. 2012b) so we think that emigration rate is unlikely to have increased after 1996 when no trapping occurred.

Residents.—$ARS_{H/W}$ was <1 for all ages of 1992 year-class residents, and the difference in ARS between H and W residents was statistically significant for all ages except 3 and 7 (Table 5). Sample size was lowest for age-7 and second lowest for age-3, and the $ARS_{H/W}$ values for these ages were similar to those for the other ages. $ARS_{H/W}$ was significantly lower for age-1 than for age-0 residents of the 1992 year-class, but thereafter $ARS_{H/W}$ did not change significantly between successive ages (Figure 10). The

frequency of H relative to W age-0 residents of the 1992 year-class was higher near the lower and upper bridges than near the middle bridge (Figure 4; $P<0.05$, 2x3 *G*-test of independence). We do not know what caused this difference among sites but by age-1, H relative frequency had decreased considerably at the lower and upper bridges (Figure 4), reinforcing the conclusion of an overall decrease in $ARS_{H/W}$ from age-0 to age-1.

$ARS_{H/W}$ for 1993 year-class residents was significantly >1 at age-0 but was significantly <1 at each of ages 3-6 (Table 5). $ARS_{H/W}$ for residents decreased significantly from age-0 to age-1 and from age-2 to age-3 (Figure 10).

The entire length of stream between the barrier falls and the weir (lower trap site) was electrofished for the first time in 1998 when the 1993 year-class was age-5, and nearly the entire stream between the weir and the upper bridge was also electrofished that year (Figure 5). The density of age-5 fish was much higher below the weir than above it (Figure 5), in part because fish captured above the weir in 1996 and 1997 were removed from the stream. $ARS_{H/W}$ for age-5 fish was significantly lower below the weir than above it ($P<0.01$, 2x2 *G*-test of independence). Based on the small sample of fish collected below the weir in 1996, the only year before 1998 that any part of the stream below the weir was electrofished, $ARS_{H/W}$ of age-3 fish of the 1993 year-class may also have been lower below the weir than above it (Table 5). $ARS_{H/W}$ for 1993 year-class residents was significantly lower at age-5 (total sample pooled over collections made above and below the weir) than at age-1 ($P<0.001$, 2x2 *G*-test of independence). $ARS_{H/W}$ for 1993 year-class residents was also significantly lower for samples pooled over ages 3 and 5, 4 and 5, or 3-5 than at age-1 ($P\leq0.04$); in other words, the decrease in $ARS_{H/W}$ from age-1 to age-5 remained significant even when fish that had been removed from the stream at ages 3 and 4 were included in the age-5 sample. The decrease in $ARS_{H/W}$ from age-1 to age-5 indicates that either the frequency of H relative to W fish decreased over that interval or the frequency of H relative to W fish at age-1 was lower than indicated by our sample of age-1 residents which did not include fish from below the weir.

Relative survival.—There were no age-0 migrants and $ARS_{H/W}$ of age-0 residents was <1, thus survival of the 1992 year-class from release to late summer was lower for H than for W fish. Survival from release to age-1 was also lower for H than for W fish of the 1992 year-class. Although $ARS_{H/W}$ of age-1 migrants was not significantly <1 (weighted estimate, Table 5), $ARS_{H/W}$ of age-1 residents was by a wide margin. Survival to age-1 for the total population (migrants and residents combined) was lower for H than for W fish because the number of residents approached that of migrants. A total of 3,343 age-1 migrants were trapped, mostly in the weir trap that should have caught all downstream migrants, and as noted above there were more than 2,300 age-1 residents (based on the estimate of 2,300 age-2 residents the following year). Survival of the 1992 year-class from age-1 to age-6 likely was similar between stocks based on the lack of change in $ARS_{H/W}$ of residents from age-1 to age-6 (Figure 10), the low emigration rate after age-1, and $ARS_{H/W}$ values near 1 for the few age-2 to age-4 migrants that were trapped (Table 5).

Survival of the 1993 year-class from release to late summer was higher for H than for W fish (no age-0 migrants; $ARS_{H/W}$ significantly >1 for age-0 residents). The significant decrease in $ARS_{H/W}$ from age-0 to age-1 residents of the 1993 year-class, and the similarity of $ARS_{H/W}$ between age-1 migrants and age-1 residents (Figure 10), show that survival from age-0 (late in the first summer) to age-1 was lower for H than for W fish. $ARS_{H/W}$ of residents decreased significantly from age-2 to age-3, was significantly <1 at ages 3-6, and was significantly lower at age-5 than at age-1. The decrease in the frequency of H relative to W fish from release to ages 3-6 ($ARS_{H/W}$ <1) was from either differential mortality or differential emigration. $ARS_{H/W}$ values near 1 for migrants (Table 5) and the low emigration rate after age-1 suggest that the change in relative frequency was primarily from differential mortality which in turn suggests that survival from release to ages 3-6 was lower for H than for W fish.

Dispersal.—The frequency of H relative to W fish was higher for age-1 migrants than for age-1 residents of the 1992 year-class ($P<0.001$, 2x2 *G*-test of independence using un-weighted migrants — the residents differ by less from un-weighted than from weighted migrants; Table 5). This difference indicates differential emigration rather than differential mortality. Emigration was greater for H than for W yearlings of the 1992 year-class. The frequency of H relative to W fish was similar between age-1 migrants and age-1 residents of the 1993 year-class (Table 5) indicating no difference in emigration between stocks.

Size and condition.—When significant length differences occurred, they showed H fish to be shorter than W fish of the 1992 year-class but longer than W fish of the 1993 year-class (Figure 11). Neither H nor W length was correlated with capture date of 1992 year-class age-1 migrants ($P>0.4$; AOCV with factors stock and capture date); sample sizes were too low for this comparison at older ages or for the 1993 year-class. Length differences between H and W residents were usually consistent among sites (Figures 12 and 13). Length was greater for migrants trapped in fall (October) 1995 than for spring migrants or residents captured in 1996 (true for both stocks and year-classes; Figure 11). Apparently the fall migrants were among the largest fish from their respective year-classes.

When significant differences in condition factor occurred, they always showed condition to be greater for H than for W fish regardless of year-class (Figure 14). Sub-populations (migrants or residents) and ages for which condition and (or) length differed between stocks were tested for weight differences to see if conclusions based on length alone (Figure 11) still held. Results for weight were the same as for length except that weight was greater for H than for W age-0 residents of the 1993 year-class ($P<0.05$) whereas length was not.

Sex and maturity.—Percent female (F/(F+M)) was lower for H than for W residents aged 4 and 6 of the 1992 year-class but was similar between stocks for other ages of the 1992 year-class and for all ages of the 1993 year-class (Table 11). Percent female was lower for H than for W residents of 1992 year-class ($P<0.001$) but was similar between stocks for 1993 year-class residents ($P=0.56$) when ages with sufficient data (4-6 of the 1992 year-class and 3-5 of the 1993 year-class) were analyzed together in 2-way logistic models with factors stock and age; the interaction between stock and age was not significant for either year-class ($P\geq0.29$; age treated as a categorical variable). Percent female was higher for age-3 migrants (71%) than for age-3 residents (24%) of the 1992 year-class and was also higher for age-2 migrants (74%) than for age-2 residents (39%) of the 1993 year-class ($P\leq0.007$; 2x2 *G*-tests of independence on data pooled over stocks).

Percent female above the weir declined marginally ($P=0.07$) from age-4 to age-6 for both stocks of the 1992 year-class and declined significantly from age-3 to age-5 for both stocks of the 1993 year-class (Table 12; Figure 15). Percent female was marginally higher ($P=0.07$) below the weir than above it for both stocks of the 1992 year-class at age-6 and was significantly higher below the weir than above it for both stocks of the 1993 year-class at age-5 (Table 12; Figure 15). This suggests downstream movement by females, or perhaps upstream movement by males although the latter seems less likely due to the presence of several potential barriers between the weir and the barrier falls at the lower end of our study area.

Percent precocious (percent of males with flowing milt) was similar between stocks for residents of any age from either year-class (Table 13) or when 1992 year-class ages 4-6 or 1993 year-class ages 3-5 were analyzed together in 2-way logistic models with factors stock and age ($P\geq0.79$; age treated as a categorical variable; $P_{interaction}\geq0.31$). Percent precocious above the weir increased from age-4 to age-6 for both stocks of the 1992 year-class (Table 12; Figure 16). Percent precocious above the weir increased markedly from age-3 to age-4 and then decreased from age-4 to age-5, but it was still higher at age-5 than

at age-3 ($P<0.05$; Bonferroni adjustment for three paired comparisons among ages 3-5) for both stocks of the 1993 year-class (Table 12; Figure 16). Percent precocious was similar between reaches for both stocks of the 1992 year-class at age-6 but was lower below the weir than above it for both stocks of the 1993 year-class at age-5 (Table 12; Figure 16). The increase in percent precocious with age was probably due to increasing male maturity (see below).

Length was similar among females, males with flowing milt, and males without, but condition was lower for females than for either of the male groups (with or without flowing milt) which had condition factors similar to each other (Table 14). Stock did not interact significantly with group (females, males with flowing milt, males without) in 2-way analyses of length or condition ($P>0.18$), meaning that the differences in length and condition between stocks reported above were consistent among the groups.

Ovaries weight was <0.2 g and GSI was <0.6% for all age-3 females of the 1993 year-class (Figures 17 and 18). In contrast, ovaries weight and GSI distributions were bimodal for age-5 females of the 1992 year-class, and weights and GSIs for individuals in the upper mode (61% of total age-5 females) were highly variable but on average much greater than those for individuals in the lower mode which were similar to those for all 1993 year-class age-3 females (Figures 17 and 18). Ovaries weight and GSI for age-4 females were intermediate to those for age-3 females of the 1993 year-class and age-5 females of the 1992 year-class; however, age-4 females of the 1992 year-class were more similar to the age-3 females in weight and GSI whereas age-4 females of the 1993 year-class were more similar to the age-5 females. Residents were captured in late July in 1996 and in early September in 1997. The 1.5 month difference (late July-early September) may have allowed for greater maturation in 1997. The bimodality of ovaries weight and GSI for age-4 and age-5 females caught in September suggests that individuals in the upper mode may have been maturing in preparation for spawning the following spring.

Gonad weight and GSI were much greater for males (Figures 19 and 20) than for females of a given year-class and age, providing an explanation for the higher condition of males compared to females (Table 14). Testes weight and GSI were least for age-3 males of the 1993 year-class and greatest for age-5 males of the 1992 year-class, but weights and GSIs of age-3 and age-4 males caught in July 1996 were even more similar to each other, and more different from those of age-4 and age-5 males caught the following year in September, than was the case for females. Bimodality of testes weight and GSI was hardly evident for males caught in September (very few males in the lower mode). The difference in testes weight and GSI between males with and without flowing milt was greatest for age-3 males (with>without) and then decreased with increasing age and month of capture (Figures 21 and 22).

Ovaries weight and GSI were similar between stocks for age-3 females of the 1993 year-class (Table 15). Both metrics were similar between stocks for age-4 females of the 1993 year-class when all samples were included (Table 15); however, ovaries weight (Figure 23) and GSI were significantly greater for the H than for the W stock when females with ovaries weights <0.3 g or GSIs <1% were excluded ($P\leq0.02$; cutoffs based on division between lower and upper modes in Figures 17 and 18). A lower percentage of total H than of total W females had ovaries weights <0.3 g (Figure 23) or GSIs <1%, strengthening the conclusion of greater ovaries weight and GSI for H than for W females. Ovaries weight and GSI were similar between stocks for age-4 and age-5 females of the 1992 year-class when each age was analyzed separately (Table 15); however, both metrics were significantly greater for H than for W females when the ages were analyzed together (2-way AOVs; second factor age) and age-5 females with ovaries <0.3 g or GSIs <1% were included ($P\leq0.02$; distributions of both metrics were unimodal for age-4 females of the 1992 year-class; Figures 17 and 18). When age-5 females with ovaries weights or GSIs from the lower mode were excluded and females aged 4-5 were analyzed together, GSI was significantly greater for H than for W females ($P=0.02$) but ovaries weight was not ($P=0.12$). A lower percentage of total H than of total W age-5 females had ovaries weights (Figure 23) or GSIs from the lower mode,

supporting the conclusion of greater ovaries weight and GSI for H than for W females of that age. We conclude that ovaries weight and GSI were greater for H than for W females aged ≥4 of both year-classes. This suggests that maturation rate (the speed with which maturing females reach maturity, perhaps related to time of spawning) or reproductive investment (fecundity and [or] egg size) was greater for H than for W females.

Testes weight and GSI for age-3 males of the 1993 year-class differed significantly between stocks, but the interaction between stock and milt status (flowing or not) was also significant (Table 16). Testes weight and GSI were lower for H than for W males without flowing milt but were similar between stocks for males with flowing milt (Table 15; Figure 25). Overall, testes weight and GSI were significantly lower for H than for W age-3 males (Table 16), a finding that was confirmed by 1-way analyses (data pooled over milt status). The overall difference between stocks suggests lower maturity for H than for W males. The interaction between stock and milt status may indicate that W males required a greater minimum testes weight for milt to flow than did H males.

Testes weight and GSI for age-4 males of both year-classes were similar between stocks regardless of milt status (Tables 14 and 15). Both metrics were also similar between stocks for age-5 males of the 1992 year-class, but the interaction between stock and milt status was significant (Table 16) reflecting opposite differences for males without flowing milt (H>W) compared to males with (H<W) (Table 15).

In summary, we found three main differences in sex ratio or maturity between stocks. First, percent female was lower for H than for W residents of the 1992 year-class; however, it was similar between stocks for residents of the 1993 year-class. Second, ovaries weight and GSI were greater for H than for W females aged ≥4 of both year-classes. Third, testes weight and GSI were lower for H than for W males of the 1993 year-class at age-3, and the difference between stocks was greater for males without than with flowing milt.

Discussion

Potentially confounding factors

Potentially confounding factors to our assumption of equivalent conditions for H and W progeny included unequal fitness among the *PEPA** genotypes used to genetically mark the stocks, maternal effects such as egg size or vertically transmitted diseases, and environmental differences during embryo incubation before the stocks were mixed. No effect of *PEPA** genotype was found on survival, growth, or condition of juveniles in Dworshak Hatchery, or on "loss" (from mortality or emigration), size, condition, or incidence of precocious males for naturally reared one-, two-, and three-year olds remaining in the study stream (migrants were not sampled) in a companion study (Reisenbichler et al. 2006); however, adult return rate for juveniles reared at and released from the hatchery was three times as high for males with the **110/110* genotype (H mark in the current study) as for males with the **100/100* genotype (mark for the majority of W progeny; Table 1). Return rate of males with the **100/110* genotype (mark for the rest of the W progeny) was intermediate; female return rate was unaffected by *PEPA** genotype. Lack of an effect on performance of juveniles in the hatchery or a stream suggests that the differences among crosses observed in the current study were not due to the genetic marks.

Crosses of H females and W males (HxW), in addition to HxH and WxW crosses, were included in companion studies (Rubin et al. 2012a; 2012b) to test for maternal effects. HxW progeny were maternal half siblings of HxH progeny and paternal half siblings of WxW progeny. Survival, growth, condition, precocious maturation of males, and dispersal of HxW progeny were intermediate to those for HxH and WxW progeny, not similar to those for HxH progeny, indicating that the differences among crosses resulted from additive genetic differences between the hatchery and wild populations rather than

from maternal differences between hatchery and wild females. Thus the differences between H and W fish found in the current study probably were due to genetic rather than to maternal differences.

The incubation environment of the 1993 year-class differed drastically between stocks when flow to the trays was interrupted by the power outage. Density in trays at that time was higher for H than for W alevins due to higher fecundity for H than for W females and higher percent eye-up for H than for W embryos. As a result, available oxygen was depleted more quickly by H than by W alevins, and survival of H embryos from eye-up to button-up was only 28% compared to 55% survival for W embryos. Thus the key assumption of rearing both stocks in a common environment was violated. The expected effect on subsequent comparisons was difficult to predict. The more extreme low oxygen environment experienced by H alevins may have culled weak individuals from that population, thereby favoring the H group. Conversely, lower oxygen level may have caused H survivors to be weaker than W survivors, thereby favoring the W group. Oxygen availability in 1992 was not a problem because flow was not interrupted, embryo density was only half that of 1993 (one half rather than both halves of a female's eggs per tray), and eye-up to button-up survival was uniformly high ($\geq$87%).

Incubation temperature was colder for H than for W embryos of both year-classes (Table 3) because of spawning time differences and the need to synchronize button-up between stocks. The temperature difference probably had no effect on survival in the hatchery or stream since none was found in a study designed to detect such an effect (Rubin et al. 2012g). Effects of incubation temperature on growth in that study were inconsistent, occurring for some replicates but not others. Faster growth for H than for W fish in the hatchery was observed in the current study and in a companion study where incubation temperature was similar between H and W embryos (Rubin et al. 2012a), suggesting that the growth difference in the current study was from genetic rather than from incubation temperature differences.

A potentially confounding factor that did not violate our assumption of equivalent conditions for H and W progeny was conducting the natural rearing portion of the study in Brushy Fork, a stream that neither the original North Fork wild population (from which the Dworshak Hatchery population was founded) nor the Fish Creek wild population was native to. Elevation in Brushy Fork (1707-1852 m in study reach) was greater than in Fish Creek (roughly 700-1000 m), and Brushy Fork therefore was colder and had a shorter growing season than Fish Creek (see Byrne 2001 for Fish Creek temperatures). Brushy Fork was also likely higher and colder than most areas used for juvenile rearing by the ancestral North Fork population. Although we cannot be sure if or how these environmental differences between Brushy Fork and Fish Creek or the North Fork influenced our comparisons between H and W progeny, we doubt that they were responsible for our finding of higher survival for W than for H progeny. Natural spawning and juvenile rearing of steelhead occurred in Brushy Fork downstream from the anadromous barrier at the lower end of our study area. Brushy Fork upstream from the barrier had colder-than-average temperatures for juvenile steelhead but was nonetheless inhabitable and certainly more environmentally similar to rearing areas in Fish Creek and the North Fork than to Dworshak Hatchery.

One factor that the cold temperatures in Brushy Fork may well have affected is residualization rate for both stocks. Brannon et al. (2004b) presented a conceptual model of temperature effects on steelhead life history expression which states that cold rearing temperatures tend to favor resident over anadromous life histories.

Juveniles at the hatchery

Survival of our experimental fish from ponding to smolt release was 18% for the 1992 year-class and 0% for the 1993 year-class, much lower than is typical for production fish. Low survival of the 1992 year-class was due to an outbreak of Ich and perhaps also from co-habiting a Burrows pond with production fish that were twice as heavy and 10 times as numerous when first mixed. Zero survival of the

1993 year-class, which occupied an entire Burrows pond, was due to outbreaks of Ich and IHNV that started five months after ponding and five months before the intended smolt release date. Survival was 3.8 times as high for H as for W fish of the 1992 year-class to smolt release, 10 months after ponding, and was 5.2 times as high for H as for W fish of the 1993 year-class to six months after ponding when only 1% of the original fry were still alive. The 1993 result indicates that W fish succumbed to the epizootic earlier than did H fish. A companion study comparing Dworshak Hatchery steelhead to a different wild stock (Selway River) under several different treatments (one or two years of hatchery rearing with stocks in sympatry or allopatry) found that survival to release was either similar between stocks, or was higher for H than for W progeny but by a smaller amount ($\leq$1.6 times as high) than in the current study; however, disease outbreaks did not occur and overall survival of experimental fish to release was much higher ($\geq$57%) in the companion study (Rubin et al. 2012a).

Susceptibility to disease appeared to be lower for H than for W fish. Lower susceptibility could have been from generally better health due to faster growth, social superiority (Rubin et al. 2012a), or other factors, but it may also have resulted from some level of evolved resistance to specific diseases such as IHNV. This disease first caused epizootics at the hatchery in 1981 (Howell et al. 1985) and has been a chronic problem ever since.

Juveniles in the stream

Relative survival.—Survival of the 1992 year-class in Brushy Fork was lower for H than for W fish. Survival was significantly lower for H than for W fish from release to age-0 parr (i.e., during the two month interval to the end of the first summer) and from release to age-1. Thereafter, survival was similar between stocks but $ARS_{H/W}$ remained well below 1 supporting the conclusion of lower survival for H than for W fish in Brushy Fork.

Survival of the 1993 year-class from release to age-0 was significantly higher for H than for W fish. In contrast, survival after age-0 was significantly lower for H than for W fish. Our results suggest that net survival from release to ages 3 and older was also lower for H than for W fish. $ARS_{H/W}$ was significantly <1 for residents aged 3-6. $ARS_{H/W}$ for the pooled sample of residents aged 3-6 (n=1575) was 0.69. $ARS_{H/W}$ estimates for migrants were near 1 (Table 5), supporting differential mortality rather than differential emigration as the cause for the change in relative frequency from release to ages 3-6. The problem is that the migrant traps were only operated for part of the spring migration period in 1994 and 1995 (Table 6) so we could have missed differences in emigration between stocks for age-1 and age-2 fish. Nonetheless, fish were actively migrating when the traps were operative (Figure 8), and migrant data from those times support lower survival for H than for W fish from release to ages 3 and older.

The survival pattern for the 1993 year-class—higher for H than for W fish from release to age-0 but lower for H than for W fish after age-0—may have been due to the density differences during the power outage at the hatchery, when mortality of more dense H alevins from limited oxygen was about twice that of W alevins. As mentioned above, the differential mortality may have culled weak individuals from the H population. If so, then weak individuals may have been culled from the W population in a compensatory fashion during the first summer after release in Brushy Fork, resulting in higher survival for H than for W fish. Thereafter, survival of the 1993 year-class in Brushy Fork was lower for H than for W fish, similar to the survival difference between stocks observed for the 1992 year-class from release.

Dispersal.—Emigration rate (proportion of the population that emigrated) was zero at age-0, highest at age-1, and progressively decreased with age thereafter. Mean lengths of naturally produced smolts in the Clearwater River drainage during 1993-1999 ranged from 155 to 189 mm among seven tributaries (Byrne 2001). Based on this, most migrants in the current study were too small to be smolts migrating to the ocean (Figure 11) and therefore were simply dispersing downstream.

Emigration rate at age-1 was higher for H than for W fish of the 1992 year-class but similar between stocks for the 1993 year-class (Table 5). We do not think that this inconsistency stemmed from the inconsistency between year-classes in size of H relative to W fish (length and weight less for H than for W residents of the 1992 year-class at ages 0 and 1; weight greater for H than for W residents of the 1993 year-class at those ages; length greater for H than for W residents of the 1993 year-class at age-1 but not at age-0). A companion study found that emigration at age-1 was not driven by competitive displacement of small by larger fish, but rather that emigrants were among the larger individuals in the population (Rubin et al. 2012f). The difference in mean length between age-1 migrants and age-1 residents in the current study appeared small given the difference in mean capture dates, especially for the 1992 year-class (Table 17), suggesting that migrants were not among the smaller individuals in the yearling population. Length distribution shape was similar between age-1 migrants and age-1 residents (data not shown); there was no evidence of truncation or skewing that might suggest emigration by a particular size class.

$ARS_{H/W}$ of residents was slightly higher below than above the weir for the 1992 year-class at ages 4 and 6 whereas it was much lower below than above the weir for the 1993 year-class at ages 3 and 5 (Figure 10). This difference between year-classes may have resulted at least in part from the difference between stocks in emigration of younger fish of the 1992 year-class and the lack thereof for the 1993 year-class. Greater downstream movement by H than by W fish of the 1992 year-class coupled with cessation of movement by at least some of the migrants between the weir, where the trap was located, and the barrier falls farther downstream could produce higher $ARS_{H/W}$ below than above the weir. No difference in downstream movement between stocks for the 1993 year-class should have produced an $ARS_{H/W}$ below the weir that was similar to $ARS_{H/W}$ above it. The fact that $ARS_{H/W}$ was lower below than above the weir indicates either lower survival for H then for W fish after downstream dispersal or greater emigration from the study reach (i.e., past the barrier falls) for H than for W fish.

Percent female was lower for H than for W residents of the 1992 year-class at ages 4-6 whereas percent female was similar between stocks for residents of the 1993 year-class. This pattern may also have resulted from the difference in emigration between stocks for the 1992 year-class and the lack thereof for the 1993 year-class. Percent female was much higher for migrants than for residents at age-3 of the 1992 year-class and age-2 of the 1993 year-class (Table 11). If the same trend (higher percent female for migrants than for non-migrants) held for both stocks at younger ages, then greater emigration for H than for W fish would result in lower percent female for H than for W residents, and similar emigration between stocks would result in similar sex ratios between H and W residents, exactly what we observed. Higher percent female for migrants than for non-migrants, when coupled with cessation of downstream movement between the weir and the barrier falls, may also have contributed to higher percent female below than above the weir for age-6 residents of the 1992 year-class and age-5 residents of the 1993 year-class, and to the decrease in percent female above the weir during the previous two years (Figure 15).

Growth and condition.—When size differences occurred, they showed H fish to be smaller than W fish of the 1992 year-class but larger than W fish of the 1993 year-class. These size differences probably represented growth differences since it seems unlikely that mortality targeted small HxH fish but was not size selective for WxW fish or only targeted large WxW fish. The size differences between stocks were apparent for age-0 residents (weight was significantly greater for H than for W age-0 residents of the 1993 year-class although length was not), prior to any emigration. Despite the inconsistency in size difference between year-classes, condition was higher for H than for W fish of both year-classes.

Maturity.—Our sampling revealed a high incidence of residualization by experimental steelhead. We electrofished intensively each year during 1996-1999 to remove as many of them and any offspring they produced as possible. Juvenile trout were collected along with our experimental fish, and samples that mostly comprised yearlings based on length frequency analysis were assayed for GPIA to determine species. Juveniles from 1997 were either cutthroat trout (*n*=334), or cutthroat-steelhead hybrids with cutthroat mothers (*n*=13; maternity confirmed with mitochondrial DNA markers; Ostberg and Rodriguez 2006), whereas juveniles from 1998 were either cutthroat trout (*n*=427), cutthroat-steelhead hybrids (*n*=30), or pure steelhead (*n*=114). These data suggest that spring 1997 was the first time that steelhead females (1992 year-class age-5 or 1993 year-class age-4) spawned, and they confirm that steelhead males (1992 year-class age-4 or 1993 year-class age-3) spawned (with cutthroat females) in spring 1996, although they do not preclude that steelhead males spawned in spring 1995 or earlier.

Females spawning at age-5 is in agreement with the relatively high ovaries weights and GSIs for upper-modal age-4 females from the 1993 year-class that were sampled in September (Figures 17 and 18). Apparently, at least some of these females were maturing in preparation for spawning at age-5 the following spring. Also, some age-4 females from the 1992 year-class apparently spawned at age-5 even though their ovaries weights and GSIs, in July of their fourth year, were relatively low. Some males may have spawned at age-3 since distributions of testes weight, GSI, and percent precocious overlapped considerably between July-sampled males (1992 year-class age-4 and 1993 year-class age-3; Figures 19-22; Table 13). Distributions of testes weight and GSI of September-sampled males (1992 year-class age-5 and 1993 year-class age-4) were nearly unimodal (Figures 19-22), and means of testes weight and GSI for those males (Table 15) were high enough to suggest that nearly all of them, even those without flowing milt, were maturing and likely capable of spawning the following spring (Schmidt and House 1979).

Ovaries weight and GSI were greater for H than for W females aged 4 and older of both year-classes, suggesting that maturation rate (speed with which maturing females reach maturity, perhaps related to time of spawning) or reproductive investment (fecundity and [or] egg size) was greater for H than for W females. This finding is consistent with differences in spawning time and fecundity between anadromous females returning to Dworshak Hatchery and to Fish Creek. The fish ladder at the hatchery is generally opened in early February and closed in early May (Burge et al. 2004). Most hatchery females enter the ladder and are spawned in March and April; few are prevented from spawning by ladder closure in May. Median capture date of upstream-migrating wild females at the Fish Creek weir ranged from 25 April-8 May, and median capture date of downstream-migrating female kelts ranged from 25 May-26 June during 2001-2005 (Byrne 2002-2006). Of course median spawning time was between those dates, likely sometime in May in most years, and clearly several weeks later than at the hatchery. Mean fecundity, standardized to an average body length of 80.7 cm, was 21% higher for females returning to Dworshak Hatchery than for wild females returning to Fish Creek in 1992 and 1993, and ovaries weight (the product of fecundity and mean egg weight) was 16% greater (Appendix).

The differences in ovaries weight and GSI between stocks for residualized females in Brushy Fork developed under common rearing and were therefore due to genetic (or, less likely, maternal) differences. This suggests that the differences in spawning time and (or) fecundity between anadromous females returning to Dworshak Hatchery and Fish Creek may have been from genetic differences, perhaps due to domestication of the Dworshak population (see below), rather than from differences between juvenile rearing or adult holding environments.

Testes weight and GSI were lower for H than for W males of the 1993 year-class at age-3, suggesting lower maturity for H than for W males at that age. Testes weight and GSI were lower for H than for W males without flowing milt but were similar between stocks for males with flowing milt, suggesting that at age-3, W males required a greater minimum testes weight for milt to flow than did H

males. Differences in male maturity and presence of flowing milt disappeared at older ages when nearly all males of both stocks were mature.

Domestication versus ancestry

We found differences between stocks in survival, growth, and condition in the hatchery, and in downstream migration after release. We also found differences between stocks in survival, dispersal, growth, condition, and maturation in a stream environment. All of these differences were likely caused by genetic differences between the stocks rather than by any of the potentially confounding factors discussed above, except that lower per capita oxygen availability and resultant higher mortality for H than for W alevins of the 1993 year-class may have affected comparisons for that year-class, particularly the comparison of first-summer survival in the stream. Genetic differences between the stocks could have resulted from divergence of the hatchery population from its wild founder population due to aspects of the hatchery program, or alternatively from ancestral differences between the wild founder population, which was native to the North Fork Clearwater River, and the wild population from Fish Creek (Figure 1).

Domestication is considered the most likely mechanism by which hatchery culture can rapidly, in few generations, cause fitness-related genetic changes to a hatchery population (Araki et al. 2008). Domestication results from natural selection for traits that are beneficial in the hatchery program and is expected to increase fitness for hatchery rearing but decrease fitness for natural rearing (Reisenbichler et al. 2004; Araki et al. 2008). Relative survivals from the current study are for the most part consistent with domestication of the hatchery population. For the 1992 year-class, survival in the hatchery and to dams on the lower Snake and Columbia rivers after release was higher for H than for W fish, whereas survival in Brushy Fork was lower for H than for W fish. Survival of the 1993 year-class in the hatchery was zero for both stocks due to outbreaks of IHNV and Ich; however; W fish succumbed to the epizootic sooner than did H fish. Survival of the 1993 year-class in Brushy Fork was higher for H than for W fish during the two months from release to age-0 parr but was lower for H than for W fish thereafter, and as discussed above our results show lower net survival from release to ages 3 and older for H than for W fish with the caveat that age-1 and age-2 migrant samples did not represent the entire spring migration period.

An analysis of presumably neutral DNA microsatellite markers (i.e., markers likely not to be under selection) found significant genetic differentiation between *O. Mykiss* from the North Fork, including Dworshak Hatchery steelhead, and wild steelhead from Lochsa River tributaries including Fish Creek, although at the watershed scale (10 watersheds and 3 hatchery complexes representing all steelhead-bearing waters in Idaho), the Dworshak Hatchery population clustered with wild steelhead from upper Clearwater River watersheds, including the Lochsa, Selway, and South Fork Clearwater River watersheds, as distinct from other Idaho watersheds (Nielsen et al. 2009). The existence of genetic differentiation based on neutral markers is neither necessary nor sufficient evidence that differences in local adaptation exist; however, they suggest the opportunity. Ancestral affinities between the Dworshak Hatchery population and wild steelhead in the Clearwater River basin are discussed in more detail in Rubin et al. (2012a; 2012b).

We suspect that local adaptation was similar between the Fish Creek population and the original North Fork population despite genetic differences at neutral markers. The North Fork Clearwater River and Fish Creek are geographically proximate and share the same geology. Based on data collected prior to initiation of the hatchery program, life history traits were similar between the original North Fork population and wild steelhead from Fish Creek, although mean smolt age may have been less for the North Fork population than for the Fish Creek population (see the "Source Populations" section above). Fish Creek and Brushy Fork were both tributaries of the Lochsa River and therefore may have been environmentally more similar to each other than to North Fork tributaries, perhaps favoring the Fish Creek population. The North Fork population may have been favored at Dworshak Hatchery because its water came from the North Fork; however, temperatures and chemistry differed from those experienced

by the ancestral North Fork population because the hatchery employed a water reuse system to provide heated and ion supplemented water during the winter. In summary, we cannot rule out ancestral differences as the cause of all or part of the survival differences we observed between H and W fish; however, our results are consistent with domestication of the hatchery stock and its expected consequence: greater fitness for hatchery rearing but reduced fitness for natural rearing for hatchery relative to wild origin fish.

Management implications

Our primary purpose was to test whether Dworshak Hatchery fish were less fit for natural rearing than local wild populations. If less fit, Dworshak fish released in areas of the Clearwater River basin supporting wild fish were likely to compromise the fitness of the wild populations through interbreeding. This study indicates that fitness of the wild populations would be reduced. During our study, local managers decided against supplementing wild steelhead populations to avoid any risk of reducing fitness. Our results support that decision.

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References

Aebersold, P. B., G. A. Winans, D. J. Teel, G. B. Milner, and F. M. Utter. 1987. Manual for starch gel electrophoresis – a method for the detection of genetic variation. NOAA Technical Report NMFS 61.

Applebee, A. E., and J. M. Tipping. 1991. Use of electronic counters for coho and Chinook salmon, steelhead, and cutthroat trout smolts. The Progressive Fish Culturist 53:195-198.

Araki, H., Berejikian, B. A., Ford, M. J. & Blouin, M. S. 2008. Fitness of hatchery-reared salmonids in the wild. Evol. Appl. 1, 342–355.

Blankenship, L., and R. Tivel. 1980. Puget Sound wild stock coho trapping and tagging 1973-1979. Progress Report No. 111, State of Washington, Department of Fisheries, Olympia, WA.

Brannon, E. L., D. F. Amend, M. A. Cronin, J. E. Lannan, S. LaPatra, W. J. McNeil, R. E. Noble, C. E. Smith, A. J. Talbot, G. A. Wedemeyer, H. Westers. 2004a. The controversy about salmon hatcheries. Fisheries 29:12-28.

Brannon, E. L., M. S. Powell, T. P. Quinn, and A. J. Talbot. 2004b. Population structure of Columbia River basin Chinook salmon and steelhead trout. Reviews in Fisheries Science 12:99-232.

Burge, H. L., Faler, M., Roseberg, R. B., Jones, R. N., and Olson, J. M. 2004. Adult steelhead returns to Dworshak NFH in 2002-2003 and prognosis for 2003-2004. Appendix B *in* Annual Report, Fiscal Year 2003, U.S. Fish and Wildlife Service, Idaho Fishery Resource Office, Ahsahka, Idaho.

Byrne, Alan. 2001. Idaho Fish and Game, fishery research - steelhead supplementation in Idaho rivers summary report 1 January 1993 - 31 December 1999. Bonneville Power Administration Report DOE/BP-01466-6. Portland, OR.

Byrne, Alan. 2001-2006. Steelhead supplementation in Idaho rivers – Annual Progress Reports 2000-2005. Project No. 1990-0500, Bonneville Power Administration Report DOE/BP-00009949-1. Portland, OR.

Cone, R. S. 1989. The need to reconsider the use of condition indices in fisheries science. Transactions of the American Fisheries Society 118:510-514.

Cytel Software Corporation. 1997. StatXact-3, Version 3.1. Copyright 1989-1997.

Howell, P., K. Jones, D. Scarnecchia, L. LaVoy, W. Kendra, and D. Ortmann. 1985. Stock assessment of Columbia River anadromous salmonids Volume II: Steelhead stock summaries stock transfer guidelines—information needs. Final Report to Bonneville Power Administration, Contract DE-AI79-84BP12737, Project 83-335, 1032 p.

Leary, R. F., F. W. Allendorf, S. R. Phelps, and K. L. Knudsen. 1987. Genetic divergence and identification of seven cutthroat trout subspecies and rainbow trout. Transactions of the American Fisheries Society 116:580-587.

Leider, S. A., P. L. Hulett, J. J. Loch, and M. W. Chilcote. 1990. Electrophoretic comparison of the reproductive success of naturally spawning transplanted and wild steelhead trout through the returning adult stage. Aquaculture 88:239-252.

Lindsay, R. B., K. R. Kenaston, and R. K. Schroeder. 1992. Steelhead production factors. Oregon Department of Fish and Wildlife, Fish Research Project F-120-R, Annual Progress Report, Portland, OR.

Martinson, R.D., R.J. Graves, R.B. Mills, & J.W. Kamps. 1997. Monitoring of downstream salmon and steelhead at federal hydroelectric facilities - 1996. Annual report. U.S. Dept. of Energy, Bonneville Power Administration, Portland, OR (U.S.A.).

Miller, W. H. 1987. A review of Dworshak National Fish Hatchery mitigation record. U.S. Fish and Wildlife Service, Dworshak Fisheries Assistance Office, Ahsahka, Idaho. FR-1/FAO-88-02.

Nielsen, J. L., A. Byrne, S. L. Graziano, and C. C. Kozfkay. 2009. Steelhead genetic diversity at multiple spatial scales in a managed basin: Snake River, Idaho. North American Journal of Fisheries Management 29:680-701.

Ostberg, C. O., and R. J. Rodriguez. 2006. Hybridization and cytonuclear associations among native westslope cutthroat trout, introduced rainbow trout, and their hybrids within the Stehekin River Drainage, North Cascades National Park. Transactions of the American Fisheries Society 135:924-942.

Ott, L. 1984. An introduction to statistical methods and data analysis, second edition. Duxbury Press, Boston.

Pettit, S. W. 1976. Dworshak Fisheries Studies. Idaho Department of Fish and Game Job Completion Report, Project DSS-29.

Peven, C. M., R. R. Whitney, and K. R. Williams. 1994. Age and length of steelhead smolts from the mid-Columbia River basin, Washington. North American Journal of Fisheries Management 14:77-86.

Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. 1982. Fish hatchery management. American Fisheries Society, Bethesda, MD.

Prentice, E.F., T.A. Flagg, C.S. McCutcheon, & D.F. Brastow. 1990. PIT-tag monitoring systems for hydroelectric dams and fish hatcheries. In: Parker, N.C., A.E. Giorgi, R.C. Heidinger, D.B. Jester, Jr., E.D. Prince, & G.A. Winans (eds), pp. 323-334. Fish-marking techniques. American Fisheries Society Symposium 7, Bethesda, MD.

Reisenbichler, R. R., M. C. Hayes, S. P. Rubin, L.A. Wetzel, and B. M. Baker. 2006. *PEPA** genotype affects return rate for hatchery steelhead. Transactions of the American Fisheries Society 135:28-39.

Reisenbichler, R. R., and S. P. Rubin. 1999. Genetic changes from artificial propagation of Pacific salmon affect the productivity and viability of supplemented populations. ICES Journal of Marine Science 56:459-466.

Reisenbichler, R. R., S. P. Rubin, L. A. Wetzel, and S. R. Phelps. 2004. Natural selection after release from a hatchery leads to domestication in steelhead, *Oncorhynchus mykiss*. Pages 371-383 *in* K. M. Leber, S. Kitada, H. L. Blankenship, and T. Svasand, editors. Stock enhancement and sea ranching, second edition. Blackwell Publishing Ltd, Oxford, UK.

Roseberg, Ralph, Howard Burge, Bill Miller, and Dan Diggs. 1992. A review of coded-wire tagged fish released from Dworshak, Kooskia and Hagerman National Fish Hatcheries, Idaho, 1976-1990. U.S. Fish and Wildlife Service, Idaho Fishery Resource Office.

Rubin, S. P., R. R. Reisenbichler, J. E. Hensleigh, L. A. Wetzel, and B. M. Baker. 2012a. Genetic differences between hatchery and wild steelhead for growth and survival in the hatchery and seaward migration after release. Chapter 2 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, J. E. Hensleigh, L. A. Wetzel, B. M. Baker, F. E. Leonetti, K. D. Stenberg, and S. L. Slatton. 2012b. Genetic differences between hatchery and wild steelhead for survival, growth, dispersal, and male maturation in a natural stream. Chapter 3 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, and S. L. Slatton. 2012c. Predicted time from fertilization to maximum wet weight for steelhead alevins based on incubation temperature and egg size. Chapter 4 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, and K. D. Stenberg. 2012f. Effect of size of unfed fry at release on survival and growth of juvenile steelhead in streams and a hatchery. Chapter 7 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, K. D. Stenberg, and B. M. Baker. 2012g. Effect of incubation temperature on post-embryonic survival and growth of steelhead in a natural stream and a hatchery. Chapter 5 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

SAS Institute Inc. 2000. SAS Online DOC^{R}, Version 8. SAS Institute, Cary, North Carolina.

Scheaffer, R. L., W. Mendenhall, and L. Ott. 1986. Elementary survey sampling, third edition. Duxbury Press, Boston.

Schmidt, S. P., and E. W. House. 1979. Precocious sexual development in hatchery-reared and laboratory-maintained male steelhead trout (*Salmo gairdneri*). Journal of the Fisheries Research Board of Canada 36:90-93.

Shaklee, J. B. F. W. Allendorf, D. C. Morizot, and G. S. Whitt. 1990. Gene nomenclature for protein-coding loci in fish. Transactions of the American Fisheries Society 119:2-15.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry, 3rd edition. W. H. Freeman and Company, New York.

Thedinga, J. F., M. L. Murphy, S. W. Johnson, J. M. Lorenz, and K. V. Koski. 1994. Determination of salmonid smolt yield with rotary-screw traps in the Situk River, Alaska, to predict effects of glacial flooding. North American Journal of Fisheries Management 14:837-851.

Van Deventer, J. S., and W. S. Platts. 1985. Computer software system for entering, managing, and analyzing fish capture data from streams. Research Note, Intermountain Research Station, USDA Forest Service, Ogden, Utah.

Wheeler, P. A. and G. H. Thorgaard. 1991. Cryopreservation of rainbow trout semen in large straws. Aquaculture 93:95-100.

Wold, E. 1970. Information pertaining to 1969 steelhead spawning operation at Dworshak National Fish Hatchery. U.S. Fish and Wildlife Service, Dworshak Dworshak National Fish Hatchery, Ahsahka, Idaho.

Zimmerman, C. E., and G. H. Reeves. 2000. Population structure of sympatric anadromous and nonanadromous *Oncorhynchus mykiss*: evidence from spawning surveys and otolith microchemistry. Canadian Journal of Fisheries and Aquatic Sciences 57:2152-2162.

APPENDIX: Difference in fecundity between Dworshak Hatchery steelhead and wild steelhead from Fish Creek, Idaho

We measured fecundity and egg size for samples of females returning to Dworshak Hatchery and to Fish Creek in 1992 and 1993. The samples included the females whose progeny were used in the study reported above; procedures for measuring fecundity and egg size were the same as described therein. Mean fecundity, standardized to an average body length of 80.7 cm, was 21% higher for Dworshak than for Fish Creek females, a significant difference (Figure A1). Mean (length-standardized) egg weight was significantly less for Dworshak than for Fish Creek females; however, the difference was inconsistent between return years as indicated by a significant stock*year interaction; mean egg weight was less for Dworshak than for Fish Creek females in 1992 ($P<0.05$ after Bonferroni adjustment for two comparisons) but was similar between stocks in 1993. Ovaries weight was computed for each female as the product of fecundity and egg weight. Mean (length-standardized) ovaries weight was 16% greater for Dworshak than for Fish Creek females, a significant difference that was driven by the fecundity difference.

Tables

Table 1. Number of adults spawned in 1992 and 1993, and resulting number of full-sib families. The eggs from each female were divided into two portions, each fertilized by a different male. Some wild males were spawned on more than one date. Number of wild males not used on a previous date is given in parentheses. *PEPA** genotype of all hatchery parents was **111/*111*. *PEPA** genotypes of parents of wild families are given in footnotes. *PEPA** genotype was **111/*111* for all hatchery progeny and either **100/*100* or **100/*111* for wild progeny.

Year	Stock	Date Spawned	Females	Males	Families
1992	H	15 Apr	11	7	17[a]
	W	16 Apr	3	2 (2)	6
		28 Apr	1[b]	3 (3)	3
		8 May	2	3 (1)	4
		20 May	1	2 (1)	2
		Total	7	(7)	15[c]
1993	H	21 Apr	25	30	50
	W	14 Apr	1	2 (2)	2
		23 Apr	6	5 (5)	12
		30 Apr	2	2 (2)	4
		13 May	1	2 (2)	2
		18 May	1	1[d] (0)	2
		21 May	1	2[e] (1)	2
		Total	12	(12)	24[f]

[a]Five families (half of the eggs from each of five females) were excluded from the study, four because the genetic mark was wrong (*PEPA** genotype of sire not **111/*111*) and one due to low survival between eye-up and button-up.
[b]Eggs divided into three rather than two portions.
[c]*PEPA** genotype of parents: both **100/*100* for 13 families; one **100/*100* and the other **100/*111* for 2.
[d]This male fertilized both halves of the female's eggs, but the halves were incubated on opposite sides of the tray divider and otherwise treated as separate families.
[e]Cryopreserved milt.
[f]*PEPA** genotype of parents: both **100/*100* for 8 families; one **100/*100* and the other **100/*111* for 13; one **100/*100* and the other **111/*111* for 3.

Table 2. Number of males siring various numbers of full-sib families.

Year	Stock	Males	Families
1992	H	1	6
		1	3
		2	1
		3	2
	W	1	1
		2	3
		4	2
1993	H	10	1
		20	2
	W	1	4
		2	3
		4	1
		5	2

Table 3. Number of swim-up fry released in Brushy Fork Creek or ponded at Dworshak Hatchery, incubation temperature, and developmental stage of fry at release or ponding as predicted from the model based on incubation temperature. Embryos from different spawning dates were incubated for different periods of time in chilled and ambient water to synchronize their development. Chilled and ambient temperature, respectively, averaged 7.4 ^{o}C (range 5.9-10.9) and 13.0 (10.7-14.9) in 1992, and 7.2 (6.1-9.3) and 12.6 (11.4-14.9) in 1993. Percent of time to MAWW = time between fertilization and release expressed as a percentage of time between fertilization and maximum alevin wet weight predicted by the temperature model. Weighted means of temperature and development were computed for the wild group where the weights were the number of fry from each spawning date.

Year	Stock	Date Spawned	Number of fry	Days on chilled water	Days on Ambient water	Mean temperature (^{o}C)	Accumulated Temperature units (^{o}C)	Percent of time to MAWW
1992	H	15 Apr	37,911	77	0	7.4	567	100.5
	W	16 Apr	13,377	76	0	7.4	559	101.1
		28 Apr	3,451	49	15	8.8	566	106.3
		8 May	4,028	30	24	10.1	545	104.6
		20 May	3,758	8	34	12.0	504	101.6
		Total or mean	24,614			8.7	550	102.5
1993	H	21 Apr	41,418	67	7	7.8	574	102.4
	W	14 Apr	901	81	0	7.2	584	101.5
		23 Apr	12,095	63	9	8.0	573	103.5
		30 Apr	4,680	49	16	8.6	558	103.3
		13 May	3,421	25	27	10.1	524	100.0
		18 May	3,468	16	31	10.8	509	98.4
		21 May	1,131	10	34	11.5	505	98.3
		Total or mean	25,696			8.9	553	102.0

Table 4. Relative survival, length, and condition of juveniles at Dworshak Hatchery; and detection rate at lower Snake and Columbia River dams of PIT-tagged fish. *N* or proportion of fry refers to fry ponded; *n* or proportion of juveniles refers to juveniles sampled. Within a year-class, means without a letter (x, y) in common differ ($P<0.05$). The 1992 year-class was mixed with 30,000 production fish to attain a density similar to 1993. A disease outbreak caused 1993 juveniles to be sampled three months earlier than those from 1992 and precluded the release of PIT-tagged fish (see text).

Year-class	Stock	*N* or proportion of fry	*n* or proportion of juveniles	Relative survival	Mean (SD) fork length (mm)	Weight[a] at common length[b] (*k*[c])	*n* PIT tagged	Detection rate (%)
1992	H	0.617	0.858	3.75 x	130 (26) x	17.7 (0.93) x	326	35.6 x
	W	0.383	0.142	1.00 y	108 (20) y	17.0 (0.89) y	53	3.8 y
	Total	3,208	423					
1993	H	0.634	0.899	5.16 x	99 (9) x	8.0 (0.83) [d]		
	W	0.366	0.101	1.00 y	94 (8) y	7.6 (0.78) [d]		
	Total	32,964	388					

[a]Weight (in grams) evaluated at common length with weight-length relations from AOCV; slopes similar between stocks ($P>0.05$) unless otherwise indicated.
[b]Geometric mean of lengths pooled over stocks.
[c]$k=100{,}000*W/L^3$; W=weight (g) at common length; L=common length (mm).
[d]Slope of weight-length relation differed between stocks ($P<0.05$) so stocks not compared.

Table 5. Apparent survival of H relative to W fish ($ARS_{H/W}$) in Brushy Fork. An asterisk indicates that $ARS_{H/W}$ differs from 1 (P<0.05). Dates associated with spring versus fall migrants are given in Figure 8. "Above" and "below" indicate that residents were captured above and below the weir trap, respectively; residents were captured above the weir unless otherwise indicated (see Figures 4 and 5).

Year-class	Sub-population	Age	*n*	Proportion H (H/(H+W))	$ARS_{H/W}$
1992	Fry	0	59,317[a]	0.606	
	Migrants	1	686[b]	0.569	0.86 *
		1, weighted[c]		0.579	0.89
		2	5	0.600	0.98
		3, spring	11	0.727	
		3, fall	3	0.333	
		3, total	14	0.643	1.17
		4	1	0.000	0.00
		Total	706	0.569	0.86 *
	Residents	0	361	0.540	0.76 *
		1	346	0.439	0.51 *
		2	194	0.459	0.55 *
		3	128	0.523	0.71
		4, above	408	0.473	0.58 *
		4, below	19	0.474	0.59
		4, total	427	0.473	0.58 *
		5	179	0.480	0.60 *
		6, above	41	0.463	0.56
		6, below	213	0.549	0.79
		6, total	254	0.535	0.75 *
		7, above	1	0.000	0.00
		7, below	9	0.556	0.81
		7, total	10	0.500	0.65

[a]Total number of fry released.

[b]A total of 3,343 fish visually identified as age-1 steelhead were trapped in 1993, 688 of these were sampled, and 686 were confirmed as age-1 steelhead; no age-0 steelhead were trapped. In subsequent years, all trapped fish visually identified as steelhead were sampled.

[c]Proportion H was estimated as a weighted mean by dividing the run into four nearly equal time periods and weighting proportion H in each period by the number of fish trapped in the period (Figure 9). $ARS_{H/W}$ was not significant by this method (P=0.13).

Table 5, continued.

Year-class	Sub-population	Age	*n*	Proportion H (H/(H+W))	$ARS_{H/W}$
1993	Fry	0	34,150[a]	0.600	
	Migrants	1	73	0.575	0.90
		2, spring	56	0.643	
		2, fall	2	1.000	
		2, total	58	0.655	1.26
		3	10	0.600	1.00
		Total	141	0.610	1.04
	Residents	0	321	0.682	1.43 *
		1	255	0.584	0.94
		2	150	0.647	1.22
		3, above	625	0.539	0.78 *
		3, below	19	0.421	0.48
		3, total	644	0.536	0.77 *
		4	523	0.535	0.77 *
		5, above	106	0.585	0.94
		5, below	277	0.397	0.44 *
		5, total	383	0.449	0.54 *
		6, above	4	0.250	0.22
		6, below	21	0.286	0.27 *
		6, total	25	0.280	0.26 *

[a]Total number of fry released.

Table 6. Dates of operation of juvenile downstream migrant traps in Brushy Fork.

Collection year	Trap	Dates
1992	Weir	1 Jul-1 Nov
1993	Kray	16 Jun-20 Sep
	Weir	7 Jul-19 Oct
1994	Weir	15-27 Jul
		21 Sep-2 Oct
1995	Screw	6 May-12 Jun
	Weir	22 Sep-18 Oct
1996	Screw	10 Apr-27 Jun

Table 7. Dates when juveniles were collected by electrofishing in Brushy Fork.

Collection year	Dates
1992	20-21 Aug
1993	31 Aug-9 Sep
1994	2-5 Aug
1995	26-30 Jul
1996	23-29 Jul
1997	4-9 Sep
1998	1-8 Sep
1999	20-27 Jul

Table 8. Type of tissue and aging structure sampled from juveniles collected from Brushy Fork. Fish sampled for muscle tissue were euthanized, frozen whole, and dissected in the lab, whereas those sampled for fin tissue were released.

Collection year	Sub-population	Fork length (mm)	Tissue	Aging structure
1992	Both	All	Muscle	None
1993	Both	All	Muscle	None
1994	Migrants	All	Muscle	Otoliths
	Residents	<90	Muscle	Scales
		≥90	Ventral fin	Scales
1995	Migrants	All	Muscle	Otoliths
	Residents	All	Ventral fin	Scales[a]
1996	Migrants	All	Ventral fin	Scales
	Residents	All	Muscle	Otoliths[a]
1997	Residents	All	Muscle	Otoliths
1998	Residents	All	Muscle	Otoliths
1999	Residents	All	Muscle	Otoliths

[a]Scales and otoliths collected from a sub-sample.

Table 9. Egg size, embryo density and survival during incubation, and final size of fry at release. A compartment was a whole tray in 1992 and half of a tray in 1993 (trays were fitted with a center divider that year). Each compartment contained a full-sib family. Within a year, means without a letter (x, y) in common differ (P<0.05).

Year	Stock	Statistic	Egg weight (mg)[a]	Number of fertilized eggs per compartment[b]	Percent eye-up[b]	Number of eyed embryos per compartment[b]	Eye-up to button-up survival (%)[c]	Fry weight (mg)[c]
1992	H	*n*	11	10	10	10	10	17
		Mean	112 x	2594 x	92.6 x	2381 x	87.1 x	234 x
		SD	8	716	8.7	621	12.9	21
	W	*n*	7	15	15	15	15	14[d]
		Mean	124 y	2233 x	75.8 y	1775 x	95.6 x	241 x
		SD	7	836	21.9	1038	20.0	21
1993	H	*n*	25	50	50	50	23	23
		Mean	105 x	3228 x	96.5 x	3120 x	27.6 x	196 x
		SD	9	670	5.2	684	20.6	18
	W	*n*	10	24	24	24	12	12
		Mean	109 x	2789 y	81.1 y	2261 y	54.7 y	190 x
		SD	13	693	22.6	838	30.6	20

[a]*n*=number of females; egg weight was not determined for 2 of 12 wild females in 1993.
[b]*n*=number of compartments (i.e., families); these variables were not measured for 7 of 17 1992 hatchery compartments because embryos had started to hatch before eyed eggs were counted.
[c]Survival was low in 1993 because a power outage interrupted flow to the incubation trays (see text), *n*=number of whole trays (i.e., maternal families) in 1993 because after the power outage families on either side of the center divider in each tray were mixed. Further, for 1993 hatchery trays fry were accidentally mixed between two whole trays and again between two other whole trays, and thus *n*=23 rather than 25.
[d]One outlier was excluded.

Table 10. Emigration rate of 1992 relative to 1993 year-class fish ($RE_{92/93}$) in 1994-1996. Proportion 92 is the proportion of total residents (or migrants) that were from the 1992 year-class.

	Residents		Migrants		
Year	*n*	Proportion 92	*n*	Proportion 92	$RE_{92/93}$
1994	449	0.432	78	0.064	0.09
1995	278	0.460	72	0.194	0.28
1996	1071	0.399	11	0.091	0.15

Table 11. The percentage of total fish (males + females) that were females. Numbers in parentheses are sample size. Percentages without a letter (x, y) in common differ (P<0.05).

Year-class	Sub-population	Age	Stock		
			H	W	Total
92	Migrants	3	77.8 (9) x	60.0 (5) x	71.4 (14)
	Residents	3	15.4 (13) x	33.3 (12) x	24.0 (25)
		4	16.3 (202) x	27.6 (225) y	22.2 (427)
		5	12.8 (86) x	16.1 (93) x	14.5 (179)
		6	16.9 (136) x	36.5 (115) y	25.9 (251)
		7	0.0 (5)	0.0 (5)	0.0 (10)
93	Migrants	2	68.4 (38) x	85.0 (20) x	74.1 (58)
	Residents	2	47.8 (23) x	23.1 (13) x	38.9 (36)
		3	37.2 (344) x	36.8 (296) x	37.0 (640)
		4	28.2 (280) x	25.9 (243) x	27.2 (523)
		5	31.6 (171) x	39.0 (210) x	35.7 (381)
		6	28.6 (7) x	33.3 (18) x	32.0 (25)

Table 12. Results of 2-way logistic model analyses for effects of stock and age or of stock and stream reach (above versus below the weir) on percent female (percent of total males and females that were females) or percent precocious (percent of total males that were flowing milt). Effects of stock and age were tested for residents from above the weir. Effects of stock and stream reach were tested for residents of a single age. The interaction term (stock*age or stock*reach) was included in the logistic models but it was never significant ($P \geq 0.21$).

Response	Year-class	Age(s)	Reach(es)	Factor	*P*-value
Percent female	1992	4-6	Above	Stock	0.80
				Age[a]	0.07
		6	Both	Stock	0.02
				Reach	0.07
	1993	3-5	Above	Stock	0.83
				Age[a]	<0.01
		5	Both	Stock	0.24
				Reach	0.01
Percent precocious	1992	4-6	Above	Stock	0.53
				Age[a]	0.02
		6	Both	Stock	0.52
				Reach	0.12
	1993	3-5	Above	Stock	0.31
				Age[b]	<0.01
		5	Both	Stock	0.66
				Reach	0.01

[a]Age treated as a continuous variable.
[b]Age treated as a categorical variable.

Table 13. The percentage of total male residents that were flowing milt. Numbers in parentheses are sample size. Percentages without a letter (x, y) in common differ (P<0.05).

Year-class	Age	Stock H	Stock W	Stock Total
92	4	71.0 (169) x	73.0 (163) x	72.0 (332)
	5	82.7 (75) x	75.6 (78) x	79.1 (153)
	6	70.8 (113) x	78.1 (73) x	73.7 (186)
	7	80.0 (5) x	80.0 (5) x	80.0 (10)
93	3	55.6 (216) x	54.5 (187) x	55.1 (403)
	4	83.1 (201) x	84.4 (180) x	83.7 (381)
	5	65.0 (117) x	60.9 (128) x	62.9 (245)
	6	100.0 (5) x	91.7 (12) x	94.1 (17)

Table 14. Length and condition factor (k) of female residents and of male residents with and without flowing milt. Means without a letter (x, y) in common differ (P<0.05).

Year-class	Age	Sex and milt status	n	Mean (SD) fork length (mm)	Mean k
92	4	Females	95	134.9 (12.9) x	1.249 x
		Males without	93	135.9 (10.3) x	1.305 y
		Males with	239	133.6 (11.3) x	1.311 y
	5	Females	26	158.7 (11.1) x	1.143 x
		Males without	32	162.4 (9.7) x	1.234 y
		Males with	121	161.5 (11.0) x	1.245 y
	6	Females	62	172.1 (11.7) x	1.093 x
		Males without	45	172.4 (10.8) x	1.152 y
		Males with	128	175.4 (11.5) x	1.155 y
93	3	Females	237	123.4 (10.1) x	1.243 x
		Males without	181	124.1 (9.1) x	1.284 y
		Males with	222	125.3 (8.3) x	1.311 y
	4	Females	142	158.2 (12.0) x	1.122 x
		Males without	62	152.3 (13.4) y	1.233 y
		Males with	319	154.5 (11.7) y	1.231 y
	5	Females	125	171.0 (12.6) x	1.074 x
		Males without	80	172.7 (11.1) x	1.130 y
		Males with	127	169.9 (10.1) x	1.146 y

Table 15. Length, gonad weight, and gonadosomatic index (GSI) of female residents and of male residents with and without flowing milt. Gonad weight was $\log_{10}$ transformed and compared between stocks with AOCV; $\log_{10}$ transformed length was the covariate. GSI was arcsine square root transformed and compared between stocks with AOV. Two-way analyses were used for males (Table 16). Means without a letter (x, y) in common differ (P<0.05). Length was not tested. Standard deviations are asymmetric due to back-transformations from log of gonad weight and arcsine-square root of GSI.

Year-class	Age	Sex and milt status	Stock	*n*	Mean (SD) fork length (mm)	Mean (SD) gonad weight (g)	Mean (SD) GSI (%)
92	4	Females	H	22	131.7 (14.7)	0.117 (0.048, 0.082) x	0.412 (0.207, 0.277) x
			W	48	135.7 (12.3)	0.092 (0.043, 0.081) x	0.340 (0.168, 0.225) x
		Males without	H	27	136.3 (10.1)	0.630 (0.249, 0.412) x	2.044 (0.854, 1.078) x
			W	28	133.6 (11.5)	0.742 (0.225, 0.323) x	2.416 (0.878, 1.070) x
		Males with	H	59	134.6 (13.0)	0.800 (0.257, 0.379) x	2.593 (0.932, 1.133) x
			W	60	134.4 (10.9)	0.766 (0.212, 0.293) x	2.497 (0.694, 0.803) x
	5	Females	H	10	157.6 (10.8)	0.694 (0.498, 1.760) x	2.008 (1.491, 2.436) x
			W	13	159.2 (12.7)	0.343 (0.259, 1.066) x	1.110 (0.899, 1.594) x
		Males without	H	10	161.4 (7.6)	5.691 (0.836, 0.980) x	10.643 (1.165, 1.225) x
			W	17	163.0 (11.5)	4.660 (1.238, 1.687) x	9.156 (2.119, 2.366) x
		Males with	H	5	162.0 (14.2)	4.695 (0.987, 1.249) x	8.435 (1.772, 1.960) x
			W	6	159.5 (7.4)	5.653 (0.761, 0.879) x	11.025 (1.466, 1.557) x

Table 15, continued.

Year-class	Age	Sex and milt status	Stock	*n*	Mean (SD) fork length (mm)	Mean (SD) gonad weight (g)	Mean (SD) GSI (%)
93	3	Females	H	95	125.1 (11.2)	0.060 (0.013, 0.017) x	0.259 (0.063, 0.072) x
			W	82	123.0 (8.8)	0.058 (0.014, 0.018) x	0.246 (0.062, 0.071) x
		Males without	H	66	125.4 (10.2)	0.257 (0.171, 0.510) x	1.292 (0.877, 1.353) x
			W	58	124.7 (7.7)	0.467 (0.205, 0.366) y	1.986 (0.864, 1.104) y
		Males with	H	65	126.2 (8.9)	0.685 (0.249, 0.391) x	2.744 (1.089, 1.354) x
			W	59	125.9 (6.8)	0.742 (0.216, 0.304) x	2.922 (0.927, 1.097) x
	4	Females	H	72	158.7 (12.4)	0.312 (0.226, 0.827) x	1.048 (0.889, 1.658) x
			W	60	156.7 (11.1)	0.258 (0.173, 0.525) x	0.807 (0.635, 1.097) x
		Males without	H	29	152.0 (12.6)	3.945 (0.684, 0.828) x	9.221 (1.428, 1.534) x
			W	27	151.1 (13.9)	3.944 (0.708, 0.863) x	9.366 (1.518, 1.637) x
		Males with	H	20	156.6 (11.4)	4.243 (1.081, 1.451) x	9.241 (2.080, 2.315) x
			W	17	152.5 (13.2)	4.358 (1.181, 1.620) x	9.701 (1.172, 1.239) x

Table 16. *P*-values of 2-way analyses for effects of stock, milt status, and their interaction on $\log_{10}$ transformed testes weight (AOCV, covariate $\log_{10}$ length) and arcsine square root transformed GSI (AOV) of resident males. Values <0.05 are bolded.

Year-class	Age	Source of variation	Testes weight	GSI
92	4	Stock	0.315	0.329
		Milt status	**0.018**	**0.034**
		Stock*milt status	0.089	0.112
	5	Stock	0.936	0.423
		Milt status	0.750	0.779
		Stock*milt status	**0.035**	**0.006**
93	3	Stock	**0.000**	**0.001**
		Milt status	**0.000**	**0.000**
		Stock*milt status	**0.003**	**0.027**
	4	Stock	0.752	0.393
		Milt status	0.464	0.616
		Stock*milt status	0.743	0.657

Table 17. Mean (SD) capture date and length of age-1 migrants and residents. SD of capture date is in days.

Year-class	Sub-population	*n*	Capture date	Fork length (mm)
1992	Migrants	683	26 Jul (17.0)	74.5 (6.8)
	Residents	346	4 Sep (3.4)	77.8 (8.0)
1992	Migrants	70	21 Jul (1.9)	75.1 (4.5)
	Residents	255	3 Aug (1.0)	77.3 (6.4)

Figures

Figure legends

Figure 1. Study area.

Figure 2. Length frequency of parents. Within year-class, sex, and stock, relative frequencies sum to 1. Vertical dashed lines indicate boundaries between 1-, 2-, and 3-salt length classes based on historical length frequencies of known-age (coded wire tagged) returns to Dworshak Hatchery (Ralph Roseberg, U.S. Fish and Wildlife Service, Idaho Fishery Resource Office, personal communication).

Figure 3. Survival of the 1993 year-class from eyed embryo to button-up fry for each incubation tray in relation to initial density of eyed embryos. The regression line was fit to data pooled over stocks.

Figure 4. Number (*n*) or density index (*n*/m) of 1992 year-class residents aged 0-7 at electrofishing sites in Brushy Fork in relation to distance upstream from the weir trap. Ages are indicated at the left in each panel; note that ages 0-7 correspond to collection years 1992-1999. Density index=number of fish caught on the first pass per m stream length. Data are plotted at site midpoints; horizontal error bars indicate the length of stream sampled when known. Vertical dashed lines designate the location of the weir trap (0 m) and the three bridges (Figure 1); note that the barrier falls was 3,945 m downstream from the weir.

Figure 5. Number (*n*) or density index (*n*/m) of 1993 year-class residents aged 0-6 at electrofishing sites in Brushy Fork in relation to distance upstream from the weir trap; note that ages 0-6 correspond to collection years 1993-1999. All else is the same as in Figure 4.

Figure 6. Lengths at tagging of all juveniles of the 1992 year-class released from Dworshak Hatchery, and of the subset of juveniles detected at the dams. Within a category (released or detected) relative frequencies sum to 1. Hatchery juveniles are shown in the upper panel and wild in the lower.

Figure 7. Travel time for the 1992 year-class from release at Dworshak Hatchery to detection at Lower Granite Dam (Granite; distance=116 km) or Little Goose Dam (Goose; distance=176 km) versus length at tagging. Only two wild fish were detected, both at Little Goose Dam.

Figure 8. Number of Brushy Fork migrants caught each day in each trap, and the proportion of total daily catch (traps combined) belonging to the H group. Trapping did not occur on dates without entries (Table 6). Text in each panel designates year-class and age. In 1996, the screw trap started operation on 10 April but no fish were caught during that month.

Figure 9. Number of age-1 migrants of the 1992 year-class trapped in Brushy Fork in 1993, proportion of total catch that was sampled, and proportion of sampled fish that were H fish. Data are grouped into four nearly equal time periods. The Kray-Meekin trap was operated during the first period and the screen weir trap during the last three.

Figure 10. Apparent survival of H relative to W fish ($ARS_{H/W}$) for the 1992 (top) and 1993 (bottom) year-class at successive ages in Brushy Fork. Above = above the weir trap and below = between the barrier falls and the weir trap. Sample sizes are indicated by numbers near data points (residents) or at bar bases (migrants). An asterisk near a data point indicates that $ARS_{H/W}$ of residents differed significantly from 1 ($ARS_{H/W}$ of migrants never did), and an asterisk between data points indicates a significant change in $ARS_{H/W}$ between the points ($P<0.05$). Bars are shown only for ages where the sample of migrants exceeded 14 (Table 5).

Figure 11. Mean (+SD) length of fish at successive ages and seasons in Brushy Fork. Text in each panel designates year-class and sub-population (migrants or residents). Spr=spring; fal=fall. An asterisk indicates that H and W differ for that pair of bars ($P<0.05$).

Figure 12. Mean length of 1992 year-class residents at electrofishing sites in Brushy Fork in relation to distance from the weir trap. Ages are indicated at the left in each panel; note that ages 0-7 correspond to collection years 1992-1999. Data are plotted at site midpoints; horizontal error bars indicate the length of stream sampled when known. Vertical dashed lines designate the location of the weir trap and the three bridges (Figure 1); note that the barrier falls was 3,945 m downstream from the weir.

Figure 13. Mean length of 1993 year-class residents at electrofishing sites in Brushy Fork in relation to distance from the weir trap. Ages are indicated at the left in each panel; note that ages 0-6 correspond to collection years 1993-1999. All else is the same as in Figure 12.

Figure 14. Mean condition factor of 1992 (top) and 1993 (bottom) year-class fish at successive ages in Brushy Fork. An asterisk indicates that H and W differ for that pair of bars ($P<0.05$).

Figure 15. Percent female for 1992 (top) and 1993 (bottom) year-class residents at successive ages in Brushy Fork. Above = above the weir trap; below = between the weir trap and the barrier falls. Sample sizes are given next to points.

Figure 16. Percentage of total males that were flowing milt (percent precocious) for 1992 (top) and 1993 (bottom) year-class residents at successive ages in Brushy Fork. Above = above the weir trap; below = between the weir trap and the barrier falls. Sample sizes are given next to points.

Figure 17. Ovaries weight distributions for 1993 (top) and 1992 (bottom) year-class resident females. Within a year-class and age, bar heights sum to 1.

Figure 18. Gonadosomatic index (GSI) distributions for 1993 (top) and 1992 (bottom) year-class resident females. Within a year-class and age, bar heights sum to 1.

Figure 19. Testes weight distributions for 1993 (top) and 1992 (bottom) year-class resident males. Within a year-class and age, bar heights sum to 1.

Figure 20. Gonadosomatic index (GSI) distributions for 1993 (top) and 1992 (bottom) year-class resident males. Within a year-class and age, bar heights sum to 1.

Figure 21. Testes weight distributions for male residents with and without flowing milt. Text in panels designates year-class and age. Within a year-class, age, and milt type, bar heights sum to 1.

Figure 22. Gonadosomatic index (GSI) distributions for male residents with and without flowing milt. Text in panels designates year-class and age. Within a year-class, age, and milt type, bar heights sum to 1.

Figure 23. Ovaries weight plotted against length for resident females. Large text in panels designates year-class and age. Horizontal dashed lines indicate the cutoff (0.3 g) between the lower and upper modes of ovaries weight (Figure 17); modes were not apparent for 1992 year-class age-4 females. Regression lines were fit with lower-modal females excluded. Small text above and below the dashed lines indicates the percentage of total H and the percentage of total W females in the upper and lower

modes. Note that tests for differences between stocks were conducted with $\log_{10}$ ovaries weight (covariate $\log_{10}$ length), not untransformed values as shown here (see text).

Figure A1. Means (+SDs) of fecundity, egg weight, and ovaries weight (the product of fecundity and egg weight) for females returning to Dworshak Hatchery and wild females returning to Fish Creek in 1992 and 1993. Means (and SDs) were standardized to the mean fork length among all 164 females examined (80.7 cm). Numbers indicate sample size; *n* differs between fecundity and egg weight due to missing egg weight measurements for a few females. Symbols preceding and succeeding the comma indicate significance for the difference between stocks and the stock*year interaction, respectively; * = $P<0.05$, ns = $P>0.2$. Statistics are from 2-way AOCVs with factors stock and return year, and covariate length. Neither the dependent variable nor the covariate was transformed for the analyses. Length never interacted with stock, return year, or stock*return year ($P>0.30$).

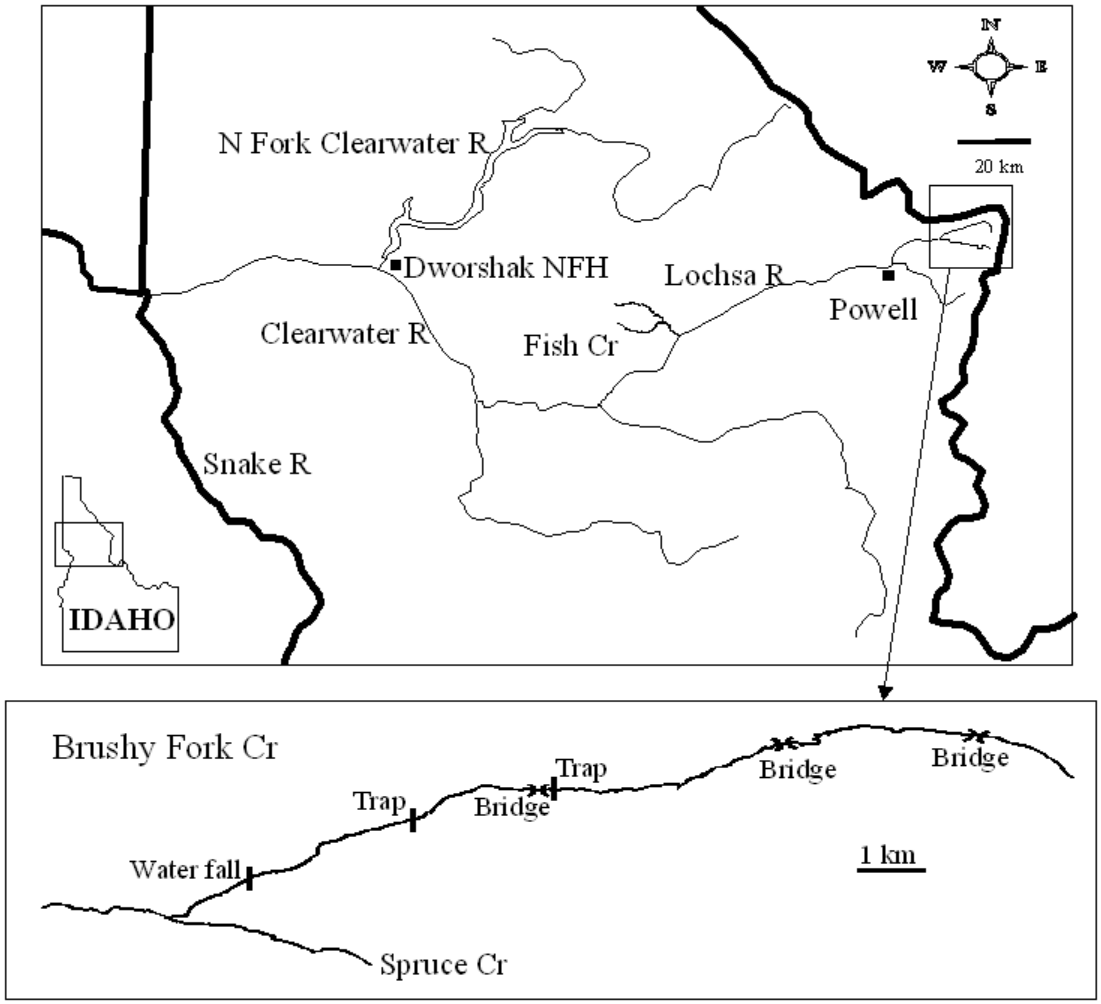
N
W
E
S
20 km
N Fork Clearwater R
Dworshak NFH
Lochsa R
Powell
Clearwater R
Fish Cr
Snake R
IDAHO
Brushy Fork Cr
Trap
Trap
Bridge
Bridge
Bridge
Water fall
1 km
Spruce Cr

Figure 1.

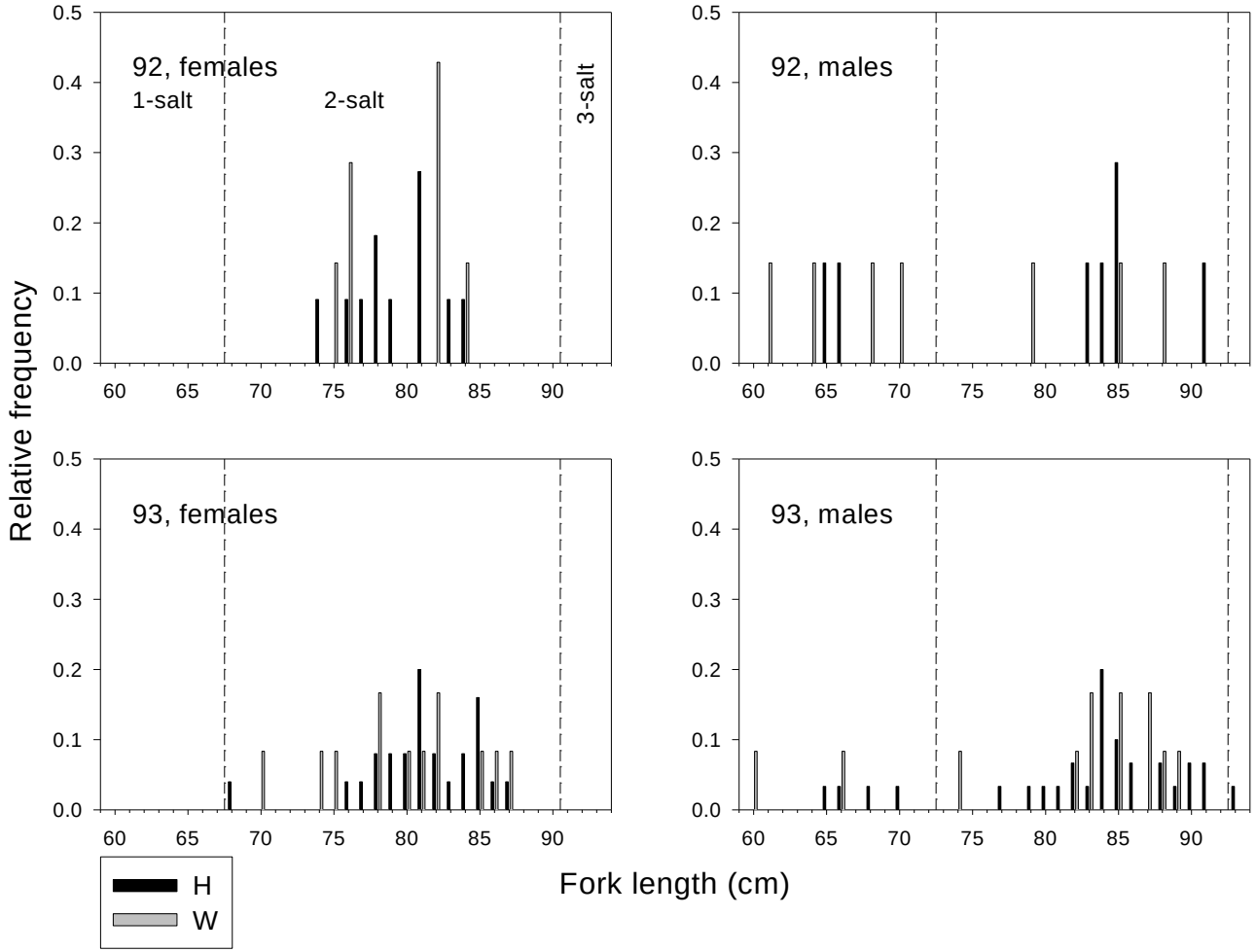
92, females
1-salt
2-salt
3-salt
92, males
93, females
93, males
Relative frequency
Fork length (cm)
H
W

Figure 2.

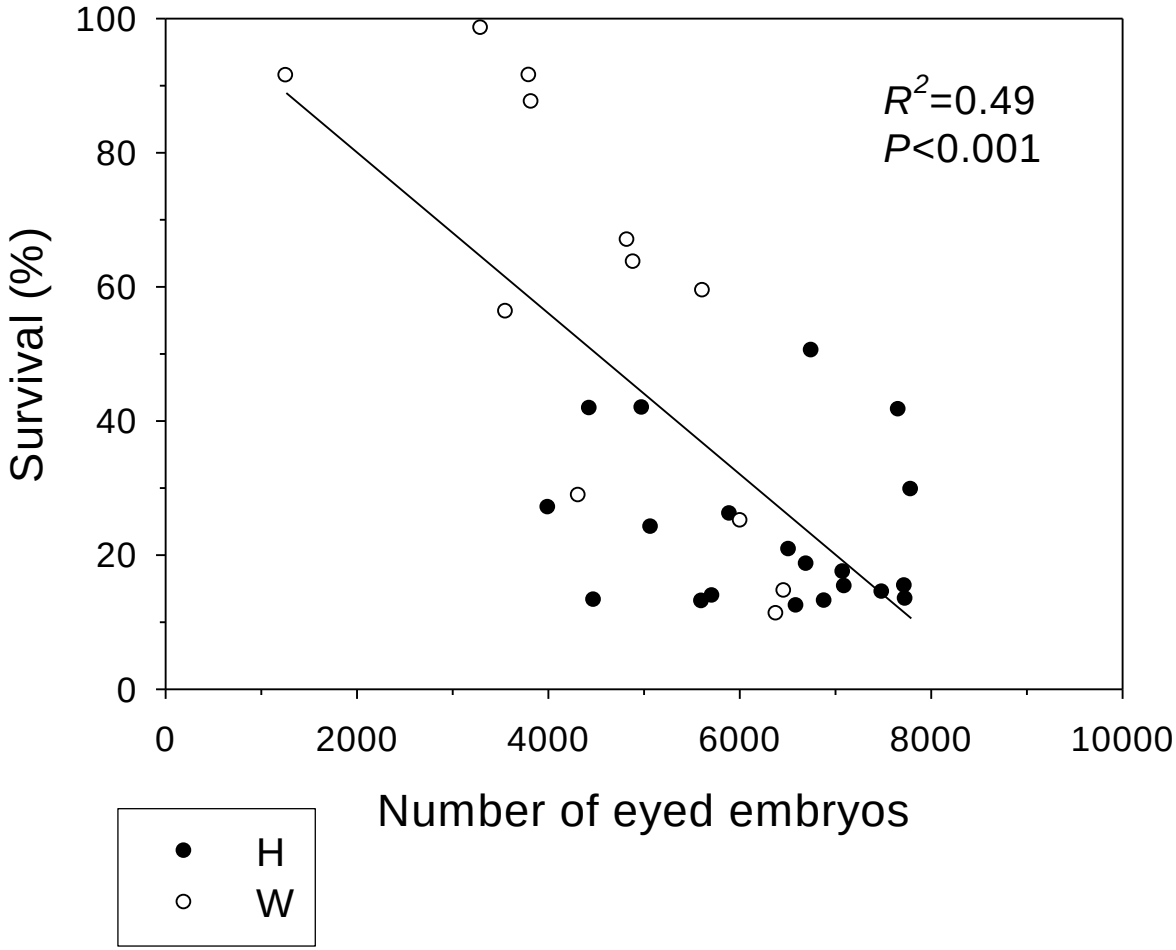
100
80
60
40
20
0
Survival (%)
0
2000
4000
6000
8000
10000
Number of eyed embryos
R^2=0.49
P<0.001
H
W

Figure 3.

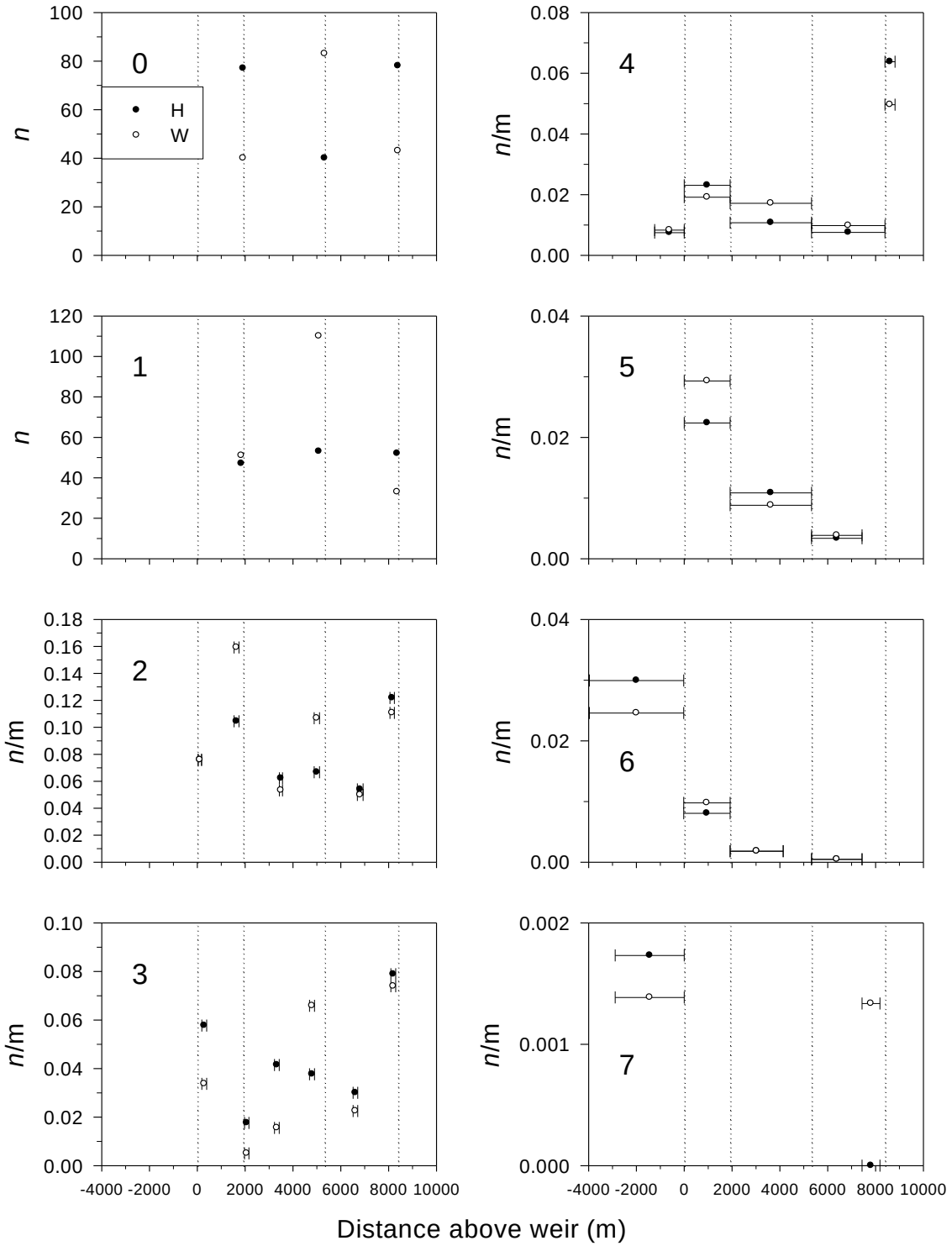

Figure 4.

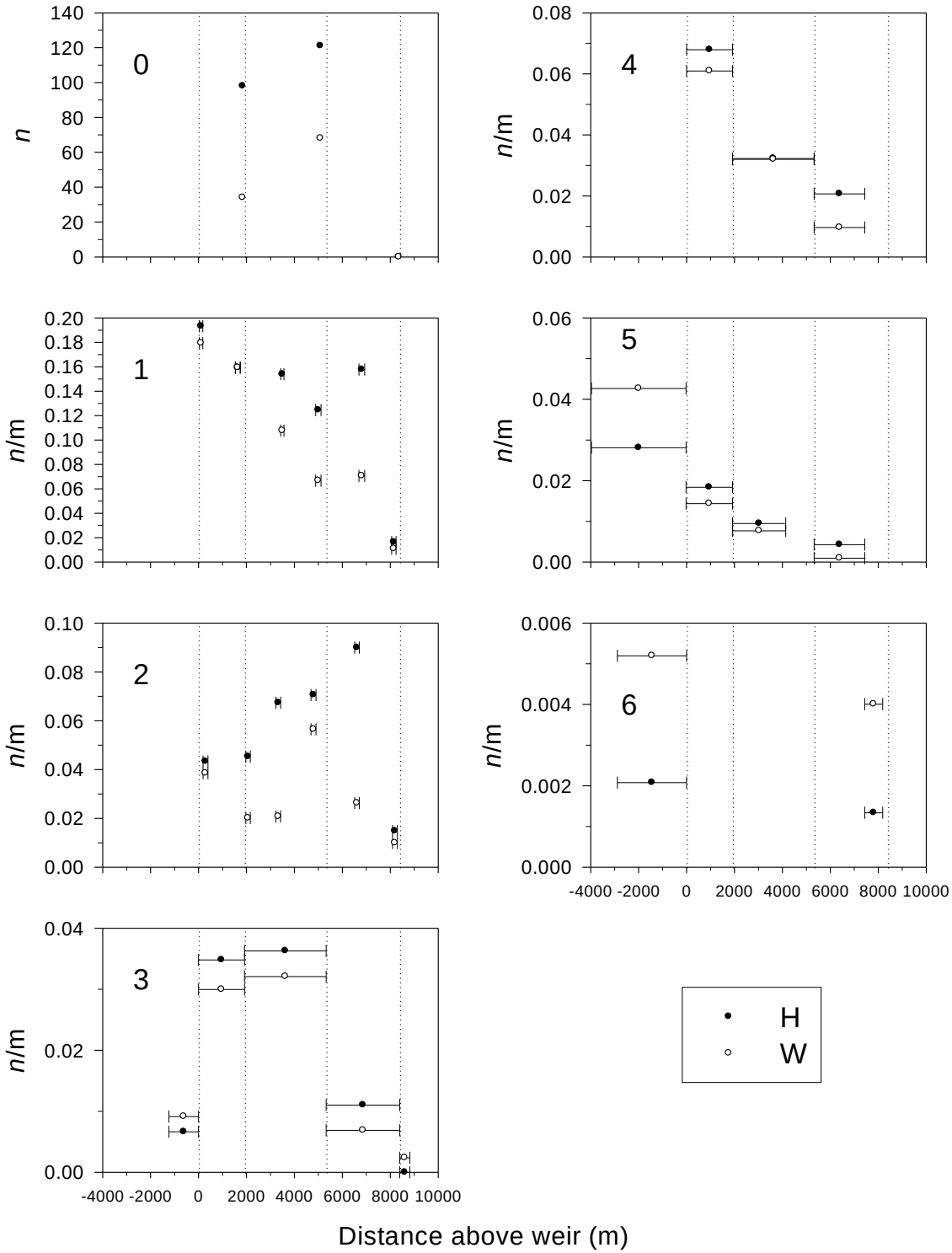

Figure 5.

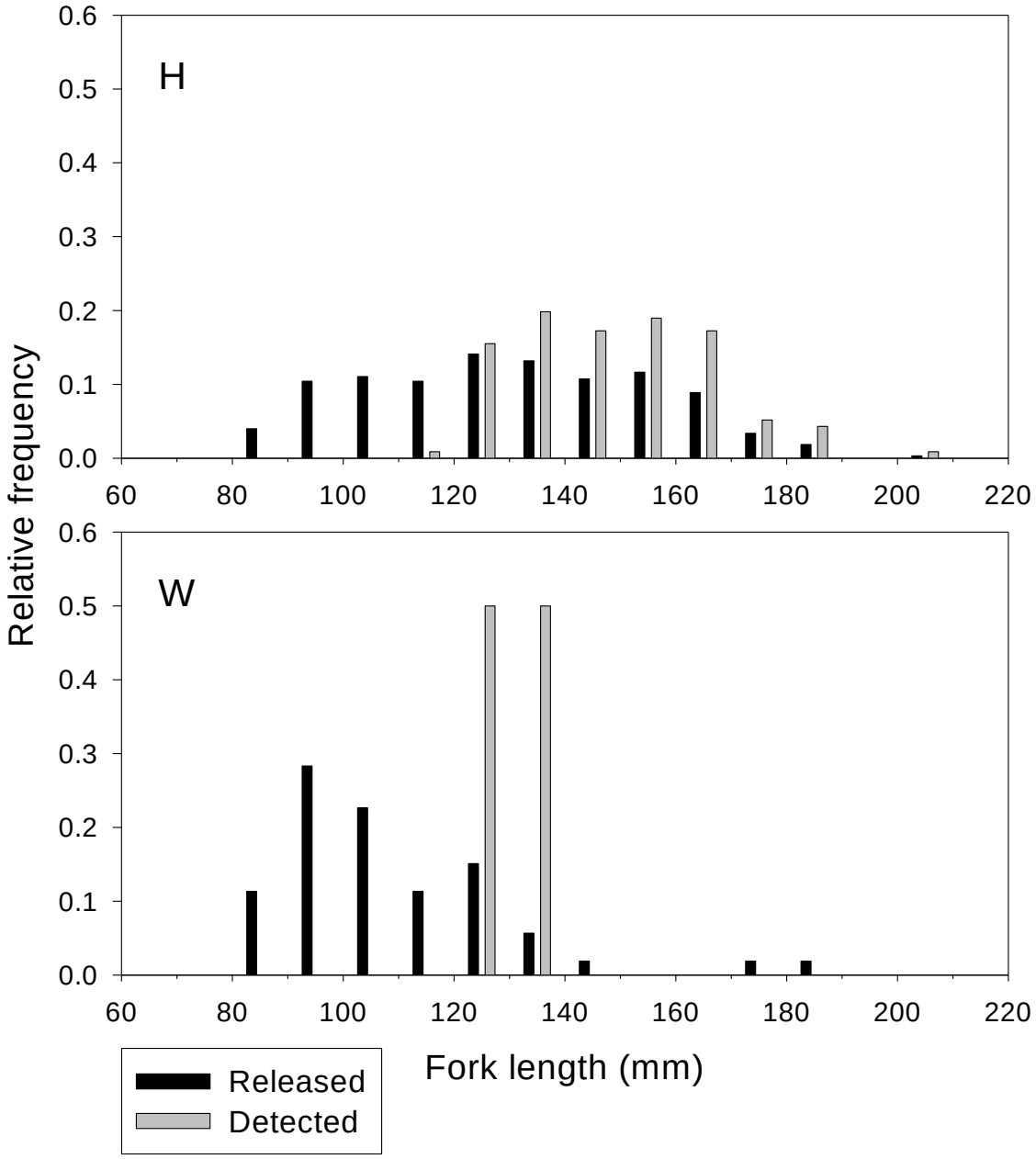
H
W
Relative frequency
Fork length (mm)
Released
Detected
0.0
0.1
0.2
0.3
0.4
0.5
0.6
60
80
100
120
140
160
180
200
220

Figure 6.

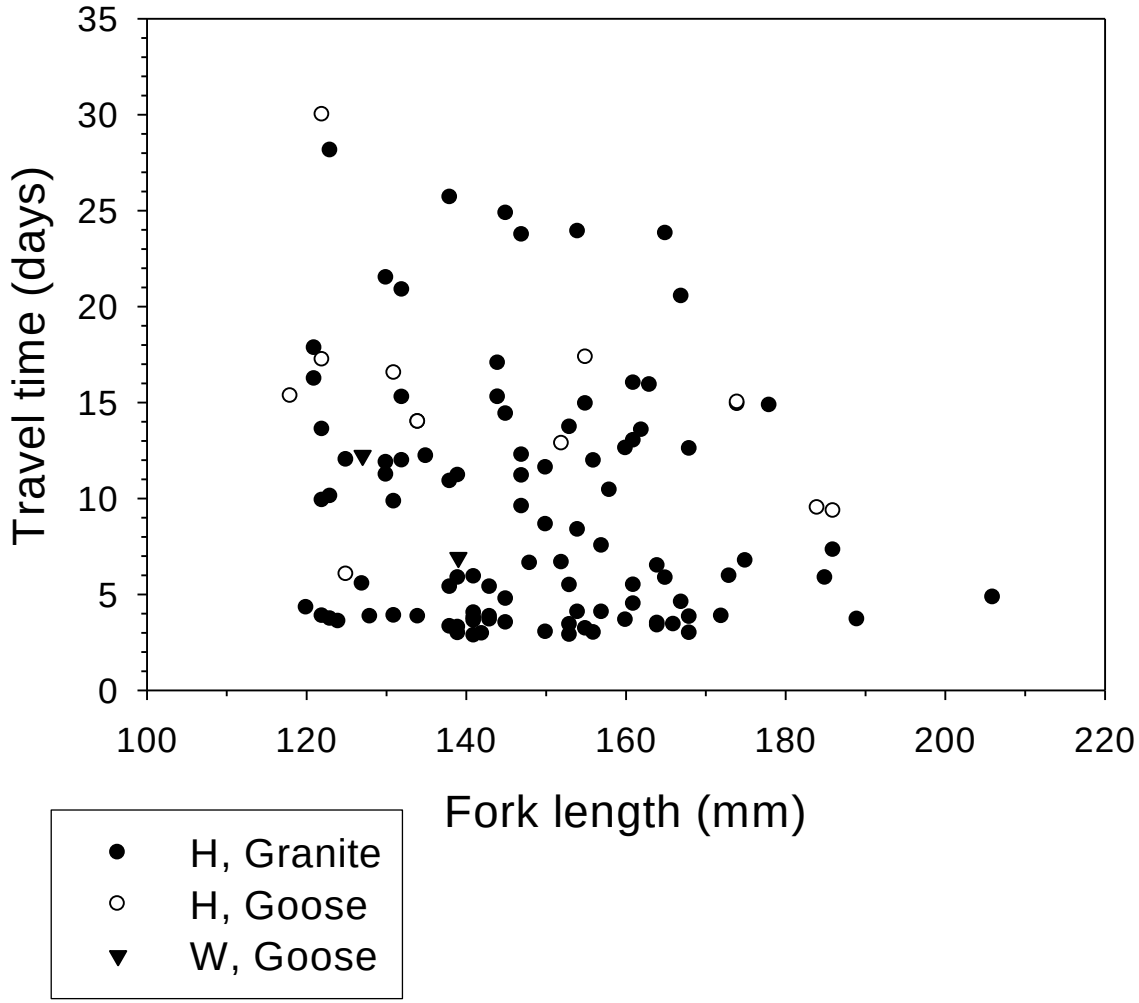
35
30
25
20
15
10
5
0
Travel time (days)
100
120
140
160
180
200
220
Fork length (mm)
H, Granite
H, Goose
W, Goose

Figure 7.

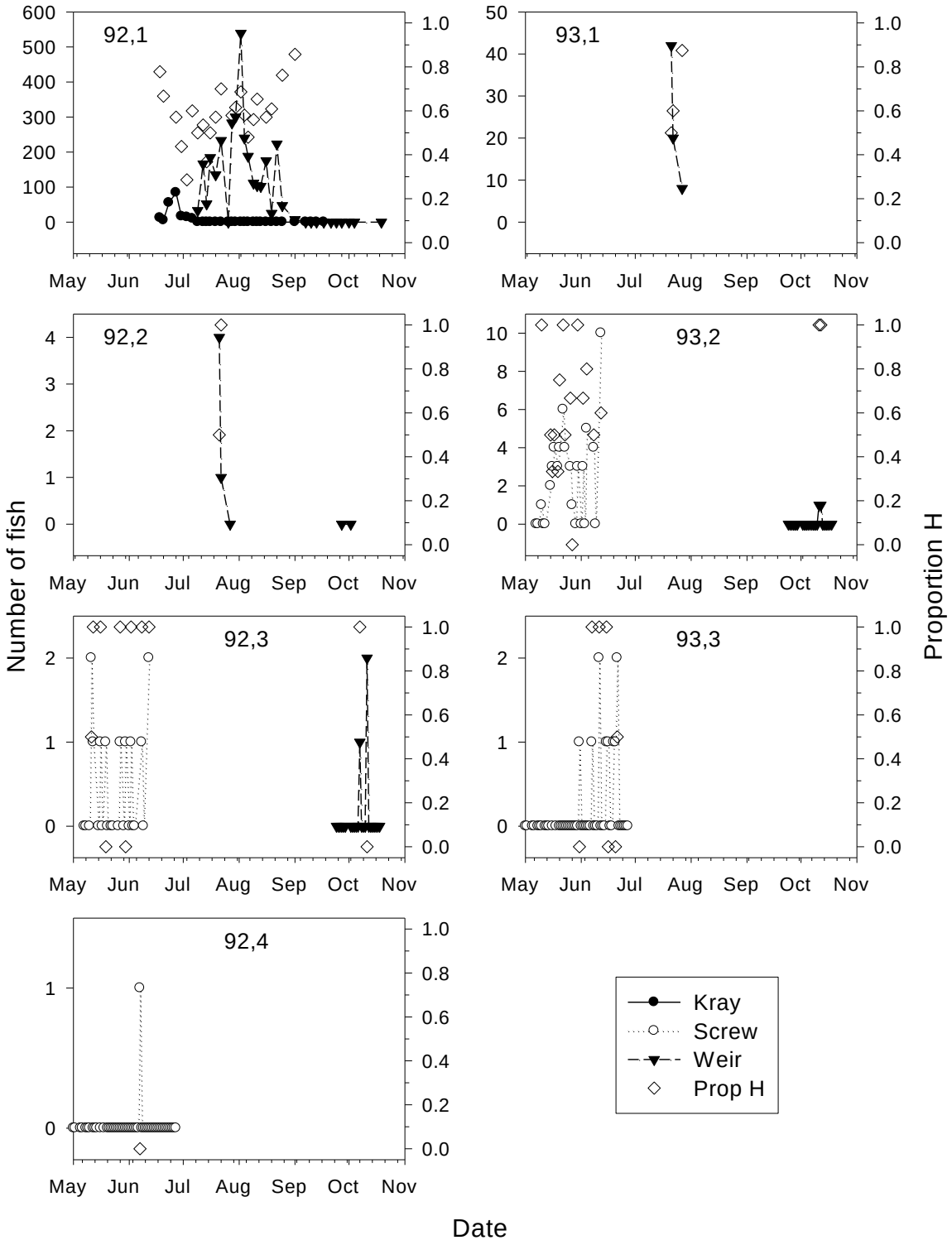
92,1
93,1
92,2
93,2
92,3
93,3
92,4
Number of fish
Proportion H
Date
May
Jun
Jul
Aug
Sep
Oct
Nov
Kray
Screw
Weir
Prop H

Figure 8.

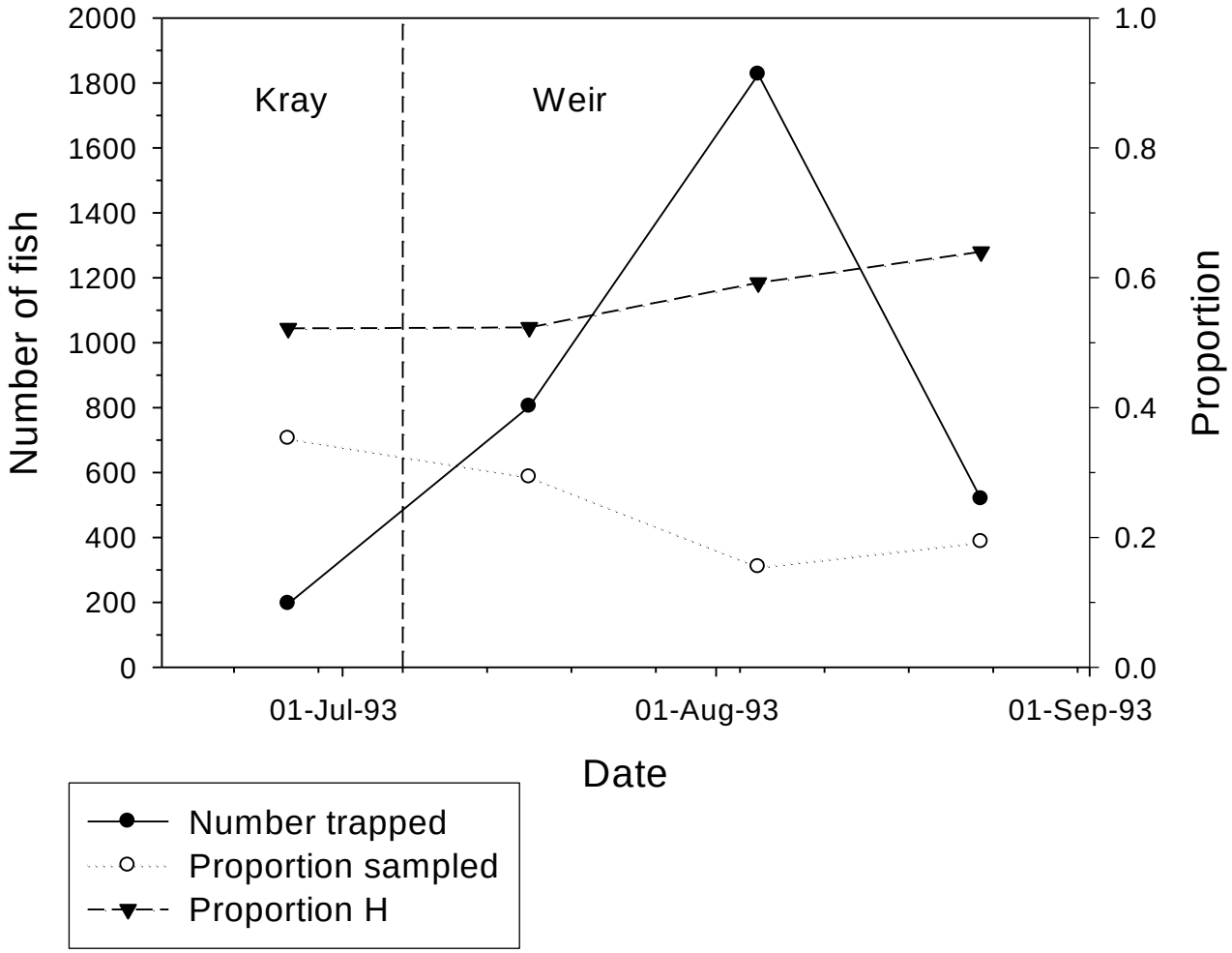
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Number of fish
1.0
0.8
0.6
0.4
0.2
0.0
Proportion
Kray
Weir
01-Jul-93
01-Aug-93
01-Sep-93
Date
Number trapped
Proportion sampled
Proportion H

Figure 9.

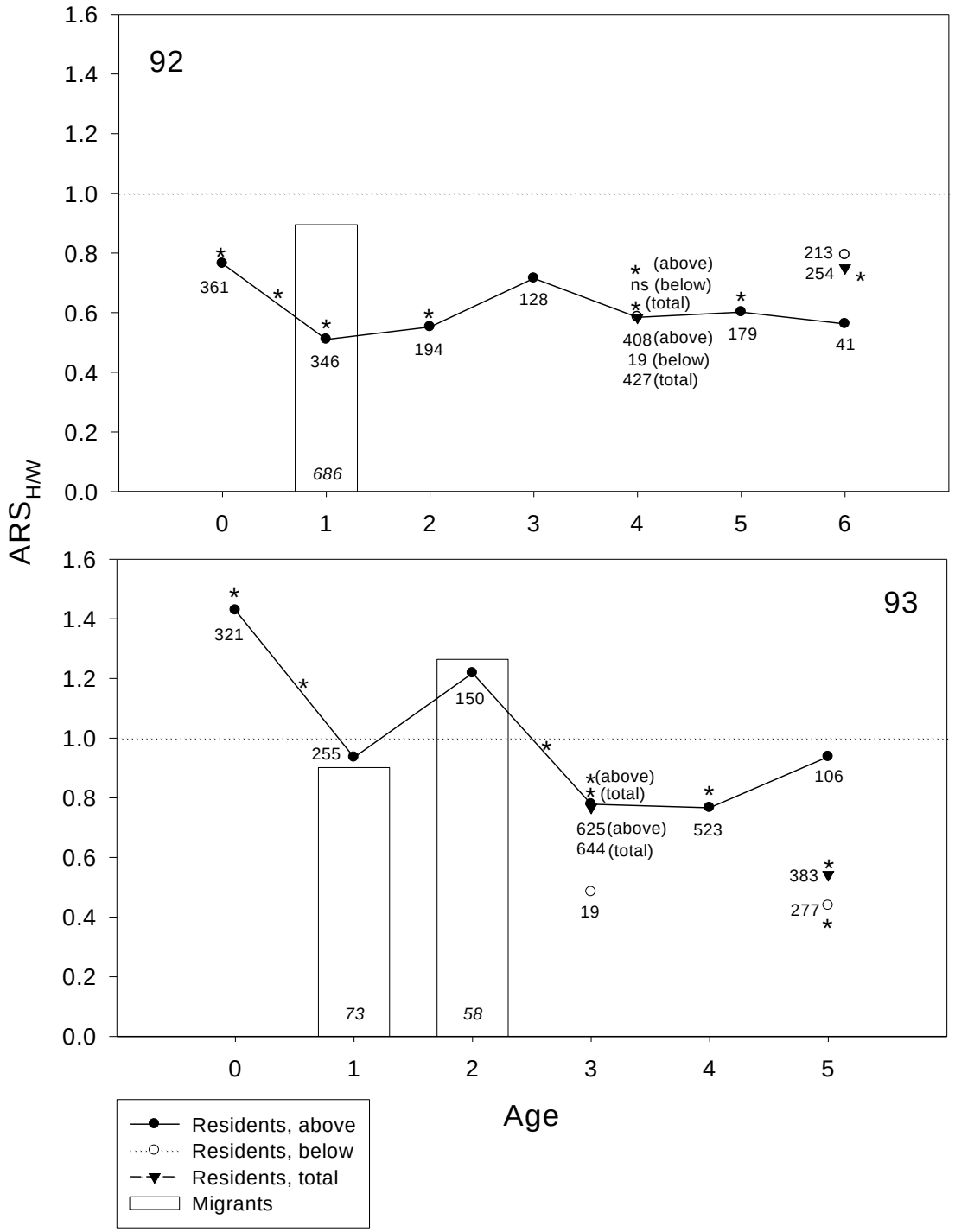
92
93
ARS H/W
Age
361
346
194
128
408 (above)
19 (below)
427 (total)
179
41
213
254
686
321
255
150
625 (above)
644 (total)
523
106
383
277
19
73
58
Residents, above
Residents, below
Residents, total
Migrants

Figure 10.

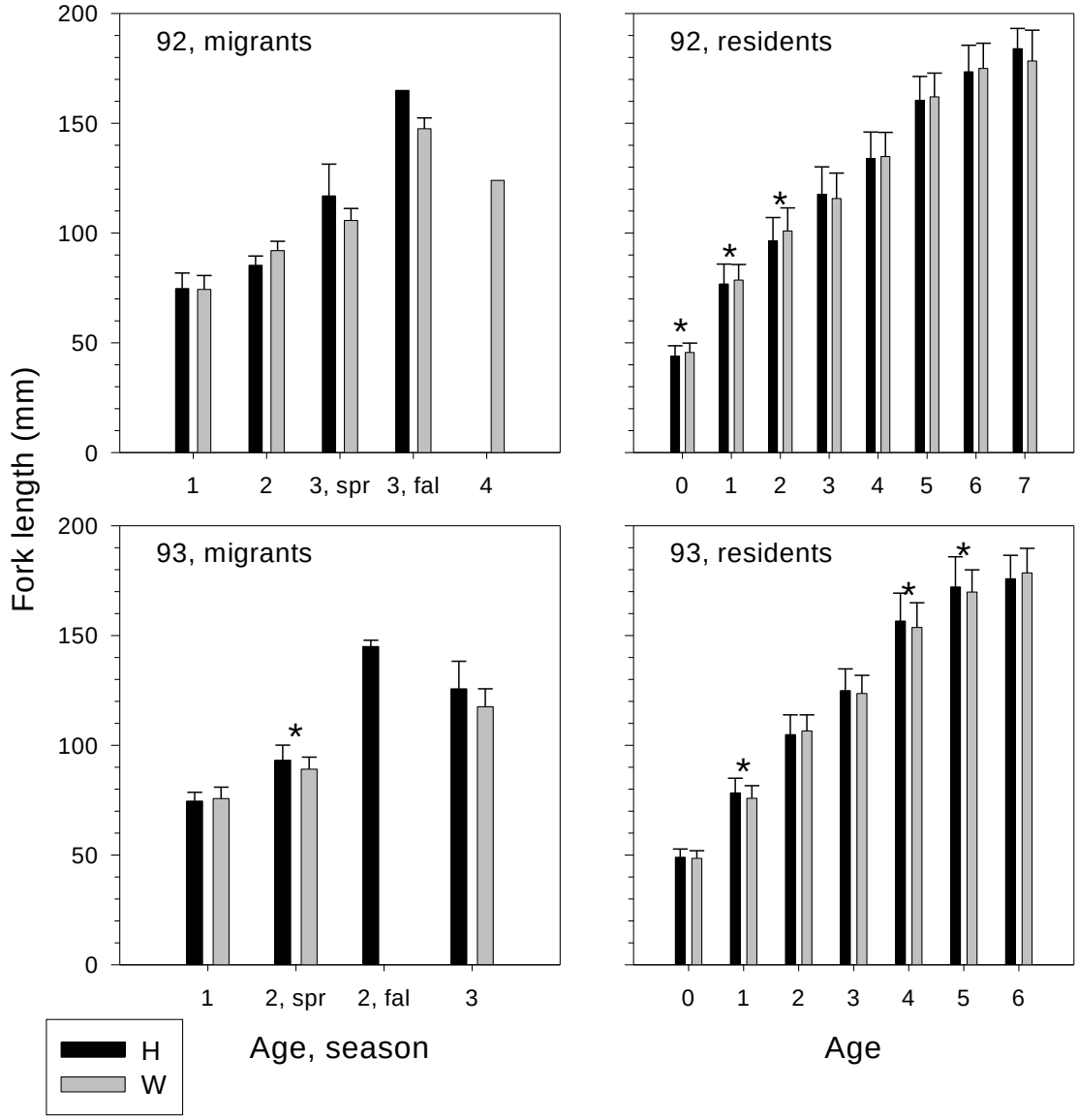
92, migrants
92, residents
93, migrants
93, residents
Fork length (mm)
200
150
100
50
0
1
2
3, spr
3, fal
4
0
1
2
3
4
5
6
7
2, spr
2, fal
Age, season
Age
H
W
*

Figure 11.

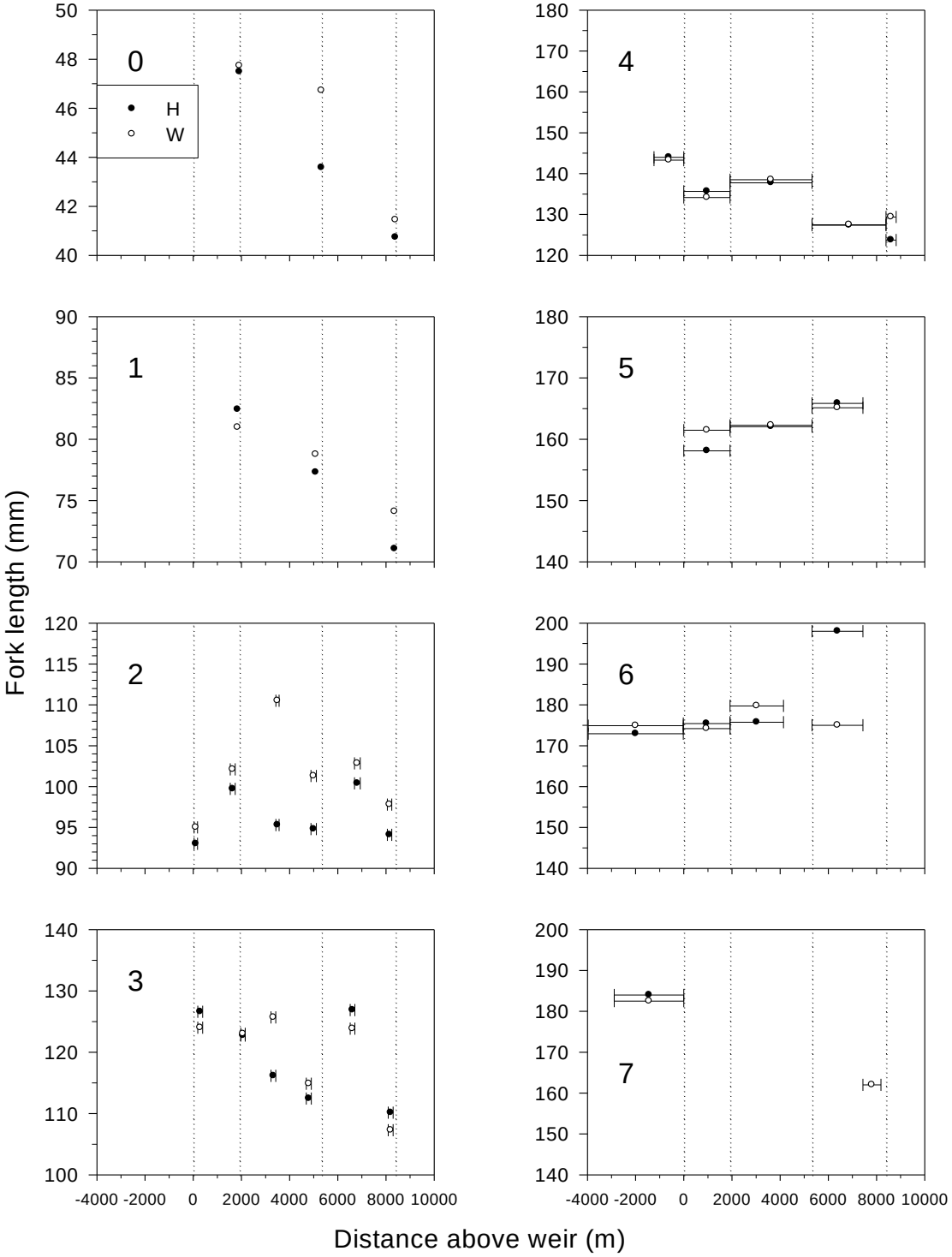

Figure 12.

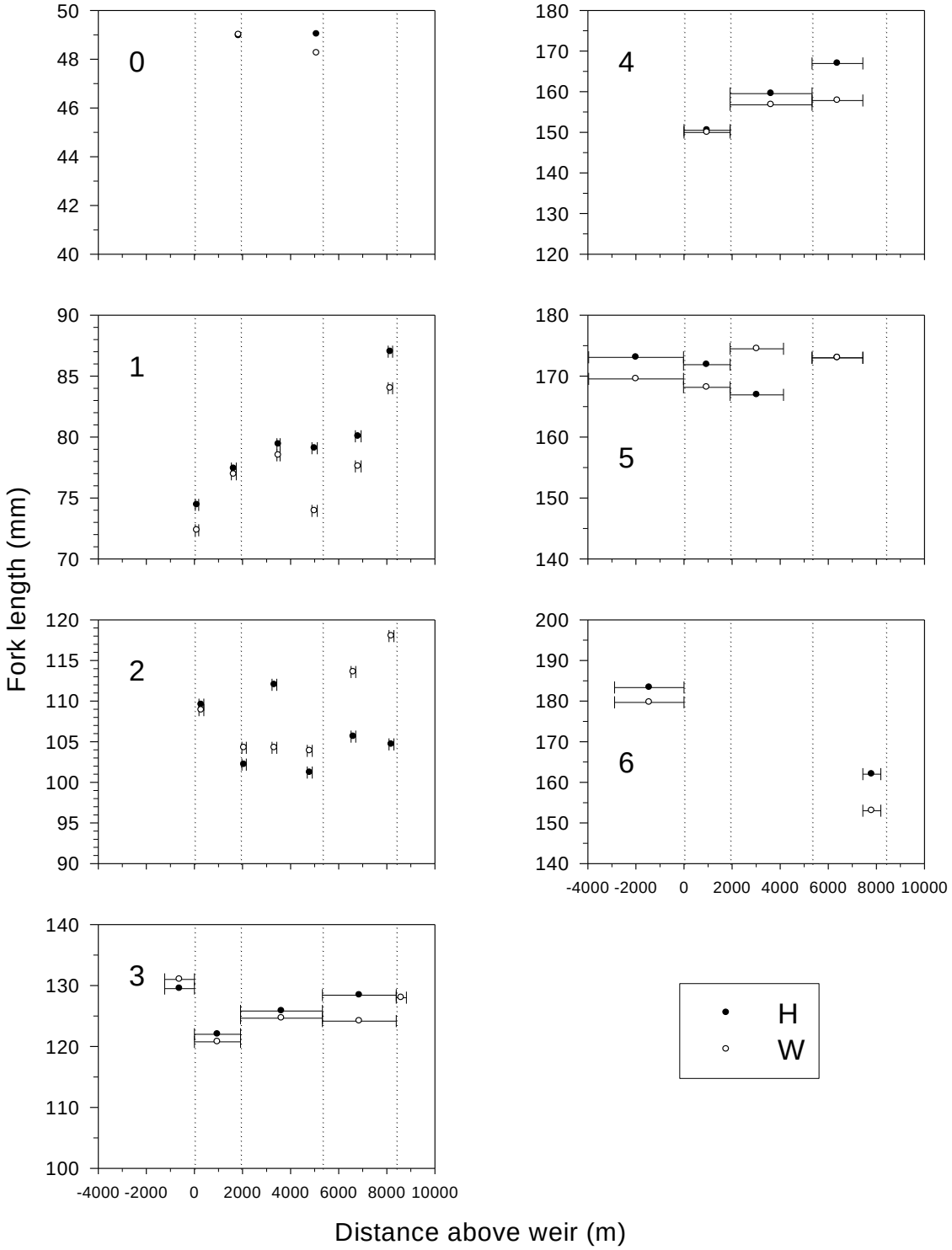

Figure 13.

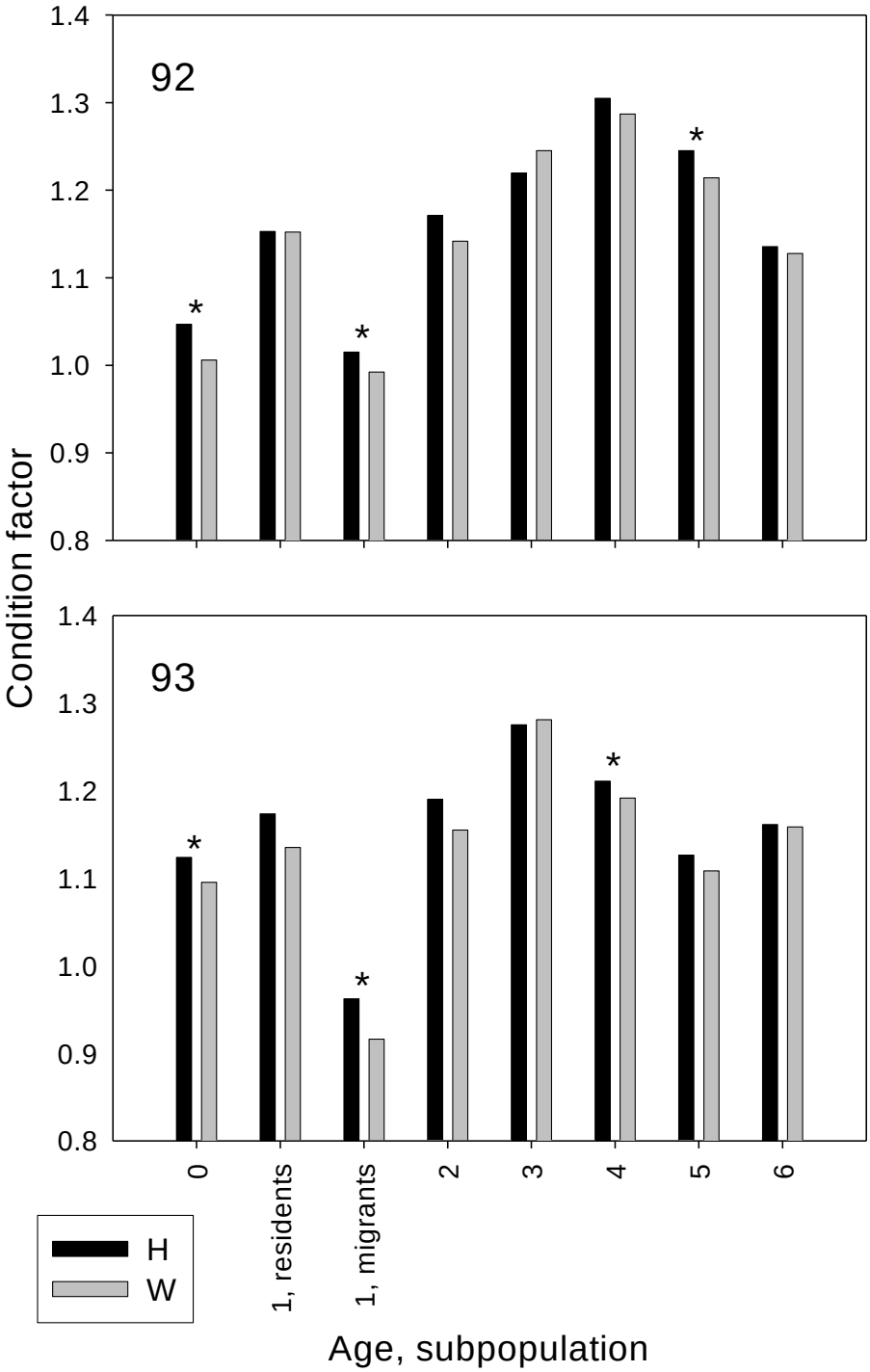
92
93
Condition factor
1.4
1.3
1.2
1.1
1.0
0.9
0.8
*
0
1, residents
1, migrants
2
3
4
5
6
H
W
Age, subpopulation

Figure 14.

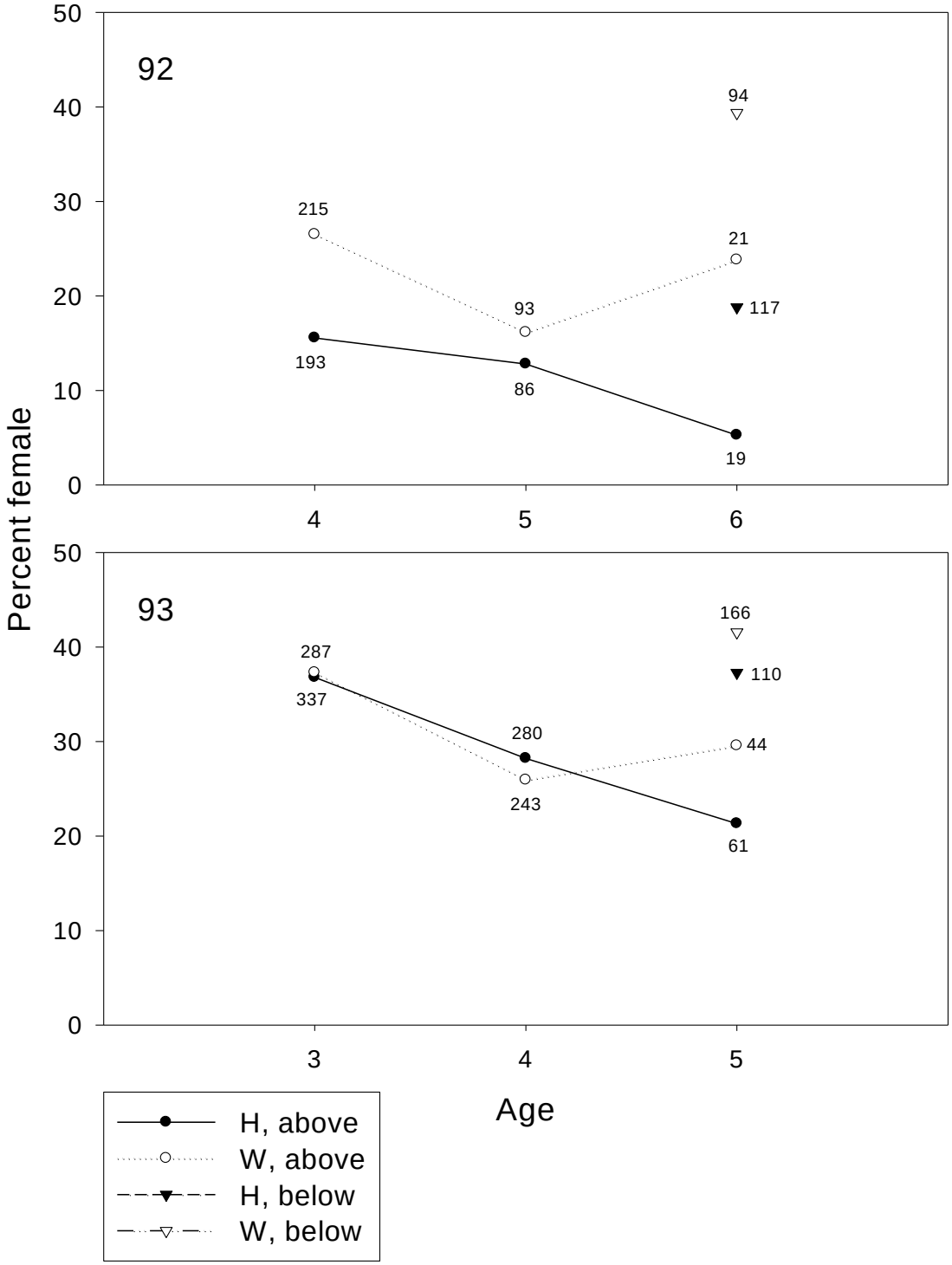
92
94
215
21
93
117
193
86
19
93
166
287
110
337
280
44
243
61
Percent female
Age
H, above
W, above
H, below
W, below

Figure 15.

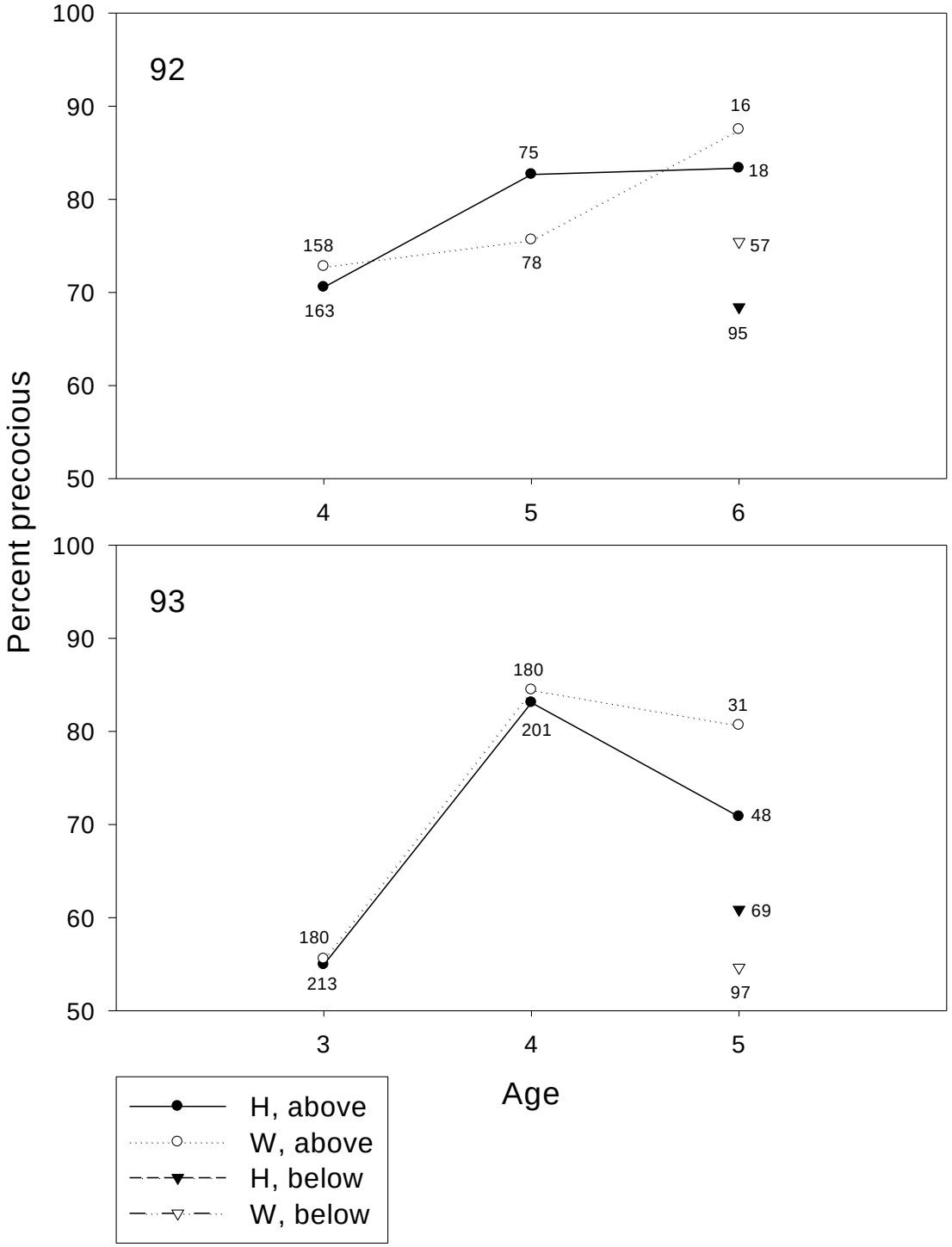
92
93
Percent precocious
Age
100
90
80
70
60
50
4
5
6
3
158
163
75
78
16
18
57
95
180
213
201
31
48
69
97
H, above
W, above
H, below
W, below

Figure 16.

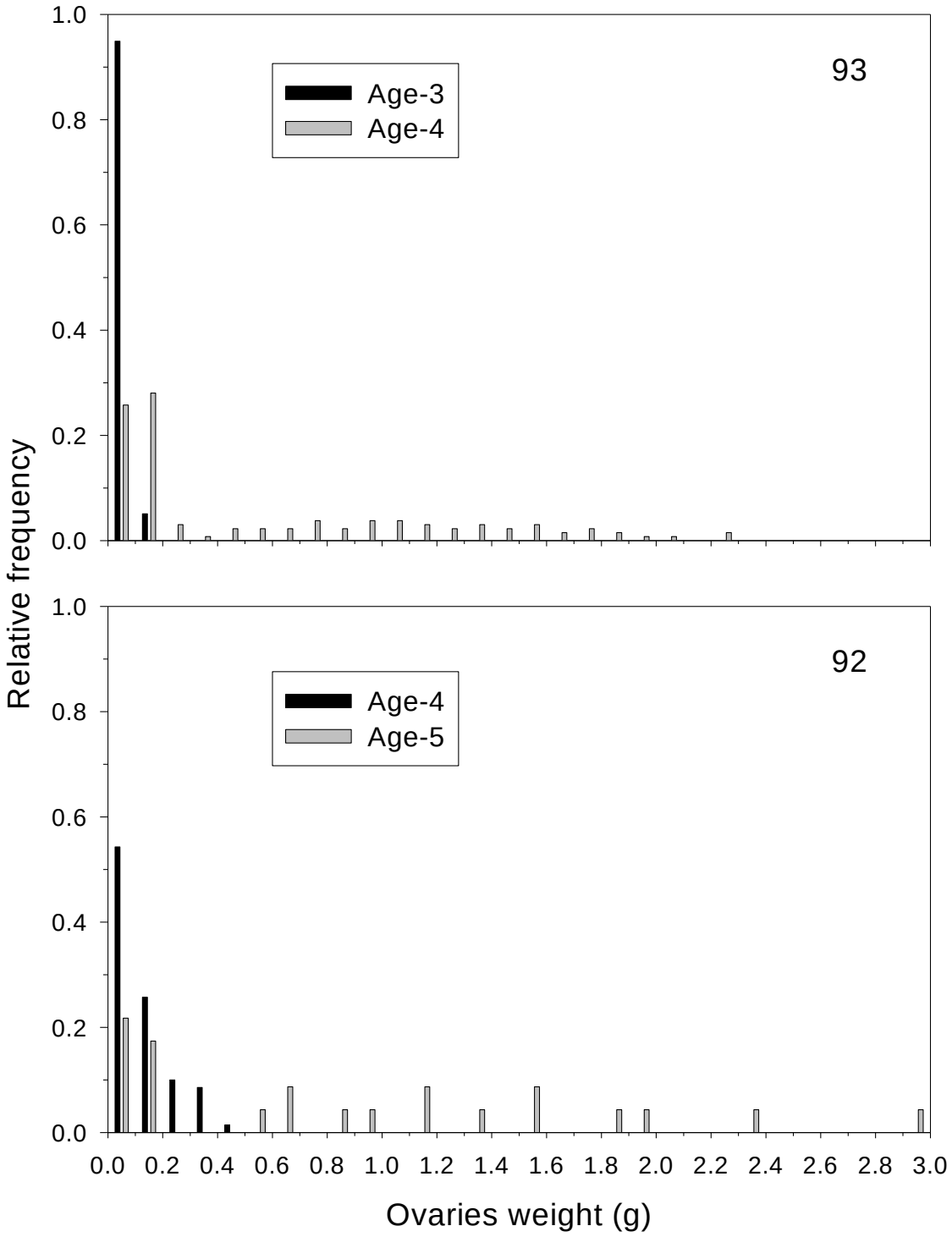
93
Age-3
Age-4
92
Age-4
Age-5
Relative frequency
Ovaries weight (g)
0.0
0.2
0.4
0.6
0.8
1.0
0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0

Figure 17.

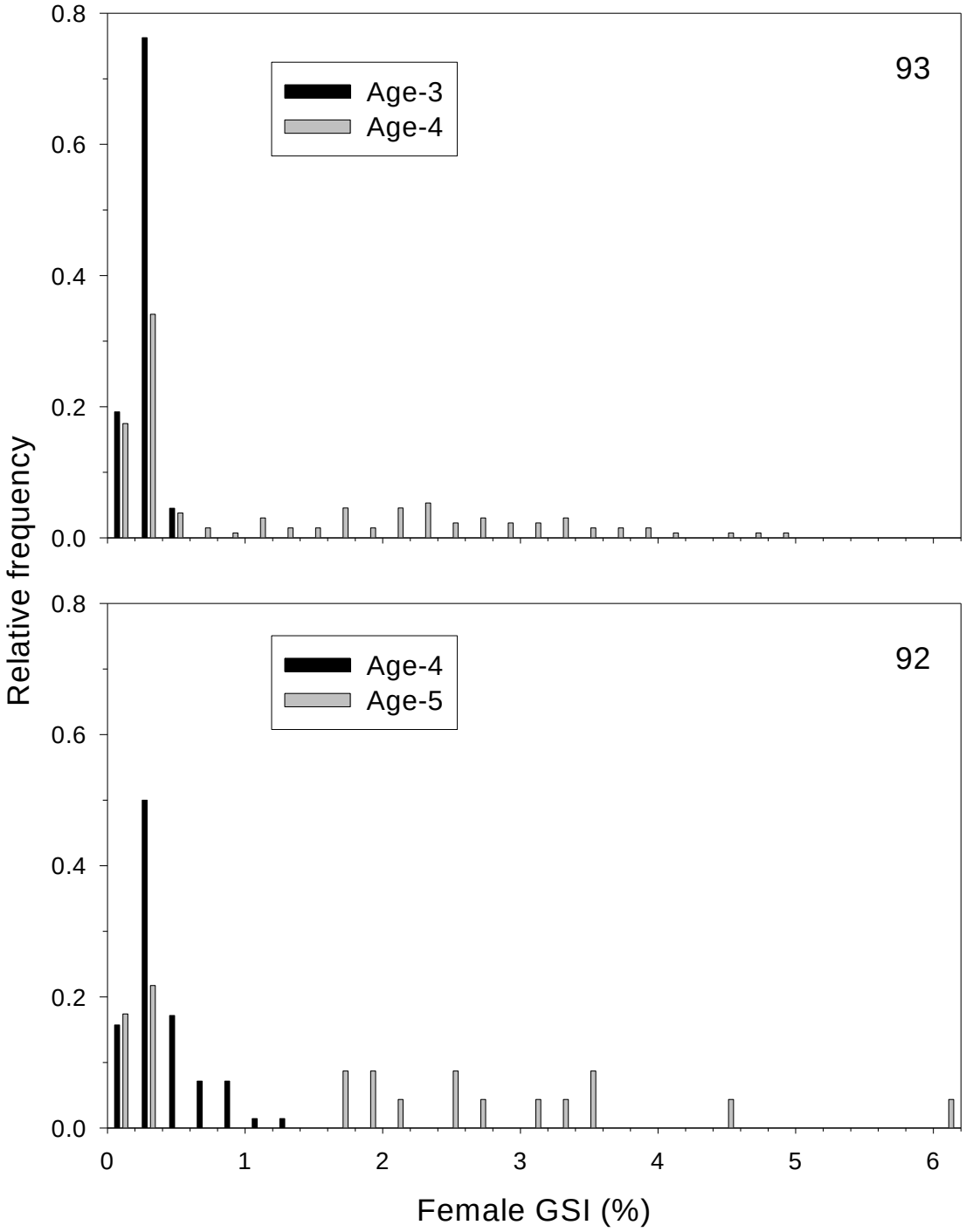
0.8
0.6
0.4
0.2
0.0
Age-3
Age-4
93
Relative frequency
0.8
0.6
0.4
0.2
0.0
Age-4
Age-5
92
0
1
2
3
4
5
6
Female GSI (%)

Figure 18.

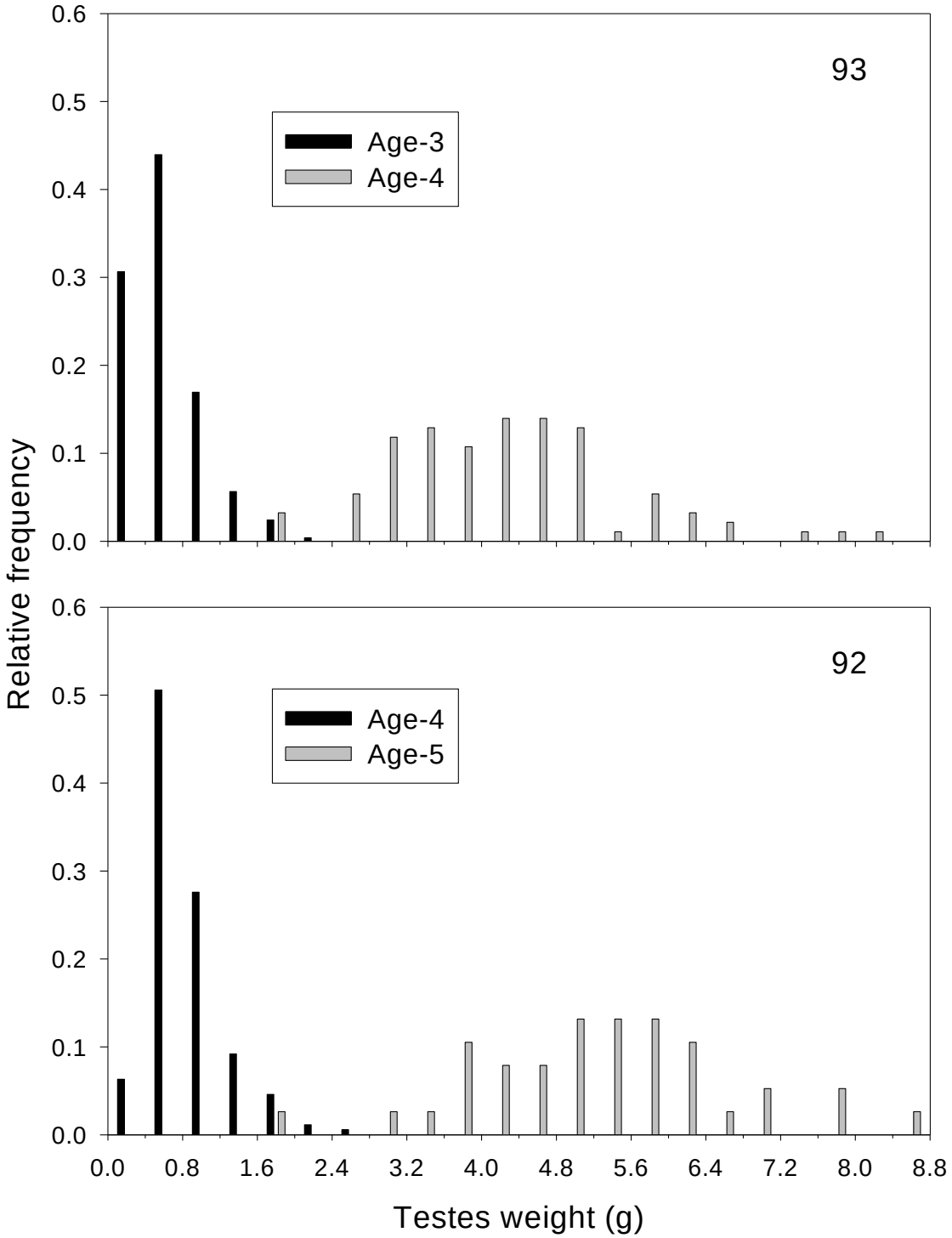
93
Age-3
Age-4
92
Age-4
Age-5
Relative frequency
Testes weight (g)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.0
0.8
1.6
2.4
3.2
4.0
4.8
5.6
6.4
7.2
8.0
8.8

Figure 19.

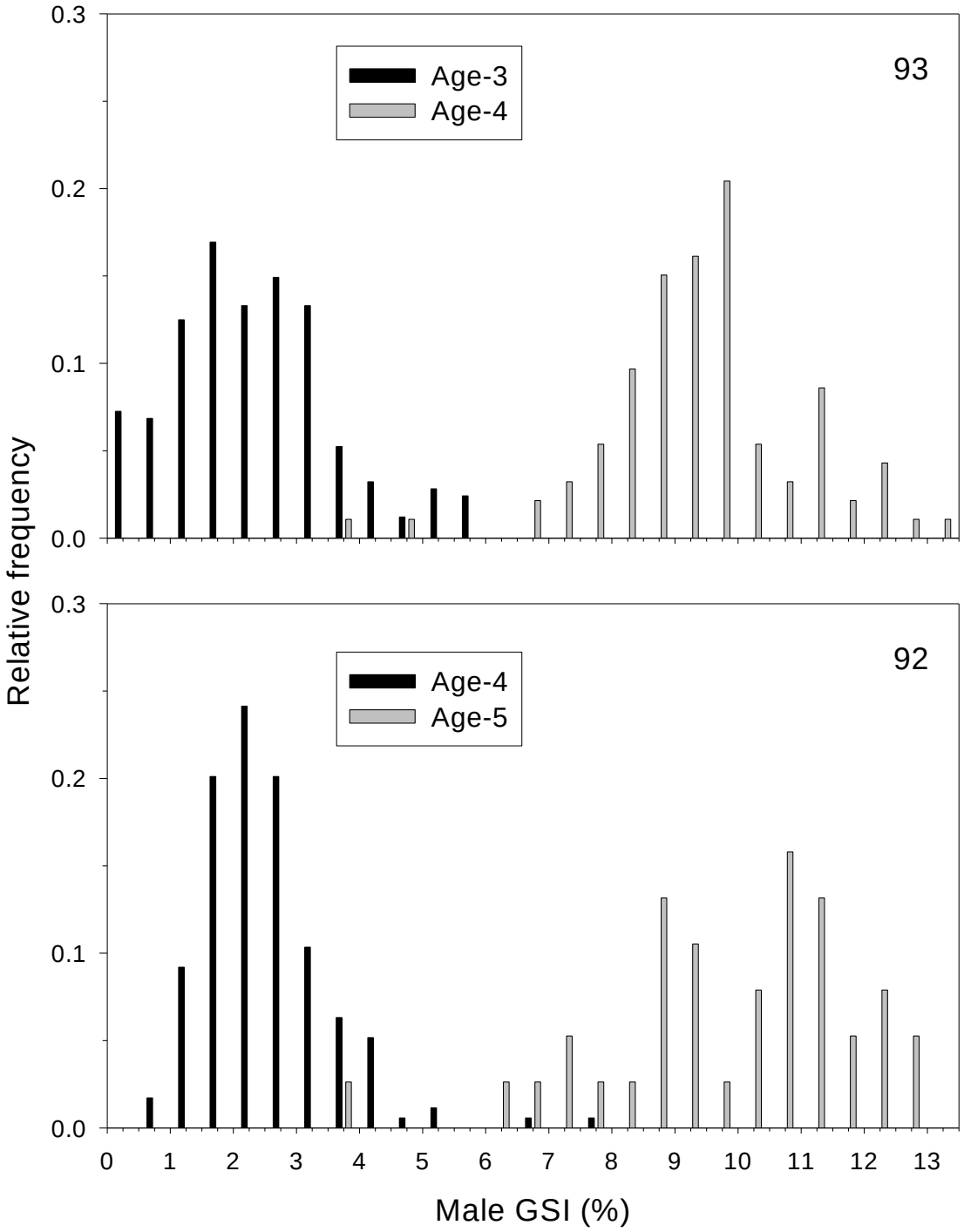
0.3
0.2
0.1
0.0
Age-3
Age-4
93
Relative frequency
0.3
0.2
0.1
0.0
Age-4
Age-5
92
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Male GSI (%)

Figure 20.

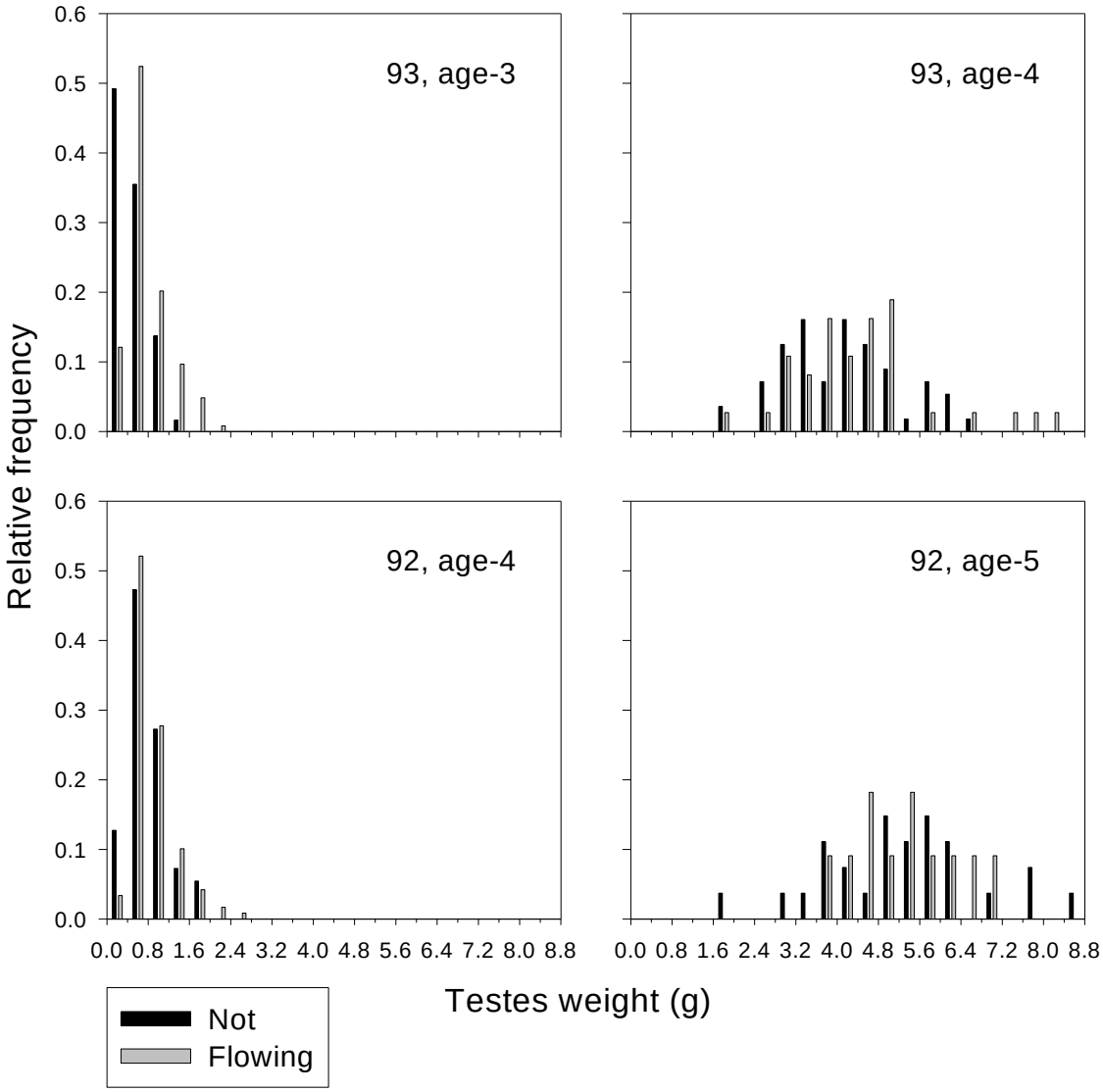
93, age-3
93, age-4
92, age-4
92, age-5
Relative frequency
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.0 0.8 1.6 2.4 3.2 4.0 4.8 5.6 6.4 7.2 8.0 8.8
Testes weight (g)
Not
Flowing

Figure 21.

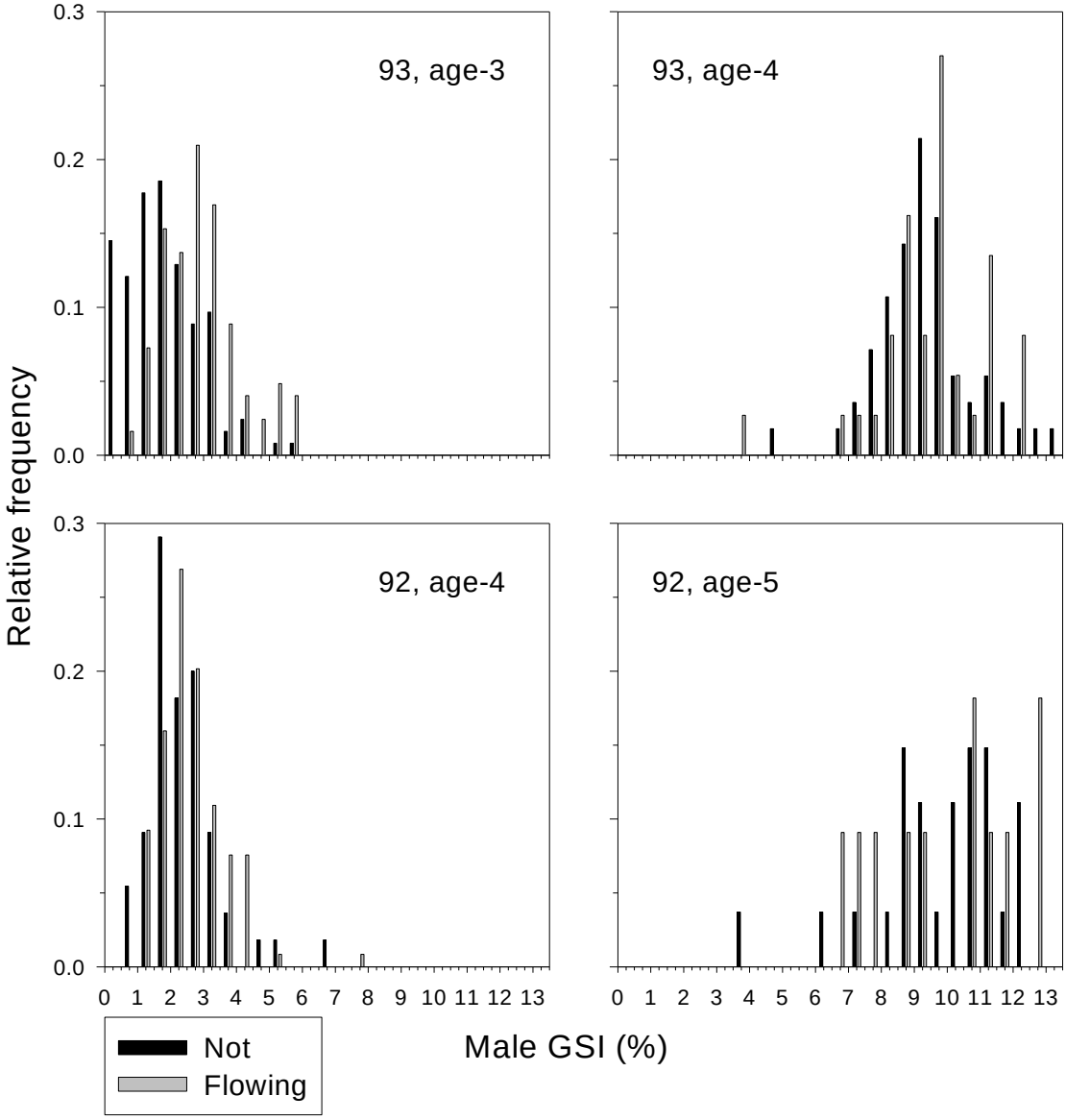
93, age-3
93, age-4
92, age-4
92, age-5
Relative frequency
0.0
0.1
0.2
0.3
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Male GSI (%)
Not
Flowing

Figure 22.

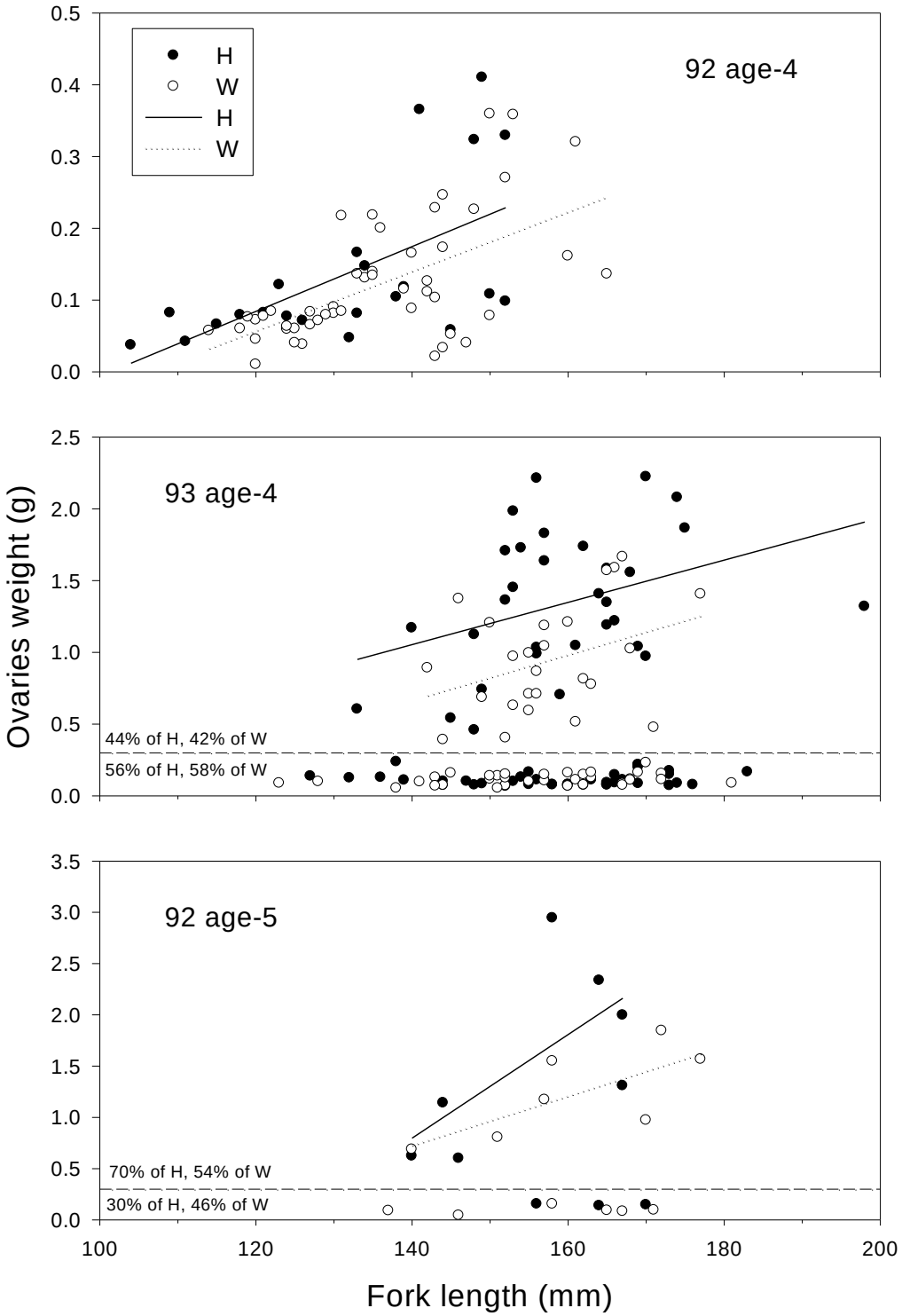
H
W
H
W
92 age-4
93 age-4
92 age-5
44% of H, 42% of W
56% of H, 58% of W
70% of H, 54% of W
30% of H, 46% of W
Ovaries weight (g)
Fork length (mm)
0.0
0.1
0.2
0.3
0.4
0.5
0.5
1.0
1.5
2.0
2.5
0.5
1.0
1.5
2.0
2.5
3.0
3.5
100
120
140
160
180
200

Figure 23.

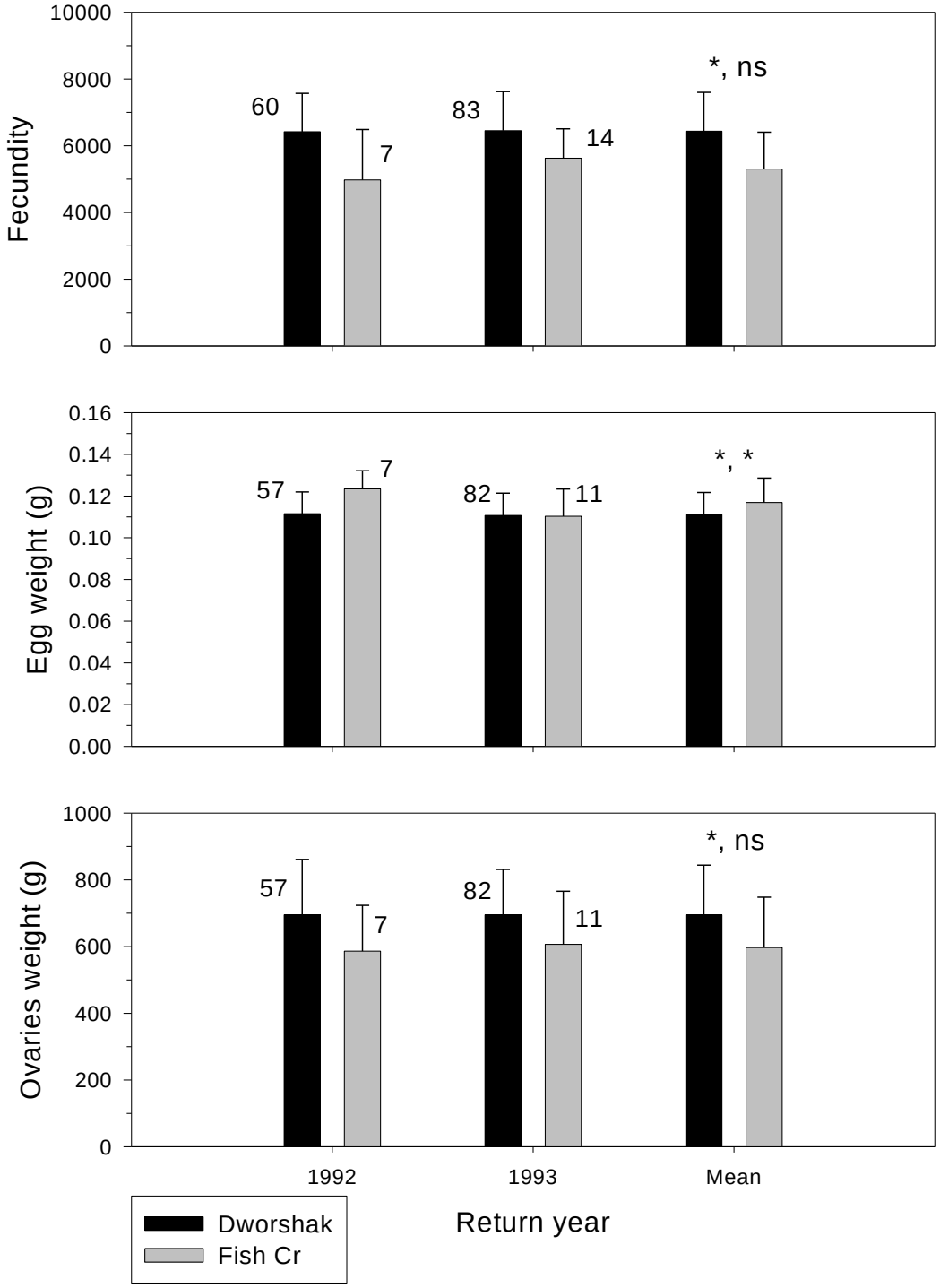
Fecundity
10000
8000
6000
4000
2000
0
60
7
83
14
*, ns
Egg weight (g)
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
57
7
82
11
*, *
Ovaries weight (g)
1000
800
600
400
200
0
57
7
82
11
*, ns
1992
1993
Mean
Return year
Dworshak
Fish Cr

Figure A1.

Chapter 2: Genetic differences between hatchery and wild steelhead for growth and survival in the hatchery and seaward migration after release *(Study sites: Dworshak Hatchery and Clearwater Hatchery; Stocks: Dworshak hatchery and Selway River wild; Year classes: 1994 and 1995)*

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Abstract

Various studies suggest that sea ranching of anadromous salmonids can result in domestication (increased fitness in the hatchery program) and a loss of fitness for natural production; however, the mechanism has not been characterized adequately. We artificially spawned hatchery and wild steelhead *Oncorhynchus mykiss* from the Clearwater River, Idaho, reared the resulting genetically marked (at the *PEPA* allozyme locus) progeny (HxH, HxW from hatchery females and wild males, and WxW) in hatcheries, and tested for differences in survival, growth, early maturation, downstream migration, and adult returns. Rearing treatments were mixed (crosses reared together) and separate (crosses reared separately from each other) at the hatchery of origin for the hatchery population where smolts are produced in one year, and at a nearby hatchery employing lower rations, lower winter temperatures, and two years of rearing to more closely mimic the natural life history (natural smolt age = 2-4 years). The hatchery population had been artificially propagated for six generations at the onset of our study. We found little or no difference in survival in the hatchery but substantially higher rates of growth and subsequent downstream migration for HxH than for WxW fish. Faster growth for HxH fish resulted in greater size at release which contributed to their higher migration rate, but other as yet uncharacterized traits also affected migration since the migration difference between crosses was apparent even within size classes. Growth of WxW fish was slower in the mixed than in the separate treatment indicating that WxW fish were competitively inferior to HxH fish in the hatchery environment. Incidence of precocious males was higher for WxW than for HxH fish in the separate but not in the mixed treatment. Incidence of HxH precocious males was similar between treatments. Apparently, the presence of HxH fish suppressed high incidence of early maturation by WxW males. A direct effect beyond the suppression of WxW growth by HxH fish was involved because the effect persisted within size categories. In-hatchery survival and growth of WxW relative to HxH fish may have been better with two-year rearing than in the standard one-year program (differences were consistent but small and non-significant); however, performance remained substantially worse for progeny of wild fish. Greater downstream migration for HxH than for WxW fish was primarily due to greater residualization for WxW than for HxH fish near the smolt release site rather than to immediate differential mortality. By August the residuals had lost condition compared to their condition in the hatchery the previous March, a month before release, and the residuals produced almost no smolts the following spring. Adult return rate was higher for HxH than for WxW fish for one year-class, consistent with the difference in downstream migration; only three adults (all WxW) returned from the other. Intermediate performance by HxW fish on growth, early maturation, downstream migration, and adult returns corroborated the genetic basis of the stock differences. Natural selection after release from the hatchery favored fish that performed well in the hatchery (grew fast, didn't mature early, and excelled in other as yet uncharacterized traits) and genetically changed (domesticated) the wild population to resemble the hatchery population.

Introduction

Artificial propagation of salmon and steelhead can result in genetic divergence from wild ancestors and loss of fitness for natural rearing (Reisenbichler and Rubin 1999). This loss has been documented even in sea-ranched populations, which are released from the hatchery each generation to

rear at large in the ocean, and presumably is accompanied by increased fitness for hatchery rearing (Reisenbichler and McIntyre 1977; Kallio-Nyberg and Koljonen 1997). Domestication (natural selection for traits that are beneficial in the hatchery program) is considered to be a predominant factor in causing genetic differences between hatchery and wild populations, and apparently occurs even though most mortality for sea-ranched salmon and steelhead occurs in the natural environment after release (Reisenbichler and Rubin 1999). The presumed mechanism is low survival after release for fish that perform poorly (e.g., grow slow) in the hatchery; however, neither domestication nor the mechanism has been adequately demonstrated (Reisenbichler 2005).

We assessed domestication by testing for differences between progeny of sea-ranched (henceforth referred to as hatchery) and wild steelhead *Oncorhynchus mykiss* from the Clearwater River, Idaho (Dworshak National Fish Hatchery and Selway River, respectively; Figure 1). We evaluated survival and growth during rearing in the hatchery, downstream migration after release, and adult size and return rate. The progeny of hatchery fish and wild fish were reared together in hatcheries so that any differences in performance would result from genetic differences or possibly maternal effects (such as vertically transmitted disease, egg size, or egg quality) not environmental differences. Hybrid crosses of hatchery females and wild males were included for comparison with maternally identical crosses of hatchery females and hatchery males to assess maternal effects.

This study was designed and funded because managers considered using hatchery steelhead from Dworshak Hatchery to supplement wild steelhead populations in the Clearwater River drainage, including the Lochsa and Selway Rivers, but lacked data comparing the genetic fitness of Dworshak fish and wild fish. The Lochsa and Selway populations are believed to have had almost no introgression from hatchery fish (Rubin et al. 2012d). Differences in ancestry between the hatchery and wild populations generally were considered inconsequential because the Dworshak stock had been developed exclusively with fish from a similar stream in the same basin – the North Fork Clearwater River. Possible genetic differences caused by the hatchery program, however, were recognized as a potential problem and motivated support for this study.

Additional objectives were to test for differences in domestication between hatchery programs and for competition between progeny from hatchery and wild fish. Clearwater Hatchery is located just across the river from Dworshak Hatchery (Figure 1) but employed a different rearing strategy. Steelhead at Dworshak Hatchery were provided relatively high rations and elevated winter temperatures to produce smolts in one year. Steelhead at Clearwater Hatchery were reared for two years to more closely mimic the natural life history in the Clearwater River (where wild steelhead rear 2-4 years before migrating to sea; Byrne 2001). We reared fish at both hatcheries to assess whether the difference in performance between progeny from hatchery and wild parents would be less at Clearwater Hatchery.

Hatchery steelhead and Atlantic salmon *Salmo salar* sometimes grow faster (Reisenbichler and McIntyre 1977; Einum and Fleming 1997; Fleming and Einum 1997; Kallio-Nyberg and Koljonen 1997; Fleming et al. 2002) and behave more aggressively (Berejikian et al. 1996; Fleming and Einum 1997) than their wild counterparts in hatchery-like environments. We tested for competition by assessing whether progeny from wild fish survived or grew better in the hatchery when reared separately rather than together with progeny of hatchery fish.

An ancillary objective was to monitor the growth and emigration of juveniles that remained in the stream where they were released instead of immediately migrating downstream. Such residuals might smolt and migrate seaward in later years. We were able to compare growth and emigration of the residuals to siblings of theirs that we had released two years earlier as unfed fry to rear naturally in the same stream, thereby controlling for any habitat limitations.

Reisenbichler et al. (2004) presented some results from the 1995 year-class reared at Clearwater Hatchery. This study extends those results by (1) including a second year-class, (2) reporting on adult returns, (3) testing for differences in severity of domestication between two hatchery programs, (4) testing for competition among progeny groups reared together, and (5) evaluating growth and subsequent emigration of residuals.

Methods

Source populations

Dworshak Hatchery was built to perpetuate North Fork Clearwater River steelhead after completion of Dworshak Dam blocked access to spawning grounds. The hatchery stock was started with wild adults trapped at the dam site in 1969-1972 and had been artificially propagated for six generations (average generation time = 4 years; Burge et al. 1994) without importation of other stocks when we collected adults to initiate our study. Further description of the Dworshak Hatchery population is given in Rubin et al. (2012d).

Wild adults used in our study were captured at Selway Falls on the Selway River (Figure 1). Hatchery fish have never been released in the Selway River, and few hatchery adults stray there – e.g., none of the 73 adults that we captured at Selway Falls in 1994 and 1995 were hatchery fish.

Experimental crosses

Hatchery adults were collected during 4-13 April 1994 and 27 March-27 April 1995 when they entered Dworshak Hatchery. They were recognizable because the adipose fin is removed from all juveniles before release from the hatchery. Wild adults, identified by an intact adipose fin, were taken from the fish ladder at Selway Falls (Figure 1) with dipnets during 17-31 March 1994 and 9 March-5 April 1995. Wild adults were transported to Kamiah ponds (Figure 1) and held there until their gametes were collected and transported to Dworshak Hatchery. Hatchery and wild adults were screened for their genotypes at the cytosol non-specific dipeptidase locus (*PEPA**; Shaklee et al. 1990). Each adult was marked with a unique tag, and a small piece of fin tissue was excised, frozen on dry ice, and analyzed electrophoretically following Aebersold et al. (1987). Alleles at the *PEPA** locus were designated ***100* and ***110*, reflecting their relative electrophoretic mobilities. Hatchery (H) adults homozygous for the ***110* allele and wild (W) adults homozygous for the ***100* allele were spawned to create three genetically marked progeny groups: HxH = ***110*/***110*, HxW (1995 year-class only) = ***100*/***110*, and WxW = ***100*/***100*. The genetic marks allowed us to identify cross for individuals taken from groups of fish that had been mixed as fry.

Spawning took place during April-May (Table 1). Gametes were stripped and held in containers cooled with ice until fertilization (<8 hours). Males were individually tagged and returned to holding ponds to provide milt on subsequent spawning days. In 1994 the eggs from each female were divided into two equal portions and each portion was fertilized with milt from a different male. In 1995 the procedure remained the same for WxW crosses; however, HxH and HxW crosses were made by fertilizing half of the eggs from each hatchery female with milt from a hatchery male and the other half with milt from a wild male. Thus maternal contribution was identical between the HxH and HxW groups. Paternal contribution was similar but not identical between HxW and WxW crosses because not every wild male contributed equally to those groups (Table 2). Samples of unfertilized eggs were preserved in 10% buffered formalin and weighed $\geq$90 days later to determine mean egg weight for each female.

We measured fork length for each adult and removed the otoliths from wild adults to determine freshwater and saltwater age (Figure 2). Historical length frequencies of known-age adults compiled by hatchery personnel were used to estimate the age of our hatchery adults.

The fertilized eggs from each female were incubated in a separate tray in vertical incubators at Dworshak Hatchery. A divider in the middle of each tray separated the two full-sib families. We established two (1994) or three (1995) groups of embryos based on spawning date (Table 1). Temperatures during early incubation (before eye-up) were manipulated to synchronize stage of development among embryos from different spawning dates within each group. Embryos from later spawning dates within a group were held for more days at 12°C and fewer days at 7°C than those from early dates. Mean temperature was nearly identical for the different crosses (Table 3).

When the embryos reached the eyed stage, dead individuals were counted and removed. We sampled each full-sib family proportionately and selected an equal number of eyed embryos from each cross (HxH; HxW; WxW) within each of the two or three groups with matched development (Table 1). These embryos were returned to trays for further incubation. Crosses were placed in separate trays but families were mixed within crosses. The number of embryos per tray was $\leq$4,200 and was identical among crosses within each group. Embryos for Clearwater Hatchery were transferred there before hatching. Dead embryos were periodically counted and removed from trays at both hatcheries. Mortality between the eyed-egg and button-up fry stages was $\leq$ 2% for each cross at each hatchery in both years. Temperature between fertilization and button-up remained within 5.9^{o}-13.4^{o}C.

Spawning in 1995 was more protracted than in 1994 and temperature manipulation wasn't possible at Clearwater Hatchery so we formed two sets of embryos to minimize differences in developmental stage within rearing vessels. Set I included all embryos from developmental group 1 and a third of the embryos from each family in group 2; set II included the remaining embryos from group 2 and all embryos from group 3. Set I was eventually placed in one of the replicate tanks for each treatment and set II in the other. Family composition was equalized among vessels for the 1994 year-class at Clearwater Hatchery and the only year-class (1995) reared past the eyed-stage at Dworshak Hatchery. We manipulated temperatures during and after the eyed stage at Dworshak Hatchery to achieve nearly simultaneous button-up, thereby eliminating the need for sets. Neither the HxW cross nor the 1994 year-class were reared at Dworshak Hatchery.

The experimental fish were transferred to rearing vessels at Clearwater and Dworshak hatcheries as button-up fry. Treatments and replicates were randomly assigned to vessels. One treatment was "mixed" – crosses were reared together in the same vessel. The other treatment was "separate" – each cross was reared separately. We used two vessels for the mixed treatment and two for each cross within the separate treatment at each hatchery. Fry were sampled for length and weight when they were transferred to rearing vessels. At least 20 fry were measured for fork length and a sample of $\geq$100 fry was weighed from each development group within each cross. Weighted means of length and weight were computed for each cross using the number of fry in each development group as the weighting factors. In 1994, the first developmental group was moved from incubators to vessels on 14 June; the second on 16 June. In 1995 at Clearwater Hatchery the first group was moved on 12 June and the second and third on 18 June; at Dworshak Hatchery all three groups were moved on 19 June (Table 4).

Survival and growth in the hatchery

Fish at Clearwater Hatchery were reared according to the hatchery's standard program. Mean monthly temperature ranged from 3-5°C during January-March to 12-14°C during July-September. At button-up the fry were moved from the incubator to indoor vats measuring 12.2 m x 1.2 m with 0.9 m water depth and a flow of 0.007 m^3/s. Automatic feeders dispensed the food. Mortalities were recorded and removed daily. Mean weight was determined in most months; ration was adjusted each month to accommodate increased fish size.

The 1994 year-class was counted, given a left ventral clip to identify them as our experimental fish when they returned as adults, and moved outside into raceways (15.2 m x 3.0 m with 1.2 m depth and 0.04 m^3/s flow) in September 1995. The raceways were screened to prevent avian predation. The fish were transported to acclimation ponds along Crooked River on 1 April 1996 (Figure 1), and released into Crooked River on 15 April 1996. The 1995 year-class was counted, given a right ventral clip, moved outside in June 1996, transported to the acclimation ponds on 7-8 April 1997, and released into Crooked River on 22 April 1997.

The replicates (and treatments) were reared separately until they were mixed in the acclimation ponds. We sampled the fish as yearlings when they were moved from vats to raceways and again in March of their second year of rearing, shortly before transport to Crooked River for release. Samples were collected with dipnets after crowding fish to one end of the rearing vessel. Each fish in the sample was measured and weighed; a small piece of fin tissue from each fish from the mixed vessels was assayed for *PEPA** to determine cross. Each fish in the March sample was injected with a uniquely coded passive integrated transponder (PIT) tag (Prentice et al. 1990) and examined for milt expression (i.e., free-flowing milt) by gently squeezing the abdomen. The 1995 year-class was sampled twice in March, once in mid-March for PIT-tagging and a second time two weeks later for lengths, weights, and fin tissue only.

The experimental fish kept at Dworshak Hatchery were reared according to the hatchery's standard program except that we used circular tanks rather than raceways because of small numbers of fish (Table 4). Fry were initially held indoors – tank diameter = 0.56 m; depth = 0.36 m; flow = 7 l/min. In September the fish were counted and moved outside to 1.22 m diameter tanks (depth = 0.61 m, flow = 23 l/min). This sequence mimicked the standard procedure of ponding fry in rectangular tanks indoors and then moving them outside into Burrows ponds in late summer. A water re-use system supplied heated water to most production steelhead during the winter. Our outside tanks received re-use water so mean monthly temperature ranged from 10-12°C during October-March. Temperature during July-September was 11-13 ^{o}C.

Ration was adjusted at 1-3 week intervals after samples of fish were counted and weighed as a group to determine mean weight. All fish in each tank were counted and weighed in mid September when they were moved outside. Food was dispensed by automatic feeders and mortalities were removed daily as at Clearwater Hatchery. Live fish were removed from some tanks on two occasions to equalize densities among tanks -- once, within a week of ponding, when 20 fish escaped from one of the tanks, and a second time in January. In late September, shortly after the fish were moved outside, all of the fish in one of the two separate HxH tanks died suddenly of an unknown cause. There were no deaths in any of the other tanks at that time nor did the fish in the other tanks appear stressed.

In mid-February, all fish were measured, weighed, PIT-tagged, and their left ventral fins clipped. Fin tissue from fish in the mixed treatment was analyzed for *PEPA** to determine cross. In early April all fish were measured, weighed, examined for milt expression, and their PIT-tag codes recorded. On 15 April 1996 the fish were transported and released into Crooked River near the acclimation ponds.

Survival in vessels at Clearwater Hatchery was computed by subtracting the number of mortalities between marking and release from the number of yearlings marked, and dividing that quantity by the number of fry ponded. Survival in vessels at Dworshak Hatchery was computed by adding the number of fish removed for density equalization in January to the number of fish alive in April, and then dividing by the number of fry ponded minus the number removed for density equalization a week later. In mixed vessels, survival of the HxH cross relative to the WxW cross ($RS_{HH/WW}$) was estimated from samples of fish as:

$$RS_{HH/WW} = (P_{HH2}*P_{WW1})/(P_{HH1}*P_{WW2});$$

P_{HH1} = proportion of HxH fish at ponding, P_{HH2} = proportion of HxH fish in the sample, P_{WW1} = proportion of WxW fish at ponding, and P_{WW2} = proportion of WxW fish in the sample (Chilcote et al.1986). $RS_{HW/WW}$ was computed similarly. Relative survival in separate treatments was computed as mean survival for HxH (or HxW) divided by mean survival for WxW.

Differences in ration (g food per fish per d) among vessels, when less than satiation levels, could cause growth differences. Ration to be delivered to each vessel was computed periodically (see above) at both hatcheries. We estimated the maximum ration that could be consumed (i.e., satiation level) for each of these periods using equation 4 of Elliott (1975; for brown trout) and the food's moisture content (manufacturer's specification). We computed the ratio of actual ration to maximum ration for each period and weighted these values by the number of days in the period to derive a mean ratio for the entire time in the hatchery. We calculated an index of relative ration for each vessel each month to account for differences in ration among vessels that received less than satiation levels:

Ration index = $(r_{ij} - r_j)/r_j$;

r_{ij} = ration in the *i*th vessel in the *j*th month, r_j = ration averaged across all vessels in the *j*th month. Ration index for the entire rearing period was obtained by weighting the indices for each month by the number of days in the month.

Growth of HxH relative to WxW fish ($RG_{HH/WW}$) was computed as:

$RG_{HH/WW} = (L_{HH2}-L_{HH1})/(L_{WW2}-L_{WW1})$;

L_{HH1}, L_{HH2}, L_{WW1}, and L_{WW2} = mean length of HxH fry at ponding, HxH juveniles in the final pre-release sample, WxW fry at ponding, and WxW juveniles in the final sample, respectively. $RG_{HH/WW}$ was used for comparisons between Clearwater and Dworshak hatcheries. The validity of the comparison depends on growth in length being linear with respect to time at each hatchery.

Downstream migration

Detections of PIT-tagged fish at six downstream dams on the Snake and Columbia rivers were recorded by the PIT Tag Information System (Martinson et al. 1997). Detection rate was computed as the number of fish detected at least once at any dam divided by the number of tagged fish released. We estimated survival from release to successive Snake River dams and detection efficiency at each dam using formulae of Smith et al. (1994). Data were pooled across treatments for estimating survival and detection efficiency. Estimates of survival counted live fish that did not emigrate ("residuals") as not surviving. Detection efficiency was the proportion of fish passing a dam that were detected there. Survival and detection efficiency were estimated for three river segments: Crooked River (release site) to Lower Granite Dam (LGR; segment length=280 km), LGR to Little Goose Dam (LGO; 60 km), and LGO to Lower Monumental Dam (LMN; 46 km).

Crooked River

Historical anadromous fish runs were eliminated from Crooked River by construction of Harpster Dam in 1927 near the mouth of the South Fork Clearwater River (Kiefer and Lockhart 1999). Hatchery origin steelhead and spring Chinook salmon *Oncorhynchus tshawytscha* were introduced following dam removal in 1962 and currently use Crooked River for spawning and juvenile rearing. Elevation at the mouth is 1,140 m. The acclimation ponds were located near river km 15. Mean gradient between the mouth and the ponds is 1.1%. Mean wetted width between the mouth and the ponds in summer 1995 was 12 m, and flows measured from 1991 through 1994 ranged from 0.3 to 6.9 m^3/s (Kiefer and Lockhart

1999). Mean monthly temperatures during October 1995-October 1996 ranged from 0.1^{o}C in January to 12.1^{o}C in July.

We released 19,000 each of HxH and WxW unfed button-up fry throughout the lower 16 kilometers of Crooked River on 15 June 1994, two years prior to the first release of hatchery-reared juveniles. The unfed fry were identical in family composition to the experimental fry ponded at Clearwater Hatchery in 1994. We thermally marked the otoliths of the former when they were embryos (Volk et al. 1990) so that we could distinguish our experimental fish from naturally produced *O. mykiss*.

Idaho Department of Fish and Game operated a floating scoop trap at river km 0.2 during April-mid June of 1996 and 1997 to capture juvenile outmigrants (Kiefer and Lockhart 1999). Trapped fish, including our hatchery-reared juveniles released in those years (Table 5), were scanned for PIT-tags and released. In 1996, 300 untagged hatchery-reared juveniles, identified by left ventral fin clip, were PIT-tagged when captured at the trap but were not assayed for cross. We included these juveniles when estimating the overall proportion of fish that did not leave Crooked River (see below).

The proportion of experimental fish that did not leave Crooked River was estimated as:

$1-(R_{12}*M_2/(M_1*(R_{22}+1))$;

M_1=number of fish tagged at the hatchery; M_2=number of tagged fish detected at the trap; R_{12}=dam detections of fish tagged at the hatchery; and R_{22}=dam detections of fish detected at the trap (expression 5.3 from Ricker 1975; variance given in expression 5.2). This non-migrant proportion was estimated by cross and overall; the number of fish tagged at the trap was added to M_2, and dam detections of those fish were added to R_{22}, for the 1994 year-class overall estimate (Table 5). The proportion of juveniles that left Crooked River but didn't arrive at LGR was computed as one minus the proportion that did not emigrate from Crooked River minus the proportion that arrived at LGR (Table 5).

Juvenile steelhead were captured by angling, backpack electrofishing, and seining from sites in the lower 16 km of Crooked River in late August 1996. Each fish was measured, weighed, examined for fin clips, and scanned for PIT tags. Untagged hatchery-reared fish (identifiable by fin clips) were PIT-tagged and sampled for fin tissue to determine cross. Unmarked fish were PIT-tagged and sampled for fin tissue and scales. All fish were released back into Crooked River. Scales were subsequently aged for about 20 unmarked fish from each 10 mm length class between 111 and 180 mm. Previous work indicated that most age-2 fish fell within this length range. Similar methods had been used to sample fish from Crooked River during August 1995, except that 200 unmarked fish were sacrificed and their otoliths extracted and processed to determine which of them were from our 1994 fry release. Of the 200 fish, 144 were yearlings, and 86% of the yearlings were from the fry release.

A freezer malfunction precluded assays for the *PEPA** genotypes of unmarked fish captured in 1996. We knew which of the fish that we had aged were two-year olds. Based on otolith analysis of yearlings captured in 1995, we knew that most of the two-year olds were from our fry release, but we did not know which of the two-year olds were HxH or WxW. We will refer to age-2 unmarked fish captured in 1996 as belonging to the "naturally reared" group.

Adult returns

Idaho Department of Fish and Game operated a picket weir in lower Crooked River, 20 m above the scoop trap, to catch all upstream migrating adults during March-June of 1998-2001. Marked adults from our experiment (left or right ventral clip, intact adipose fin) were measured, sexed, sampled for fin tissue to determine cross, and passed above the weir. All adults returning to Dworshak Hatchery with our identifying marks during spring 2000 and 2001 were sampled as at Crooked River and then hauled and

released into Crooked River above the picket weir. Adults were unable to access Clearwater Hatchery because it lacks a fish ladder – all smolts are released offsite; embryos are obtained from offsite adult collection facilities or Dworshak Hatchery each year. Most of our experimental adults released above the weir were recovered again in Crooked River as spawned out carcasses. Otoliths were extracted to determine saltwater age and confirm identity as an experimental fish by whether it had reared for two years in a hatchery. Fry-to-adult survival of HxH or HxW fish relative to WxW fish was computed using the formula given above for relative survival in the hatchery.

Statistical analyses

Statistical tests were conducted separately for the 1994 year-class at Clearwater Hatchery, the 1995 year-class at Clearwater Hatchery, and the 1995 year-class at Dworshak Hatchery because the experiments differed between year-classes and hatcheries. The HxW cross was included only for the 1995 year-class at Clearwater Hatchery, and only the 1995 year-class was reared at Dworshak Hatchery. For tests involving three crosses (1995 year-class at Clearwater), two paired comparisons were made – HxH versus WxW and HxW versus HxH – using the sequential Bonferroni method to determine significance (Sokal and Rohlf 1995).

Survival in the hatchery for mixed treatments was tested with *G*-tests for goodness of fit (Sokal and Rohlf 1995). The expected frequency for each vessel was the proportion of each cross from the original number of fry ponded (Table 4). Differences in the relative frequency of the crosses between age-1 and age-2 samples from a vessel at Clearwater Hatchery were tested with *G*-tests of independence (Sokal and Rohlf 1995). Exact *P*-values of goodness of fit and independence tests were obtained from StatXact-3 (Cytel Software Corporation 1997) when required by small sample size. Detection rate of (PIT-tagged) fish from mixed treatments was evaluated with log-linear analyses of three-way contingency tables (Sokal and Rohlf 1995). We tested for independence among cross, vessel, and detection status (i.e., whether detected). Incidence of mature males (hereafter termed maturity), identified by flowing milt, in mixed treatments was also evaluated with log-linear analyses. Fry-to-adult survival was tested with *G*-tests for goodness of fit. Expected frequencies were the proportion of each cross from the original number of fry ponded (i.e., after summing over vessels; Table 4).

Differences among crosses for survival in the hatchery, detection rate, and sexual maturity in the separate treatment (allopatry), and for size and condition factor in both treatments were tested with analysis of variance (AOV; Table 6). AOVs were also used to test for differences in fish size between treatments within a cross (i.e., to test for competition), and for differences in the survival or growth of HxH relative to WxW fish between hatcheries (Table 6). Vessel was a random effect in the AOVs; all other factors were fixed effects. Unlike the 1994 year-class at Clearwater Hatchery and the 1995 year-class at Dworshak Hatchery, the 1995 year-class at Clearwater Hatchery had been partitioned into two sets differing in spawning date, incubation temperature, and family composition. Preliminary analyses indicated that set did not affect survival or growth; therefore it was not included as a factor in the AOVs. Condition factor before release from hatcheries was evaluated with relative condition factor (K_n):
$K_n = (W/W')$;

W is the weight of the individual and W' is the length-specific mean weight predicted from a weight-length relation fit to that population (Anderson and Neumann 1996). Separate relations were fit for each treatment within each year-class and hatchery. Unlike for fish sampled at the hatcheries, the exponent of the weight-length relation (b in equation $W=aL^b$) was consistently 3.0 for residuals and naturally reared fish in Crooked River; thus for these fish condition factor was evaluated with Fulton's condition factor (K):

$K = 10^5 * W/L^3$;

W = weight (g); L = fork length (mm).

Variation in length changed from the first to the second sampling date at the hatcheries. Whether this change differed among crosses was tested using the difference in standard deviation of length between sampling dates as the response variable (Table 6).

Logistic regression was used to test whether differences among crosses in detection rate or maturity existed independent of differences in size among crosses. Data were pooled over replicate vessels. The dependent variable was detection or maturity; class variables were cross and treatment; and the covariate was length. A model that included the two- and three-way interaction terms involving length was initially fit. Any non-significant terms ($P>0.05$) were dropped and the model was fit again. Models that didn't fit the data ($P<0.05$; Hosmer and Lemeshow goodness of fit test; SAS 2000) were rejected. Satisfactory fit required that we exclude fish from the lower end of the length distribution where rates of detection and maturity were close to zero. We excluded successively larger fish (by 10 mm length increments) until satisfactory fit ($P>0.05$) was achieved. Analysis of covariance (AOCV) was used to explore relations among cross, fish size, and travel time. The latter was defined as the time between release in Crooked River and detection at dams, considering only fish that were detected in the year of release.

Results

Eggs and fry

Egg size was greater for hatchery than for wild females in 1994 (Table 3). HxH fry were longer than WxW fry in 1994 and longer than HxW or WxW fry in 1995 (Table 3). Siblings of these 1995 year-class fry held for a different experiment showed a different pattern of length differences. HxH fry were longer than WxW fry but similar in length to HxW fry (mean fork length = 29.3, 29.2, and 28.4 mm for HxH, HxW, and WxW, respectively; n=60 per cross; the difference between HxH and WxW was significant ($P<0.05$)). Thus we cannot make the general conclusion that incubation in a common environment resulted in greater length for HxH than for HxW fry which would have implied a genetic difference in embryo development rate or yolk conversion efficiency.

Survival and growth in the hatchery

Survival to release ranged from 66-93% among vessels at Clearwater Hatchery and from 52-79% at Dworshak Hatchery (excluding the tank where all fish died; Table 4). Mortality between the first and second sampling dates for the mixed treatment (sympatry) at Clearwater Hatchery was ≤ 2% so we considered the two samples to be nearly equivalent indicators of relative survival in the hatchery (only about 4% of the fish sampled at age-2 had also been sampled previously as yearlings). Surprisingly, there was significant heterogeneity between the first and second samples in two of the four mixed treatment vessels (vessel 1, 1994 year-class and vessel 1, 1995 year-class; $P<0.05$). Further, both mixed vessels of the 1995 year-class showed higher survival for HxH than for WxW on the first date but no survival difference on the second (Table 4). Inconsistency between samples prevents us from drawing conclusions about relative survival for the mixed treatment at Clearwater Hatchery. At Dworshak Hatchery all of the fish in each tank were sampled on each sampling date so we used the second sample, collected in April shortly before release, to evaluate survival. We found significantly higher survival for HxH than for WxW fish (Table 4); HxW fish were not included at this hatchery.

Survival in the separate treatment (allopatry) was similar between HxH and WxW fish of the 1994 year-class but was higher for HxH than for WxW fish of the 1995 year-class at Clearwater Hatchery ($P<0.05$) and may have been higher at Dworshak Hatchery ($0.05<P<0.10$; Table 4). The power of the latter test was compromised because one of the two HxH replicates was lost in late September when all of

the fish died from an unknown cause. Survival to mid September, when HxH fish were doing well in both tanks, was significantly higher for HxH fish than for WxW fish (relative survival was 78%/60% = 1.3; $P = 0.039$). The survival of HxW fish (1995 year-class at Clearwater Hatchery) was intermediate; the difference between HxH and HxW fish was marginally significant ($0.05<P<0.10$; Table 4).

Ration always exceeded satiation levels at Dworshak Hatchery but was frequently below satiation at Clearwater Hatchery (Table 7). We evaluated whether differences in ration among vessels could have caused differences in growth at Clearwater Hatchery. Ration index ranged from –0.09 to 0.05 among all vessels at Clearwater Hatchery except for one containing WxW fish from the 1995 year-class in the separate treatment (Figure 3). Within the restricted range, differences in ration among vessels appeared not to affect final fish size (Figure 3). The exceptional vessel (containing WxW) received a ration 16% above average, and the fish grew larger than those in the other vessel containing the same year-class of WxW fish (Figure 3). We believe that the difference in growth between this pair of vessels primarily resulted from the large difference in ration so we excluded the "high ration" WxW vessel (vessel 8 in Table 4) from statistical analyses of fish size.

Mean length on the second sampling date, shortly before release, was greater for HxH fish than for WxW fish in both treatments for both year-classes at both hatcheries (Table 4). Mean length of HxW fish was intermediate and significantly less than that of HxH fish in the mixed treatment but similar to that of HxH fish in the separate treatment (Table 4). Differences in weight among crosses were similar to those for length and are not reported here.

The length of WxW fish was less in mixed treatments than in separate treatments – the difference was statistically significant for the second sample of the 1994 year-class at Clearwater Hatchery and for both samples of the 1995 year-class at Dworshak Hatchery, and the pattern was consistent for the other samples (Table 4). In contrast, the length of HxH fish was significantly greater in mixed treatments than in separate treatments for both year-classes at Clearwater Hatchery (Table 4). No such difference between mixed and separate treatments was observed for HxH fish at Dworshak Hatchery, perhaps because food was always provided in excess.

Variability (standard deviation) in length increased more from the first to the second sampling date for WxW fish than for HxH fish, and by an intermediate amount for HxW fish (Table 4; Figure 4). For the 1994 year-class (at Clearwater Hatchery), the increase was significantly greater ($P<0.05$) for WxW than for HxH fish in both treatments, and for the 1995 year-class it was significant in the mixed treatment at Clearwater Hatchery and in the separate treatment at Dworshak Hatchery. These differences resulted in greater variability in length for WxW than for HxH fish near release (i.e., on the second sampling date) at Clearwater Hatchery but not at Dworshak Hatchery (Table 4; Figure 4).

No comparisons of condition factor on the second sampling date, near release, were statistically significant; however, condition was marginally higher for HxH than for HxW or WxW fish for four of eight comparisons ($0.05<P<0.10$) and the pattern was consistent for the other four (Table 4). The exponent of the weight-length relation (b in the equation weight=a*length^b) was 3.0 for each treatment and year-class at Clearwater Hatchery but 3.2 for each treatment at Dworshak Hatchery (data pooled over crosses; linear regression with $\log_e$ transformed weight and length). The difference among crosses in condition was less on the first sampling date (data not shown) than on the second. Condition was always higher for mature males than for other fish ($P<0.001$); however, excluding mature males had little effect on comparisons of condition factor among crosses.

Incidence of mature males in the separate treatment at Clearwater Hatchery was higher for WxW fish than for HxH fish (($P=0.081$ for the 1994 year-class; $P<0.05$ for the 1995 year-class; Table 4). Incidence of mature males for HxW fish in the separate treatment was intermediate and was significantly

higher than for HxH fish (Table 4). Differences among crosses were not apparent in mixed treatments. Mature males were infrequent at Dworshak Hatchery (Table 4).

Differences in length among crosses were less for mature males than for immature fish probably indicating a minimum size threshold for maturation and slowed growth for maturing fish as they diverted energy from growth to production of gametes (Thorpe 1987) (Figure 5). The difference in maturation between WxW and HxH fish was greater in the separate treatment than in the mixed treatment even after we partitioned the data by length category (Figure 6). Apparently the presence of HxH fish suppressed maturation of WxW males independently of effects on fish size. Statistical tests confirmed these competitive effects. Logistic regressions required exclusion of fish shorter than 160 mm (1994 year-class) or 170 mm (1995 year-class) to obtain satisfactory model fit. A significant interaction between cross and treatment for the 1994 year-class (P=0.005), but not between either of those factors and length, required that we make comparisons separately within each treatment. We found that incidence of maturation was higher for WxW than for HxH fish in the separate treatment (P<0.001) but not in the mixed treatment. Incidence of maturation of WxW fish was higher in the separate treatment than in the mixed treatment (P=0.028 after Bonferroni adjustment for two comparisons); HxH fish were similar between treatments. The interaction between cross and length was significant for the 1995 year-class (P=0.042) so we compared treatments within each cross separately. Differences in maturation between treatments approached significance only for the WxW cross (P=0.055 after Bonferroni adjustment for three comparisons). We also considered treatments separately and found a significant interaction between cross and length within the mixed treatment but not within the separate treatment. Within the separate treatment, maturation for WxW fish was greater than for HxH fish, as was maturation for HxW fish (P<0.001). Note that maturation of the 1995 year-class at Clearwater Hatchery increased from mid March, when fish were PIT tagged, to late March when fish were re-sampled for size and maturity. In mid March incidence of maturity was 8, 12, and 17% in the separate treatment and 12, 12, and 9% in the mixed treatment for HxH, HxW, and WxW fish, respectively, whereas in late March it was higher (Table 4).

Survival and growth of HxH relative to WxW fish were consistently but not significantly higher at Dworshak than at Clearwater Hatchery (Table 8). Mean estimates of relative survival were not available for the mixed treatment at Clearwater Hatchery due to inconsistencies between the first and second samples (see above); however, mean relative survival for HxH fish in the mixed treatment at Dworshak was higher than three of the four sample-specific estimates for the 1995 year-class mixed treatment at Clearwater (Table 4). Growth in length was approximately linear with respect to time (Figure 7); thus comparisons of relative growth were valid despite the difference in rearing period between hatcheries.

Downstream migration

Detection rate of PIT-tagged fish at dams on the lower Snake and Columbia rivers was at least twice as high for HxH fish as for WxW fish for both treatments of both year-classes (Table 4). Detection rate for HxW fish was intermediate and was significantly less than for HxH fish. Of the 396 mature males that were PIT-tagged, only 1% were detected. Excluding the tagged mature males increased detection rate (the greatest increase was to 54, 42, and 18% for HxH, HxW, and WxW fish from the 1995 year-class separate treatment at Clearwater) but had little effect on differences in detection rate among crosses. Differences in detection rate primarily developed between Crooked River and the first dam (LGR; Table 5). Arrival at LGR ranged from 15-63% among crosses released from Clearwater Hatchery and was significantly higher for HxH than for HxW fish and was also significantly higher for HxW than for WxW fish; survival from LGR to LGO and from LGO to LMN was similar among crosses and $\geq$69% (Table 5). Detection efficiency didn't differ significantly among crosses at any dam (Table 5) so we assumed that detection rate was an unbiased measure of differences among crosses in downstream migration. Too few fish from Dworshak Hatchery were detected to provide reliable estimates for cross-

specific survival or detection efficiency. Only three fish – one HxH, one HxW, and one of unknown cross (the *PEPA** genotype couldn't be resolved for this fish from the mixed treatment) – were detected one year after release and none were detected in subsequent years.

Differences in detection rate among crosses were partially due to length differences among crosses and a lower detection rate for short compared to longer fish. Few fish shorter than 170 mm were detected (Figure 8). Higher percentages of WxW than of HxH fish were below this threshold; HxW fish were intermediate (Figures 4 and 8). However, differences in detection rate among crosses persisted even when we partitioned the data by length class (Figure 9). We used logistic regression to control for the effect of length on detection rate differences among crosses. The regressions required exclusion of fish shorter than 170 mm (1994 year-class) or 180 mm (1995 year-class) to achieve satisfactory model fit. For the 1994 year-class, the slope of the regression line for detection rate versus length only differed marginally between crosses (P=0.085), and the difference in detection rate between crosses tended to decrease with increasing fish length (Figure 9). Even so, detection rate was significantly higher for HxH than for WxW fish for common lengths $\leq$193 mm (P<0.05). For the 1995 year-class, regression slopes were similar among crosses (P>0.1), and detection rate at any given length was higher for HxH than for WxW fish (P<0.001) and was intermediate for HxW fish (P=0.001 for the comparison with HxH fish). For neither year-class was the interaction between cross and treatment (mixed versus separate) significant in the logistic regressions (P>0.1). The lack of interaction indicates that rearing the crosses together had no effect on relative detection rates beyond the effect of exaggerated size differences between HxH and WxW fish. Differences among crosses in length-specific detection rates persisted when mature males were excluded.

Travel time was less for HxH fish than for WxW fish from Clearwater Hatchery ($P\leq$0.002; two-way AOVs with cross and detection site as factors; data were pooled across replicate vessels and treatments) and was intermediate for HxW fish (P=0.045 for the comparison with HxH). Travel time to LGR averaged 26.2 and 28.1 days for 1994 year-class HxH and WxW fish, respectively, and 16.7, 18.3, and 23.8 days for 1995 year-class HxH, HxW, and WxW fish. Travel time was inversely related to fish length (P<0.001); however, the difference in travel time between HxH and WxW fish persisted after we accounted for length with AOCV ($P\leq$0.006).

Non-migrant fish

We estimate that of the 46,000 1994 year-class fish released in Crooked River, 38% did not emigrate from Crooked River (Table 5). Eighty-six percent of the 1995 year-class fish from Dworshak Hatchery and 51% of the 1995 year-class fish from Clearwater Hatchery didn't emigrate from Crooked River (Table 5). Approximately 18,300 ($\pm$ 3,700; 95% CI) fish from 1996 releases and 39,100 ($\pm$ 8,100) from the 1997 release didn't emigrate. Most of the fish that left Crooked River arrived at LGR (Table 5).

Our sample of age-1 and older steelhead collected from Crooked River in August 1996 consisted of hatchery fish released before 1996 (8%), residuals from our 1996 releases (54%), and unmarked fish from natural production or from our fry release in June 1994 (38%). Residuals from fish reared at Clearwater Hatchery and released in 1996 (1994 year-class) comprised 25% HxH and 75% WxW (n=262). Only five residuals from Dworshak Hatchery (1995 year-class released in 1996) were captured; three were HxH and two were WxW.

Condition factor was higher for naturally reared two-year olds than for either HxH or WxW residuals from Clearwater Hatchery (first, third, and fifth groups from the left in Figure 10; P<0.001; one-way AOV). Four of the naturally reared two-year olds were recaptures of fish PIT tagged in Crooked River in August 1995. The recaptures showed an increase in length (mean=28 mm) and little change in condition factor (mean=0.02) since tagging. Twenty-four of the WxW residuals were recaptures of fish PIT-tagged at Clearwater Hatchery in mid March 1996. These recaptures showed an increase in length but a marked decrease in condition relative to measurements taken at tagging (last two groups in Figure

10; $P<0.001$ for condition, paired *t*-test). Measurements at tagging of WxW fish not subsequently detected at the dams provided an estimate of pre-release size and condition for "potential" WxW residuals. This group (undetected fish) wasn't directly representative of the residuals because it included undetected migrants and non-migrants that died before August. Nevertheless, results from this group were similar to those from tagged recaptures – gain in length but considerable loss of condition between sampling at the hatchery and in Crooked River (fourth and fifth groups in Figure 10; $P<0.001$, unpaired *t*-test) – suggesting little confounding from migrants and mortalities. About 22% of fish that migrated past the dams in 1996 were undetected (based on the product of detection efficiencies at each dam; Table 5), and 20% of WxW fish released from Clearwater Hatchery in 1996 arrived at LGR (Table 5); thus only about 4% of total undetected WxW fish were migrants. Only two HxH residuals were recaptures of fish tagged at Clearwater Hatchery but both showed an increase in length (mean=10 mm) and a large decrease in condition (mean=-0.22) since tagging. Total HxH residuals showed a decrease in length and a large decrease in condition relative to undetected HxH fish at tagging (second and third groups in Figure 10; $P<0.001$, unpaired *t*-test). The length decrease indicated that larger fish in the undetected group either emigrated or died prior to residual sampling in August. Comparing condition factor across groups and lengths was justified because the exponent of the weight-length relation (b in the equation $\text{weight}=a*\text{length}^b$) did not differ among the groups shown in Figure 10 ($P=0.143$; ACOV with $\log_e$ transformed weight and length) and averaged 3.0 (Bolger and Connolly 1989).

The difference in condition between residuals and naturally reared two-year olds persisted after we accounted for differences in density among sites within Crooked River. Snorkel surveys (Idaho Department of Fish and Game; unpublished data) showed that the combined density of yearling and older steelhead varied greatly among sites but tended to be higher within seven km of the release site (the acclimation ponds) than farther downstream (Figure 11, upper panel). The proportion of yearling and older steelhead that were residuals was also higher close to the release site than farther downstream (Figure 11, upper panel). Apparently, most residuals stayed near the release site inflating density there. Regardless of density or distance from the release site, condition factor was substantially lower for residuals than for the naturally reared group (Figure 11, lower panel).

Rate of detection at the dams in spring 1997 for fish captured and PIT-tagged in Crooked River during August 1996 was 0.4% for residuals from Clearwater Hatchery (1 of 262 detected) and 11.3%, significantly greater, for naturally reared two-year olds (9 of 80 detected; $P<0.001$). The lone residual detected was from the WxW group with a length of 162 mm and a condition factor of 0.91 in August 1996. Mean (SD) length and condition factor of detected naturally reared two-year olds was 144 (12) mm and 1.00 (0.04), respectively.

Adult returns

Three adults returned from the 1994 year-class reared at Clearwater Hatchery, 17 from the 1995 year-class reared at Clearwater Hatchery, and none from the 1995 year-class reared at Dworshak Hatchery (Table 9). All but one of these adults returned three years after release at lengths consistent with seaward migration during the spring of release and two winters spent at sea (i.e., > 72 cm; Figure 2). The exception was a 1995 year-class HxH male that returned after two years with one winter at sea. Carcasses were recovered from 14 of the adults returning three years after release, and otolith analysis confirmed two winters of ocean residence for all 14. Of the adults returning in 2000 (all from the 1995 year-class), eight returned to Crooked River, six returned to Dworshak Hatchery, and two (PIT-tagged as juveniles before release) were detected at LGR but were not subsequently recovered at either Crooked River or Dworshak Hatchery. Crooked River was monitored for adults in 1998-2001; Dworshak Hatchery was monitored only in 2000-2001.

The three adults from the 1994 year-class were all WxW fish (Table 9). Three WxW adults compared to zero HxH adults was not a statistically significant difference. Eleven adults from the 1995

year-class were HxH fish, four were HxW fish, and three were WxW fish, a statistically significant difference between the HxH and WxW crosses (Table 9). Mean length of 1995 year-class adults was consistently greater for HxH than for WxW fish and intermediate for HxW fish, and the difference in length between HxH and either of the other crosses was significant for fish with two winters of ocean residence (Table 9). Date of capture and return site were similar among crosses (Table 9).

Discussion

Differences among crosses for growth in the hatchery and subsequent downstream migration were qualitatively consistent among year-classes, hatcheries, and treatments. The HxH fish grew faster and migrated downstream at a higher rate than did WxW fish. Performance of HxW fish was nearly always intermediate for these and other traits, and often was significantly different from that of the HxH fish. The latter result shows that the differences between hatchery fish and wild fish were largely due to genetic differences – HxW fish were maternally identical to HxH fish and experienced the same environmental conditions as HxH fish (including conditions during incubation; Table 3) so the two crosses differed only in the genetic contribution from their fathers (hatchery or wild). Differential survival in the hatchery was neither consistent nor persuasive at Clearwater Hatchery (Table 4).

Our data show that Dworshak Hatchery steelhead differ genetically from Selway River steelhead in fitness-related traits and that the former are better adapted for the hatchery program. Comparisons of the crosses from this study in a natural stream (Rubin et al. 2012b) indicate that Selway steelhead are better adapted for natural rearing. We believe that releasing Dworshak steelhead in the Clearwater River drainage to supplement wild populations, and subsequent interbreeding of hatchery and wild fish in natural spawning areas would reduce the fitness of the wild populations. Management's decision to forego supplementation to avoid compromising the fitness of wild stocks seems prudent.

The most striking observation from our study was the intense domestication selection occurring shortly after release from the hatchery. This natural (domestication) selection strongly favored individuals that had grown well in the hatchery. Most HxH fish exceeded the threshold size for successful downstream migration (Wagner et al.1963, Tipping et al. 1995; Figure 8); whereas, many WxW fish were below the threshold size, failed to migrate successfully, and therefore were precluded from genetically contributing to the next hatchery generation. Because the differences in growth were genetic, selection against slow-growing fish was genetically changing (domesticating) the (former) wild population to resemble the Dworshak Hatchery population. Overall mortality and differential mortality among crosses were much lower in the hatchery than after release, consistent with the typically low in-hatchery mortality in sea-ranched populations and the idea that selection for fish that perform well in the hatchery occurs primarily after release (Reisenbichler et al. 2004). These arguments hold whether the hatchery population was better adapted due to previous domestication or to ancestral differences (Reisenbichler et al. 2004).

Independent data suggest that domestication selection has been changing the Dworshak population and that the original North Fork population was similar to the current Selway population. The length frequency distribution of juveniles reared for two years at Dworshak Hatchery for trials conducted during the late 1970s (Bjornn and Ringe 1984) was more similar to that for our WxW fish (left-skewed and modest peak) than our HxH fish in the two-year program (Figure 12). The year-classes of the late-1970s were reared in untreated river water at temperatures similar to those experienced by our experimental fish at Clearwater Hatchery. Those early Dworshak fish were approximately two generations removed from their wild ancestors, whereas the Dworshak fish in our study were approximately six generations removed.

Survival in the hatchery for the 1995 year-class mixed treatment reared at Clearwater Hatchery sometimes differed significantly between HxH and WxW fish (Table 4); however, Reisenbichler et al.

(2004) reported no in-hatchery survival differences for these same fish. The apparent discrepancy results because this study considered both the first (age-1) and second (age-2) samples whereas Reisenbichler et al. (2004) used only the second sample (Table 4). We believe that substantial variability between the first and second samples resulted from sampling error rather than differential survival between sampling dates. Hatchery personnel monitored the experimental fish almost daily, counting and removing any dead fish. They found that the mortality between the first and second samples was only 2%. Substantial undocumented predation by otters or birds was unlikely because the raceways were protected with netting. This variability suggests the need for complete census or a sampling method that more closely approaches equal probability of capture throughout the entire population. Sampling error was not a concern for the separate treatments (allopatry) because survival estimates were based on total counts.

Mean egg size was greater from hatchery females than from wild females in both years (although significantly different only in 1994; Table 3) so we cannot exclude an additional maternal effect beyond the purely genetic differences between HxH and WxW fish. However, we believe that any effect from the difference in egg size was small because we conducted a separate experiment at Dworshak Hatchery, using only hatchery fish, and found that differences in egg size greater than the differences in this study had no detectable effect on subsequent growth of steelhead reared in the hatchery (Rubin et al. 2012f).

Differences other than growth

The incidence of mature males in separate treatments at Clearwater Hatchery showed a genetic basis (the HxW cross was intermediate) and was greatest for the WxW cross. Mature males fail to emigrate (and grow to large size at sea) and are not included in hatchery broodstocks. Thus selection against maturation as parr is intense in most hatchery programs and, as with selection for fast growth, will change a formerly wild population (e.g., from the Selway population) to resemble the Dworshak population. Mature WxW males were large relative to immature WxW fish (Figure 5) so domestication in steelhead apparently selects for fish that grow sufficiently fast in the hatchery to reach the threshold size for successful downstream migration but against fast growing males that mature as parr. The effect of this latter selection on females is unknown. The incidence of mature males has typically been lower under one-year rearing at Dworshak Hatchery than we observed under two-year rearing at Clearwater Hatchery, but probably has been high enough to result in selection against early maturation. Incidence of mature males for the 1993, 1994, and 1999 year-classes at Dworshak Hatchery was 5, 2, and 7%, respectively (Peters 1995, 1997; Larsen et al. 2003).

Domestication selection also seemed to operate on traits other than growth and maturation because detections of WxW fish were lower than for HxH fish of the same length within the size range considered optimal for smoltification of hatchery reared steelhead (>190 mm fork length; Tipping et al. 1995; Figure 9). These other traits are as yet uncharacterized but appear to affect emigration success. Perhaps a substantial proportion of smolt-sized WxW fish were genetically predetermined to express a resident life history which can be a component of wild steelhead populations (Zimmerman and Reeves 2000). The difference between HxH and WxW fish probably was only slightly influenced by males maturing as age-2 parr (rather than maturing after one or more additional years in fresh water) because differences in detection rate among crosses were apparent for smolt-sized fish after excluding mature males.

The conclusion that domestication affects traits other than growth and maturation is tentative because we cannot exclude the possibility that the genetic marks (Reisenbichler et al. 2006), or ancestral differences between the original North Fork Clearwater population and the Selway River population, were at least partially responsible for differential performance within length categories (after release). The conclusion rests on the implicit assumption that the hatchery population (derived from the North Fork Clearwater River) would be genetically equivalent to the wild population (from the Selway River) for these traits if the former had not been taken into the hatchery. The conclusion is supported but not

confirmed by the differences in downstream migration that were consistent with differences in growth before release and with the expected consequences of domestication (better performance of HxH than WxW under hatchery rearing).

Adult returns

Adult return rates were low for our experimental fish. Only three adults were captured from the 1994 year-class (all WxW) – too few to demonstrate statistically significant differences. Returns from the 1995 year-class (n = 17) showed significant differences among crosses that were consistent with the differences in detection rate when these fish migrated to sea (HxH > HxW > WxW); however, pooling adult returns over both year-classes removed the difference between HxH and WxW fish. Low return rates and the possibility of selection on the genetic marks after release from the hatchery (Reisenbichler et al. 2006) preclude firm conclusions from adult return rates.

Length at tagging of fish detected at the dams from the 1995 year-class reared at Clearwater Hatchery was similar among crosses (Figure 8), yet length of returning adults that spent two winters at sea was greater for HxH than for WxW fish, and intermediate for HxW fish (Table 9). The differences apparently developed during ocean residence and appear to be genetically determined. We note that length differences between HxH and WxW adults were consistent with those of their parents (Figure 2). Perhaps the differences in parental length were, at least in part, the product of genetic differences, and were not solely due to chance or to differences in freshwater rearing environment (i.e., stream versus hatchery).

Ancestral affinities

Analyses of allozyme markers indicated a substantial difference between North Fork Clearwater populations (resident rainbow trout from above Dworshak Dam and steelhead from Dworshak Hatchery) and 17 other Snake River populations including the Selway River (Waples et al. 1993; Robin Waples, personal communication concerning the resident rainbow trout). The North Fork populations were most distinctive for having a high frequency of the **110* allele at the *PEPA** locus, but allele frequencies at other loci were distinctive as well (Waples et al. 1993). A more recent analysis of DNA microsatellite markers (Nielsen et al. 2009) found less differentiation between Dworshak steelhead and other populations from Idaho. Population structure followed a pattern of isolation by distance. Populations from the Clearwater River, including Dworshak steelhead, clustered together, as did populations from the Salmon River and populations from Snake River tributaries. At the watershed scale (10 watersheds and 3 hatchery complexes), Dworshak steelhead clustered with other upper Clearwater River watersheds, including the Lochsa, Selway, and South Fork Clearwater rivers (98% bootstrap support). However, within the upper Clearwater River cluster the Lochsa and Selway rivers clustered together as distinct from Dworshak steelhead and the South Fork (99% bootstrap support). At the population scale (74 natural populations and 5 hatcheries), North Fork Clearwater River populations (steelhead from Dworshak Hatchery and resident rainbow trout from Collins Creek above Dworshak dam) clustered together as distinct from other Idaho populations (80% bootstrap support), a finding in agreement with results of the allozyme study and suggesting that the difference between North Fork Clearwater and other Idaho populations, as measured by neutral molecular markers, predated construction of Dworshak Dam and Hatchery. At the population scale, Clearwater River tributaries including the North Fork clustered together, but only the North Fork branch received >50% bootstrap support. A third study (Winans et al. 2004) compared structure of 32 Columbia River basin steelhead populations, including 18 from the Snake River, among three types of molecular markers – allozymes, microsatellites, and introns – and found that all three showed distinct clusterings of the 5 Snake River B-run populations including those from Dworshak Hatchery and the Lochsa and Selway rivers.

The North Fork Clearwater River and Selway River are geographically proximate, share the same geology, and are much more similar to each other than to a hatchery (Reisenbichler and Rubin 1999). Consequently we believe that the Selway and North Fork populations were genetically similar for fitness-related traits; however, the degree of similarity is unknown. This study demonstrated domestication selection for fast growth in the hatchery (via successful downstream migration) and against maturation as parr. Possible ancestral differences seem negligible but cannot be excluded as confounding factors for our other indicators of domestication.

Change in variance for length

The marked increase in length variation of WxW fish between their first and second years (Figure 4) might have been due to a physiological "decision" by some yearlings to maintain growth and become smolts the following spring and by others to slow growth and delay smoltification (Thorpe 1977). The relatively constant variation in length for HxH fish (variance similar between age-1 and age-2) may reflect prior domestication selection for smolting at an early age so that almost all yearlings "decide" to maintain fast growth. The length distributions of age-2 fish are consistent with the notion of two distinct growth strategies. For example, the distribution for WxW fish of the 1994 year-class clearly was bimodal in both separate and mixed treatments (Figure 4). The lower mode appeared to be present in the HxH group as well, but it comprised a much smaller proportion of the total population. Bjornn and Ringe (1984) noted bimodal distributions at Dworshak Hatchery during the late 1970's (Figure 12) and found as we did (Figure 4) that the bimodality developed during the second year of rearing. They further demonstrated that growth during the first year largely determined the subsequent mode for a fish – grading the yearlings for size and removing the smaller ones eliminated the lower mode in the two-year olds.

Competition

Final size was greater for HxH than for WxW fish in the separate treatment (allopatry) indicating that growth rate was intrinsically higher for HxH than for WxW fish. The faster growth for HxH fish could have resulted from greater food consumption, greater food conversion efficiency, or both (Thodesen et al. 1999). That growth of WxW fish was slower in the mixed treatment (sympatry) than in the separate treatment indicates competition between HxH and WxW fish. At Clearwater Hatchery where rations were often below satiation levels, growth of WxW fish was slower in the mixed than in the separate treatment whereas growth of HxH fish was faster in the mixed than in the separate treatment, indicating direct competition for food between the crosses. In the mixed treatment HxH fish monopolized the limited food supply, deprived WxW fish of their fair share, and grew faster than HxH fish in the separate treatment where HxH fish had to compete with each other. At Dworshak Hatchery where rations were always in excess, growth of WxW fish was again slower in the mixed than in the separate treatment but growth of HxH fish wasn't faster in the mixed than in the separate treatment. The poorer growth of WxW fish in the mixed treatment suggests social dominance by HxH fish rather than direct competition for limited food because food presumably wasn't limited.

In feeding trials conducted with siblings of our experimental fish, willingness to feed at the surface was greater for HxH fish than it was for separately reared WxW fish (Gayle Brown, personnel communication). This behavior difference could explain how HxH fish out-competed WxW fish when food was limited – the HxH fish may simply have intercepted food near the surface before it got down to the WxW fish. Size differences between juvenile steelhead often result in dominance of larger over smaller individuals (Abbott et al. 1985) so suppression of WxW growth by HxH fish even where food was unlimited (i.e., in the mixed treatment at Dworshak) could be explained by initially higher intrinsic growth rates for HxH than for WxW fish followed by the development of social dominance of HxH over WxW fish. Thus our experiments don't provide evidence of behavior differences between crosses, such

as differences in aggression, that might promote dominance of HxH over WxW fish; however, such behavior differences certainly could have existed since our experiments weren't designed to test for them.

Maturation of WxW males was suppressed when HxH and WxW fish were reared together in the two-year program. A direct effect beyond the competitive reduction in growth was involved because the effect persisted when we compared crosses within size categories. The specific mechanism of this direct effect is unknown to us.

Non-migrants

Most of the difference in detection rate among crosses resulted from residualization or mortality in Crooked River because most fish that left Crooked River arrived at the first dam (Lower Granite Dam) and survival between dams was similar for the different crosses (Table 5). Residualization rather than mortality was likely the primary cause for the difference. In spring 1996, fish arriving at Lower Granite Dam from the 1994 year-class release comprised 77% HxH fish and 23% WxW fish (Table 5). In August 1996, fish remaining in Crooked River from that release comprised 25% HxH and 75% WxW fish. This difference between migrants and residuals in frequency of HxH relative to WxW fish suggests differential emigration rather than differential mortality.

Compared to their naturally reared siblings, residuals did not perform well in Crooked River. Condition in August was markedly lower for residuals than for naturally reared fish, and residuals produced significantly less smolts the following spring than did naturally reared fish. Naturally reared fish and residuals shared the same environment, therefore habitat deficiencies were not the cause of poor performance by the residuals. However, density may have been higher than optimal. Kiefer and Lockhart (1999) estimated that the carrying capacity of Crooked River for yearling steelhead was about 0.14 fish/m^2. Mean density of residuals shortly after the 1996 releases (total non-migrants divided by total surface area) was 0.09 fish/m^2, and density was even higher considering that a number of other yearling and older steelhead resided in the stream along with the residuals. Perhaps performance of both groups (residuals and naturally reared) would have been better had density been lower.

Because length and weight were measured at the hatchery a month before fish were released in Crooked River, we can't exclude the possibility that condition decreased before rather than after release. However, recaptures indicated that tagged residuals had increased in length but lost condition relative to a month before release. This pattern is consistent with continued growth in length and weight during the final month in the hatchery (or acclimation ponds) followed by weight loss after release in Crooked River.

Many of the fish that residualized in Crooked River rather than migrating downstream were from the WxW group, and many of these WxW residuals were small at release from the hatchery and still small (<170 mm long) when captured five months later in Crooked River (Figure 10). Presumably their small size was at least partially responsible for them residualizing rather than migrating seaward because very few fish <170 mm long at tagging were detected at the dams after release (Figure 8). We had thought that the small WxW fish that residualized in Crooked River simply needed an additional year of growth before smolting, and that they would prosper in Crooked River and eventually smolt and migrate seaward, but for the most part they didn't. Apparently, the extended period of hatchery rearing somehow prevented them from adopting a lifestyle appropriate to a stream environment after release. Although we can't exclude the possibility that they matured and completed their life cycle in freshwater, their poor condition in August suggests that this was unlikely. For one thing, poor condition in fall can result in low over-winter survival where winter temperatures are severe (Smith and Griffith 1994), and winter temperature in Crooked River probably qualified (mean temperature in January 1996 = 0.1^{o}C and was probably similarly low in January 1997). We note that hatchery rearing doesn't always result in poor performance in streams after release. Rhodes and Quinn (1999) found that in-stream survival from late spring through summer was equivalent and growth was faster for coho salmon *O. Kisutch* reared in a hatchery for three

months before release in comparison to that for the same cohort of salmon released as fry in the same streams three months earlier.

Implications for attenuating domestication

One might attempt to reduce domestication selection by increasing ration to increase growth and subsequent emigration. Our results suggest that this strategy may disappoint. One of the replicates of WxW fish at Clearwater Hatchery in 1995 (vessel 8; separate treatment) inadvertently received a higher ration than did other fish in the separate treatment (Figure 3). As a result the fish in vessel 8 attained a mean length of 198 mm, nearly equivalent to the 200 mm length of HxH and HxW fish in the separate treatment (Table 4). This length is the target for steelhead released from Clearwater River basin hatcheries because of the high emigration rate for fish of that size (Bigelow et al. 1997); however, the WxW fish from vessel 8 showed little benefit – their detection rate was only 0.3 times that of mean detection rate in the HxH vessels and 0.4 times that in the HxW vessels (Table 4).

Survival and growth of WxW fish relative to HxH fish probably were lower at Dworshak Hatchery than at Clearwater Hatchery; however, any difference was too small to be statistically significant. This small difference between hatcheries suggests that domestication was only slightly less at Clearwater Hatchery where the two-year program was expected to substantially reduce domestication. Of course, rearing fish in circular tanks was not equivalent to rearing fish in Burrows ponds at Dworshak Hatchery, and our experimental fish were conceived after most other hatchery fish had been spawned so our fish had less time to grow at Dworshak Hatchery and were substantially smaller at release (including our HxH cross) than the typical size for hatchery fish (200 mm FL). Nevertheless, our results suggest a need to test whether alternative hatchery procedures or structure meet expectations for reducing domestication.

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References

Abbott, J. C., R. L. Dunbrack, and C. D. Orr. 1985. The interaction of size and experience in dominance relationships of juvenile steelhead trout (*Salmo gairdneri*). Behaviour 92:241-253.

Aebersold, P. B., G. A. Winans, D. J. Teel, G. B. Milner, and F. M. Utter. 1987. Manual for starch gel electrophoresis – a method for the detection of genetic variation. NOAA Technical Report NMFS 61.

Anderson R. O., and R. M. Neumann. 1996. Length, weight, and associated structural indices. Pages 447-482 *in* Fisheries techniques, second edition. B. R. Murphy and D. W. Willis, editors. American Fisheries Society, Bethesda, Maryland.

Bams R. A. 1970. Evaluation of a revised hatchery method tested on pink and chum salmon fry. Journal of the Fisheries Research Board of Canada 27:1429-1452.

Berejikian, B. A., S. B. Mathews, and T. P. Quinn. 1996. Effects of hatchery and wild ancestry and rearing environments on the development of agonistic behaviour in steelhead trout (*Oncorhynchus mykiss*) fry. Canadian Journal of Fisheries and Aquatic Sciences 53:2004-2014.

Bigelow, P. E. 1997. Emigration of Dworshak National Fish Hatchery steelhead. Pages III-2-III-22 *in* Interactions of hatchery and wild steelhead in the Clearwater River of Idaho. U. S. Fish and Wildlife Service and Nez Perce Tribe. U. S. Fish and Wildlife Service Report. Fisheries Stewardship Project. 1995 Progress Report. Ahsahka, Idaho.

Bjornn, T. C., and R. R. Ringe. 1984. Evaluation of a two year yearing program for steelhead trout at Dworshak NFH. A Completion Report, Contract No. DACW 68-79-C-0044, Submitted to U. S. Department of the Army Corps of Engineers, Walla Walla District. Idaho Cooperative Fishery Research Unit, University of Idaho, Moscow, Idaho.

Bolger, T., and P. L. Connolly. 1989. The selection of suitable indices for the measurement and analysis of fish condition. Journal of Fish Biology 34:171-182.

Burge, H. L., Faler, M., Roseberg, R. B., Jones, R. N., and Olson, J. M. 2004. Adult steelhead returns to Dworshak NFH in 2002-2003 and prognosis for 2003-2004. Appendix B *in* Annual Report, Fiscal Year 2003, U.S. Fish and Wildlife Service, Idaho Fishery Resource Office, Ahsahka, Idaho.

Byrne, A. 2001. Idaho Fish and Game, fishery research - steelhead supplementation in Idaho rivers summary report 1 January 1993 - 31 December 1999. Bonneville Power Administration Report DOE/BP-01466-6. Portland, OR.

Chilcote, M. W., S. A. Leider, and J. J. Loch. 1986. Differential reproductive success of hatchery and wild summer-run steelhead under natural conditions. Transactions of the American Fisheries Society 115:726-735.

Cytel Software Corporation. 1997. StatXact-3, Version 3.1. Copyright 1989-1997.

Einum, S., and I. A. Fleming. 1997. Genetic divergence and interactions in the wild among native, farmed, and hybrid Atlantic salmon. J. Fish. Biol. 50: 634-651.

Elliott, J. M. Number of meals in a day, maximum weight of food consumed in a day, and maximum rate of feeding for brown trout, *Salmo trutta* L. 1975. Freshwater Biology 5:287-303.

Fleming, I. A., T. Agustsson, B. Finstad, J. I. Johnsson, and B. Th. Bjornsson. 2002. Effects of domestication on growth physiology and endrocrinology of Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 59:1323-1330.

Fleming, I. A., and S. Einum. 1997. Experimental tests of genetic divergence of farmed from wild Atlantic salmon due to domestication. ICES Journal of Marine Science 54:1051-1063.

Kallio-Nyberg, I., and M. L. Koljonen. 1997. The genetic consequence of hatchery-rearing on life-history traits of the Atlantic salmon (*Salmo Salar* L.): a comparative analysis of sea-ranched salmon with wild and reared parents. Aquaculture, 153:207-224.

Kiefer, R. B., and J. N. Lockhart. 1999. Intensive evaluation and monitoring of chinook salmon and steelhead trout production, Crooked River and upper Salmon River sites Annual Progress Report January 1, 1995-December 31, 1995, Report to Bonneville Power Administration, Contract No. 1991BP21182, Project No. 199107300, 65 electronic pages (BPA Report DOE/BP-21182-11).

Larsen, C., P. Bigelow, and M. Faler. 2003. Characterize and quantify residual steelhead in the Clearwater River, Idaho; Annual Report 2000. Project N. 1999-01800, 16 electronic pages, (BPA Report DOE/BP-00004665-2).

Martinson, R.D., R.J. Graves, R.B. Mills, & J.W. Kamps. 1997. Monitoring of downstream salmon and steelhead at federal hydroelectric facilities - 1996. Annual report. U.S. Dept. of Energy, Bonneville Power Administration, Portland, OR (U.S.A.).

Nielsen, J. L., A. Byrne, S. L. Graziano, and C. C. Kozfkay. 2009. Steelhead genetic diversity at multiple spatial scales in a managed basin: Snake River, Idaho. North American Journal of Fisheries Management 29:680-701.

Peters, K. K. 1995. Summary of fish health assessment and condition indices. Pages 25-41 *in* Interactions of hatchery and wild steelhead in the Clearwater River of Idaho. U. S. Fish and Wildlife Service

and Nez Perce Tribe. U. S. Fish and Wildlife Service Report. Fisheries Stewardship Project. 1994 Progress Report. Ahsahka, Idaho.

Peters, K. K. 1997. Comparison of health and condition profiles of wild A-run and hatchery B-run steelhead from the Clearwater River, Idaho. Pages II-1-II-23 *in* Interactions of hatchery and wild steelhead in the Clearwater River of Idaho. U. S. Fish and Wildlife Service and Nez Perce Tribe. U. S. Fish and Wildlife Service Report. Fisheries Stewardship Project. 1995 Progress Report. Ahsahka, Idaho.

Prentice, E.F., T.A. Flagg, C.S. McCutcheon, & D.F. Brastow. 1990. PIT-tag monitoring systems for hydroelectric dams and fish hatcheries. In: Parker, N.C., A.E. Giorgi, R.C. Heidinger, D.B. Jester, Jr., E.D. Prince, & G.A. Winans (eds), pp. 323-334. Fish-marking techniques. American Fisheries Society Symposium 7, Bethesda, MD.

Reisenbichler, R. R. 2005. Uncertainty and research needs for supplementing wild populations of anadromous Pacific salmon. Pages 263-275 in Nickum, M. J., P. M. Mazik, J. G. Nickum, and D. D. MacKinlay, editors. Propagated fish in resource management. American Fisheries Society, Symposium 44, American Fisheries Society, Bethesda, Maryland.

Reisenbichler, R. R., M. C. Hayes, S. P. Rubin, L.A. Wetzel, and B. M. Baker. 2006. *PEPA** genotype affects return rate for hatchery steelhead. Transactions of the American Fisheries Society 135:28-39.

Reisenbichler, R.R., & J.D. McIntyre. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, Salmo gairdneri. J. Fish. Res. Board Can. 34: 123-128.

Reisenbichler, R. R., and S. P. Rubin. 1999. Genetic changes from artificial propagation of Pacific salmon affect the productivity and viability of supplemented populations. ICES Journal of Marine Science 56:459-466.

Reisenbichler, R. R., S. P. Rubin, L.A. Wetzel, and S. R. Phelps. 2004. Natural selection after release from a hatchery leads to domestication in steelhead, *rhynchus mykiss*. Pages 371-383 In K.M. Leber, H.L. Blankenship, S. Kitada, and T. Svåsand [editors] Stock Enhancement and Sea Ranching: developments, pitfalls, and opportunities. 2nd edition. Blackwell Science Ltd, Oxford.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Fisheries Research Board of Canada Bulletin No. 191.

Rhodes, J. S., and T. P. Quinn. 1999. Comparative performance of genetically similar hatchery and naturally reared juvenile coho salmon in streams. North American Journal of Fisheries Management 19:670-677.

Rubin, S. P., R. R. Reisenbichler, J. E. Hensleigh, L. A. Wetzel, B. M. Baker, F. E. Leonetti, K. D. Stenberg, and S. L. Slatton. 2012b. Genetic differences between hatchery and wild steelhead for survival, growth, dispersal, and male maturation in a natural stream. Chapter 3 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, J. E. Hensleigh, F. E. Leonetti, B. M. Baker, S. L. Slatton, and K. D. Stenberg. 2012d. Differences in survival and growth in hatchery and stream environments, and in maturation of residuals in a stream, between progeny of hatchery and wild steelhead. Chapter 1 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, and K. D. Stenberg. 2012f. Effect of size of unfed fry at release on survival and growth of juvenile steelhead in streams and a hatchery. Chapter 7 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon.

Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.
SAS Institute Inc. 2000. SAS Online DOC^{R}, Version 8. SAS Institute, Cary, North Carolina.
Shaklee, J. B. F. W. Allendorf, D. C. Morizot, and G. S. Whitt. 1990. Gene nomenclature for protein-coding loci in fish. Transactions of the American Fisheries Society 119:2-15.
Smith, R. W., and J. S. Griffith. 1994. Survival of rainbow trout during their first winter in the Henrys Fork of the Snake River, Idaho. Transactions of the American Fisheries Society 123:747-756.
Smith, S. G., J. R. Skalski, J. W. Schlechte, A. Hoffmann, and V. Cassen. 1994. Introduction to SURPH.1 analysis of release-recapture data for survival studies. Report to Bonneville Power Administration, Contract No. DE-BI79-90-BP02341, Project No. 89-107.
Sokal, R. R., and F. J. Rohlf. 1995. Biometry, 3rd edition. W. H. Freeman and Company, New York.
Tipping, J. M. 1995. Length and condition factor of migrating and nonmigrating hatchery-reared winter steelhead smolts. 1995. The Progressive Fish-Culturist 57:120-123.
Thodesen, J., B. Grisdale-Helland, S. J. Helland, and B. Gjerde. 1999. Feed intake, growth and feed utilization of offspring from wild and selected Atlantic salmon (*Salmo salar*). Aquaculture 180:237-246.
Thorpe, J. E. 1977. Bimodal distribution of length of juvenile Atlantic salmon (*Salmo salar* L.) uner artificial rearing conditions. Journal of Fish Biology 11:175-184.
Thorpe, J. E. 1987. Smolting versus residency: developmental conflict in salmonids. American Fisheries Society Symposium 1:244-252.
Volk, E. C., S. L. Schroder, and K. L. Fresh. 1990. Inducement of unique banding patterns as a practical means to mass-mark juvenile pacific salmon. American Fisheries Society Symposium 7:203-215.
Wagner, H. H., R. L. Wallace, and H. J. Campbell. 1963. The seaward migration and return of hatchery-reared steelhead trout, *Salmo gairdneri* Richardson, in the Alsea River, Oregon. Transactions of the American Fisheries Society 3:202-210.
Waples, R.S., O.W. Johnson, P.B. Aebersold, C.K. Shiflett, D.M. Van Doornik, D.J. Teel, and A.E. Cook. 1993. A genetic monitoring and evaluation program for supplemented populations of salmon and steelhead in the Snake River basin. U.S. Department of Energy, Bonneville Power Administration report DOE/BP-00911-2, Portland, Oregon.
Winans, G. A., M. M. Paquin, D. M. Van Doornik, B. M. Baker, P. Thornton, D. Rawding, A. Marshall, P. Moran, and S. Kalinowski. 2004. Genetic stock identification of steelhead in the Columbia River basin: an evaluation of different molecular markers. North American Journal of Fisheries Management 24:672-685.
Zimmerman, C. E., and G. H. Reeves. 2000. Population structure of sympatric anadromous and nonanadromous *Oncorhynchus mykiss*: evidence from spawning surveys and otolith microchemistry. Canadian Journal of Fisheries and Aquatic Sciences 57:2152-2162.

Tables

Table 1. Number of adults spawned in 1994 and 1995, and resulting number of full-sib families. The eggs from each female were divided into two portions, each fertilized by a different male. Males were crossed with a variable number of females (Table 2). Males not used on a previous date are listed in parentheses. Embryos from adjacent spawning dates were grouped. Temperatures were adjusted so that all embryos within a group reached the eyed stage almost simultaneously.

	Date	Females		Males		Families			
Year	spawned	Hatchery	Wild	Hatchery	Wild	HxH	HxW	WxW	Group
1994	7 Apr	0	3	0 (0)	3 (3)	0		6	1
	11 Apr	15	2	14 (14)	3 (1)	30		4	1
	14 Apr	0	5	0 (0)	5 (2)	0		10	1
	18 Apr	10	3	11 (6)	3 (0)	20		6	2
	21 Apr	0	2	0 (0)	4 (1)	0		4	2
	25 Apr	0	3	0 (0)	3 (0)	0		6	2
	Total	25	18	(20)	(7)	50		36	
1995	10 Apr	6	1	6 (6)	5 (5)	6	6	2	1
	13 Apr	0	3	0 (0)	3 (3)	0	0	6	1
	17 Apr	1	0	1 (1)	1 (0)	1	1	0	1
	17 Apr	4	0	4 (2)	3 (1)	4	4	0	2
	20 Apr	0	3	0 (0)	4 (0)	0	0	6	2
	24 Apr	4	1	3 (2)	5 (0)	4	4	2	2
	27 Apr	2	0	2 (2)	2 (0)	2	2	0	2
	27 Apr	4	1	2 (0)	5 (0)	4	4	2	3
	1 May	2	1	1 (1)	3 (0)	2	2	2	3
	Total	23	10	(14)	(9)	23	23	20	

Table 2. Number of males producing various combinations of full-sib families.

			Number of families		
Year	Male stock	Males	HxH	HxW	WxW
1994	Hatchery	2	1	--	--
		2	3	--	--
		5	4	--	--
		11	2	--	--
	Wild	1	--	--	3
		1	--	--	4
		1	--	--	5
		4	--	--	6
1995	Hatchery	1	4	--	--
		6	2	--	--
		7	1	--	--
	Wild	1	--	1	3
		1	--	3	2
		1	--	5	3
		2	--	1	2
		2	--	2	2
		2	--	4	2

Table 3. Mean (SD) egg size; survival, temperature, and density during incubation from fertilization to the eyed stage; and final size and developmental index (k_D; Bams 1970) of fry when moved to rearing vessels. In 1995, egg weight was the same for HxH and HxW crosses because the same hatchery females were used in both crosses. Likewise, incubation temperature and number of eggs per tray were the same for HxH and HxW crosses because each tray contained both crosses from a single hatchery female, separated by a divider. Fry data for 1994 are from siblings released in Twentymile Creek (Rubin et al. 2012b); fry retained at the hatchery that year weren't sampled. Letters (x, y, z) indicate significance of comparisons between crosses within a year-class. Means without a letter in common are different (P<0.05).

	1994		1995		
Variable	HxH	WxW	HxH	HxW	WxW
Egg weight (g)[a]	0.119 (0.014) x	0.102 (0.008) y	0.107 (0.012) x	0.107 (0.012) x	0.102 (0.011) x
Survival to eye-up (%)[b]	84.2 (17.5) x	73.6 (31.6) x	95.3 (7.2) x	93.4 (9.8) x	97.3 (3.0) x
Incubation temperature (^{o}C)	8.5	9.0	8.5	8.5	8.3
Number of eggs per tray[a]	6761 (1292) x	4846 (831) y	6893 (1549) x	6893 (1549) x	4778 (707) y
Fry length (mm)[c]	29.9 (1.0) x	29.0 (0.8) y	28.4 (0.9) x	27.8 (0.8) y	27.6 (1.0) y
Fry weight (g)[d]	0.194	0.163	0.213	0.201	0.184
k_D[e]	1.94	1.88	2.10	2.10	2.06

[a]Standard deviation among females; *n* females given in Table 1.
[b]Standard deviation among families; *n* families given in Table 1.
[c]Standard deviation among individuals; *n*=60 per cross.
[d]At least 300 fry per cross were counted and collectively weighed.
[e]$k_D = 10*W^{1/3}*L^{-1}$; W = mean weight (mg); L = mean fork length (mm).

Table 4. Performance of experimental fish in the hatchery and after release. Dates of the first and second samples were 11-13 September 1995 and 11-13 March 1996 for the 1994 year-class, 10-12 June 1996 and 25-27 March 1997 for the 1995 year-class at Clearwater, and 15-16 February and 4 April 1996 for the 1995 year-class at Dworshak. Letters indicate paired comparisons: w=HxH versus WxW, x=HxW versus HxH, y=HxH_{mixed} versus $HxH_{separate}$, and z=WxW_{mixed} versus $WxW_{separate}$. Asterisks indicate significance adjusted for multiple comparisons: ** = P<0.05, * = 0.05<P<0.10, and ns = P>0.10. K_n=relative condition factor.

						Survival (%)		*n*		Relative survival	
Year-class	Hatchery	Treatment	Vessel	Cross	*N* fry	To release	Between samples	First sample	Second sample	First sample	Second sample
1994	Clearwater	Mixed	1	HxH	4,935			184	127	1.10 w ns	0.72 w **
				WxW	4,825			164	173	1.00	1.00
				Total	9,760	73.1	99.9	348	300		
			2	HxH	4,809			183	163	1.11 w ns	1.19 w ns
				WxW	4,825			166	137	1.00	1.00
				Total	9,634	78.1	99.6	349	300		
			Mean	HxH						[a]	[a]
				WxW						1.00	1.00
				Total		75.6	99.8				
		Separate	3	HxH	9,554	83.5	99.1	355	300		
			4	HxH	9,543	85.5	99.8	353	300		
			5	WxW	9,562	81.1	99.0	365	300		
			6	WxW	9,565	78.0	99.7	353	300		
			Mean	HxH		84.5	99.5				1.06 w ns
				WxW		79.5	99.3				1.00
1995	Clearwater	Mixed	1	HxH	3,991			133	133	1.69 w **	1.09 w ns
				HxW	4,007			117	120	1.48 x ns	0.98 x ns
				WxW	4,066			80	124	1.00	1.00
				Total	12,064	78.4	97.6	330	377		
			2	HxH	3,822			124	133	1.32 w *	1.11 w ns
				HxW	3,780			113	143	1.21 x ns	1.21 x ns
				WxW	3,735			92	117	1.00	1.00
				Total	11,337	81.8	98.8	329	393		
			Mean	HxH						[a]	[a]
				HxW						[a]	[a]
				WxW						1.00	1.00
				Total		80.1	98.2				
		Separate	3	HxH	11,984	90.2	99.3	203	266		
			4	HxH	11,421	93.1	98.5	203	256		
			5	HxW	11,955	82.1	98.9	218	253		
			6	HxW	11,368	83.0	99.2	235	256		
			7	WxW	12,105	76.8	97.1	207	314		
			8	WxW	11,247	66.2	98.8	202	276		
			Mean	HxH		91.7	98.9				1.28 w **
				HxW		82.6	99.0				1.15 x *
				WxW		71.5	98.0				1.00
	Dworshak	Mixed	1	HxH	222	73.4		164	163		1.97 w **
				WxW	220	37.3		88	82		1.00
				Total	442	55.4	96.8	252	245		
			2	HxH	213	66.7		142	142		1.37 w **
				WxW	211	48.8		105	103		1.00
				Total	424	57.8	99.1	247	245		
			Mean	HxH		70.0					1.63
				WxW		43.0					1.00
				Total		56.6	97.9				
		Separate	3	HxH	429	0.0[b]					
			4	HxH	415	78.8	98.7	224	221		
			5	WxW	442	54.1	96.9	225	222		
			6	WxW	397	56.9	97.7	227	216		
			Mean	HxH		78.8	98.7				1.42 w *
				WxW		55.5	97.3				1.00

[a]Means not given due to inconsistencies between first and second samples (see text).
[b]All of the fish died from an unknown cause in late September.
[c]Vessel 8 excluded because it received a higher ration than the other vessels (Figure 3).
[d]P=0.0504.

Table 4, continued.

Year-class	Hatchery	Treatment	Vessel	Cross	Mean (SD) fork length (mm) First sample	Mean (SD) fork length (mm) Second sample	Mean (SD) K_n, second sample	Mature males (%)	n PIT-tagged	Detection rate (%)
1994	Clearwater	Mixed	1	HxH	149 (18)	193 (18)	1.019 (0.056)	7.9	127	55.9
				WxW	120 (13)	154 (23)	0.977 (0.047)	5.2	173	9.2
				Total					300	
			2	HxH	142 (20)	194 (17)	1.020 (0.043)	1.2	163	59.5
				WxW	113 (10)	151 (22)	0.985 (0.046)	1.5	137	9.5
				Total					300	
			Mean	HxH	145 (19) w **	194 (18) w **	1.020 (0.049) w *	4.6 w ns		57.7 w **
				WxW	116 (11)	153 (23)	0.981 (0.046)	3.3		9.4
				Total	y *	y **				
					z ns	z **				
		Separate	3	HxH	135 (17)	177 (19)	1.010 (0.049)	5.3	300	42.7
			4	HxH	131 (17)	181 (18)	1.033 (0.049)	2.3	300	41.7
			5	WxW	127 (15)	162 (26)	0.961 (0.049)	10.0	298	17.8
			6	WxW	123 (15)	164 (25)	1.010 (0.049)	10.3	297	19.9
			Mean	HxH	133 (17) w *	179 (18) w **	1.022 (0.049) w ns	3.8 w *		42.2 w **
				WxW	125 (15)	163 (26)	0.985 (0.049)	10.2		18.8
1995	Clearwater	Mixed	1	HxH	137 (21)	220 (26)	1.023 (0.060)	9.0	143	57.3
				HxW	116 (18)	199 (27)	1.008 (0.063)	16.7	145	40.7
				WxW	98 (11)	166 (25)	0.998 (0.062)	5.6	108	5.6
				Total					396	
			2	HxH	140 (16)	216 (24)	1.012 (0.061)	16.5	141	45.4
				HxW	117 (15)	196 (27)	1.001 (0.060)	23.1	119	30.3
				WxW	109 (11)	172 (26)	0.976 (0.047)	17.9	140	7.1
				Total					400	
			Mean	HxH	139 (19) w **	218 (25) w **	1.017 (0.061) w *	12.8 w ns		51.4 w **
				HxW	117 (16) x *	197 (27) x **	1.004 (0.062) x ns	19.9 x ns		35.5 x **
				WxW	104 (11)	169 (26)	0.987 (0.055)	11.8		6.3
				Total	y **	y **				
					z ns	z ns				
		Separate	3	HxH	122 (17)	199 (20)	1.036 (0.070)	11.0	266	52.6
			4	HxH	126 (18)	200 (22)	1.019 (0.060)	12.1	265	48.3
			5	HxW	118 (15)	200 (25)	0.982 (0.075)	21.0	265	39.2
			6	HxW	117 (16)	200 (21)	0.997 (0.054)	19.9	264	35.2
			7	WxW	114 (16)	188 (26)	0.996 (0.072)	29.6	262	17.2
			8	WxW	116 (12)	198 (26)	0.988 (0.050)	30.8	262	13.4
			Mean	HxH	124 (18) w ns	200 (21) w **	1.027 (0.065) w *	11.5 w **		50.5 w **
				HxW	118 (16) x ns	200 (23) x *	0.990 (0.065) x *	20.5 x **		37.2 x **
				WxW	114 (14)[c]	188 (26)[c]	0.992 (0.061)	30.2		15.3
	Dworshak	Mixed	1	HxH	137 (19)	155 (23)	1.025 (0.056)	0.7	141	11.3
				WxW	105 (12)	112 (17)	0.984 (0.084)	0.0	69	1.4
				Total					210	
			2	HxH	137 (20)	159 (23)	1.011 (0.065)	0.0	124	11.3
				WxW	103 (12)	112 (17)	0.986 (0.075)	0.0	89	0.0
				Total					213	
			Mean	HxH	137 (19) w **	157 (23) w **	1.018 (0.060) w ns	0.4 w ns		11.3 w **
				WxW	104 (12)	112 (17)	0.985 (0.079)	0.0		0.7
				Total	y ns	y ns				
					z **	z **				
		Separate	3	HxH						
			4	HxH	137 (22)	160 (25)	1.029 (0.053)	0.5	220	14.5
			5	WxW	109 (13)	122 (18)	0.993 (0.098)	1.4	219	0.0
			6	WxW	111 (14)	125 (20)	0.958 (0.088)	0.0	217	1.4
			Mean	HxH	137 (22) w **	160 (25) w *[d]	1.029 (0.053) w ns	0.5 w ns		14.5 w ns
				WxW	110 (14)	123 (19)	0.976 (0.093)	0.7		0.7

[a] Means not given due to inconsistencies between first and second samples (see text).
[b] All of the fish died from an unknown cause in late September.
[c] Vessel 8 excluded because it received a higher ration than the other vessels (Figure 3).
[d] P=0.0504.

Table 5. Survival and detection efficiency estimates for different river segments, and the proportion of fish that didn't leave Crooked River (CR), or left CR but didn't arrive at Lower Granite Dam (LGR). Detection efficiency = proportion of tagged fish passing a dam that were detected there. LGO = Little Goose Dam and LMN = Lower Monumental Dam. Missing entries for Dworshak fish indicate an insufficient number of detections for reliable estimates.

					Proportion (SE) of *N* released that:							
								Survival (SE) between dams[a]		Detection efficiency (SE) at each dam[a]		
Year-class	Hatchery	Release year	Cross	N released	Arrived at LGR[a]	Did not leave CR[b]	Left CR but didn't arrive at LGR[c]	LGR-LGO	LGO-LMN	LGR	LGO	LMN
1994	Clearwater	1996	HxH	23,580	0.62 (0.03)	-0.04 (0.18)	---	0.94 (0.09)	0.90 (0.15)	0.46 (0.03)	0.29 (0.03)	0.39 (0.06)
			WxW	22,438	0.20 (0.02)	0.74 (0.05)	---	0.85 (0.13)	0.69 (0.17)	0.49 (0.05)	0.37 (0.06)	0.51 (0.12)
			Total	46,018	0.41 (0.02)	0.38 (0.04)	0.21	0.92 (0.07)	0.85 (0.12)	0.47 (0.02)	0.30 (0.03)	0.42 (0.05)
1995	Dworshak	1996	HxH	554	---	---	---	---	---	---	---	---
			WxW	624	---	---	---	---	---	---	---	---
			Total	1,178	0.08 (0.01)	0.86 (0.06)	0.06	---	---	---	---	---
	Clearwater	1997	HxH	28,308	0.63 (0.03)	0.33 (0.09)		0.90 (0.06)	0.78 (0.08)	0.42 (0.03)	0.46 (0.03)	0.61 (0.06)
			HxW	25,715	0.42 (0.02)	0.54 (0.07)		1.04 (0.08)	0.71 (0.09)	0.53 (0.03)	0.41 (0.04)	0.59 (0.07)
			WxW	22,157	0.15 (0.02)	0.69 (0.28)		0.94 (0.13)	0.92 (0.31)	0.48 (0.06)	0.49 (0.07)	0.42 (0.14)
			Total	76,180	0.40 (0.01)	0.51 (0.05)	0.09	0.96 (0.04)	0.76 (0.06)	0.47 (0.02)	0.45 (0.02)	0.58 (0.04)

[a]Survival from release to successive Snake River dams and detection efficiency at each dam were obtained using formulae of Smith et al. (1994).
[b]The proportion of fish that did not leave CR was obtained from formulae of Ricker (1975) (see text).
[c]Equals one minus the proportion of fish that arrived at LGR minus the proportion of fish that didn't leave CR; given for total releases only due to the greater precision of those estimates.

Table 6. Analysis of variance models used to evaluate performance in the hatchery and downstream migration after release. Asterisks indicate crossed effects and parentheses nested effects. Vessel was designated a random effect; all other factors were fixed. Recall that treatment refers to allopatry (crosses separate from each other) versus sympatry (crosses mixed).

Main effect	Response	Transformation	Conditions	Model
Cross	Survival, maturity, or detection rate	Arcsine	Separate treatment	Cross
	Length or condition factor	None	Mixed treatment; first or second sample	Cross; vessel; cross*vessel
		None	Separate treatment; first or second sample	Cross; vessel(cross)
	Difference between first and second samples in SD of length	None	Mixed or separate treatment	Cross
Treatment	Length	None	HxH or WxW; first or second sample	Treatment; vessel(treatment)
Hatchery	Relative growth	None	1995 year-class	Hatchery; treatment

Table 7. The ratio of ration (D; g dry weight/fish/day) to the maximum ration that could be consumed (D_{max}).

Hatchery	Year-class	D/D_{max} Mean	Minimum	Percent of time <1
Clearwater	1994	1.61	0.28	37.4
	1995	1.74	0.29	40.5
DNFH	1995	4.82	1.29	0.0

Table 8. Effect of hatchery program on in-hatchery survival and growth in length of HxH relative to WxW fish. Relative survival values for mixed and separate treatments are from Table 4 and are not given for mixed treatments at Clearwater Hatchery due to inconsistencies between the first and second samples (see text). The formula used to compute relative growth is given in text. For the 1995 year-class, mean relative growth was similar between hatcheries (ns = P>0.1).

Year-class	Treatment	Hatchery	Relative survival	Relative growth
1994	Mixed	Clearwater	---	1.32
	Separate	Clearwater	1.06	1.11
	Mean	Clearwater	---	1.22
1995	Mixed	Clearwater	---	1.34
		Dworshak	1.63	1.52
	Separate	Clearwater	1.28	1.07
		Dworshak	1.42	1.38
	Mean	Clearwater	---	1.21 ns
		Dworshak	1.52	1.45

Table 9. Fry-to-adult survival, length, and capture date of adult returns from Clearwater Hatchery releases. Letters indicate paired comparisons: a=HxH versus WxW and b=HxW versus HxH. Asterisks indicate significance: ** = $P<0.05$, * = $0.05<P<0.10$, and ns = $P>0.10$. Length and capture date were tested with one-way AOV. CR = Crooked River; LGR = Lower Granite Dam. Returns to LGR were of fish PIT-tagged before release from the hatchery and then detected as they moved upstream past that dam. Dworshak Hatchery was not monitored for our experimental adults in return year 1999.

							Fork length (cm)		Capture date	
Year-class	Sex	Return year	Return site	Cross	Number of fish	Relative survival	Mean	SD	Mean	SD (days)
1994	Female	1999	CR	HxH	0	0.00 a ns	---	---	---	---
				WxW	3[a]	1.00	76.3	3.5	25-Apr	0.6
1995	Female	2000	CR	HxH	1	---	82.0	---	21-Apr	---
				HxW	2	---	78.5	0.7	23-Apr	12.7
				WxW	1	---	78.0	---	15-Apr	---
			Dworshak	HxH	3	---	79.3	1.2	30-Mar	14.6
				HxW	1	---	78.0	---	4-Apr	---
				WxW	1	---	76.0	---	18-Apr	---
			Pooled	HxH	4	---	80.0	1.6	4-Apr	16.1
				HxW	3	---	78.3	0.6	16-Apr	14.2
				WxW	2	---	77.0	1.4	16-Apr	2.1
	Male	1999	CR	HxH	1	---	68.0	---	26-Apr	---
		2000	CR	HxH	3	---	82.3	2.5	21-Apr	15.3
				HxW	1	---	76.0	---	8-Apr	---
			Dworshak	HxH	1	---	85.0	---	18-Apr	---
			Pooled	HxH	4	---	83.0	2.4	20-Apr	12.6
				HxW	1	---	76.0	---	8-Apr	---
	Unknown	2000	LGR	HxH	2[b]	---	---	---	---	---
	Pooled	2000	Not LGR	HxH	8	---	81.5 a **	2.5	12-Apr a ns	15.9
				HxW	4	---	77.8 b **	1.3	14-Apr b ns	12.4
				WxW	2	---	77.0	1.4	16-Apr	2.1
		Pooled	Pooled	HxH	11	5.49 a **	---	---	---	---
				HxW	4	2.00 b ns	---	---	---	---
				WxW	2	1.00	---	---	---	---

[a]These three fish were the only 1994 year-class adult returns.
[b]Neither of these fish were encountered at CR or at Dworshak.

Figures

Figure legends

Figure 1. Study area. Wild adults were collected at Selway Falls in the lower Selway River and held at Kamiah Ponds until ripe. Hatchery adults were collected at Dworshak National Fish Hatchery. The hatchery population was initiated exclusively with wild adults returning to the North Fork Clearwater River. Progeny were reared at Clearwater Hatchery for two years or at Dworshak Hatchery for one year. The experimental fish were then released into Crooked River, tributary to the South Fork Clearwater River.

Figure 2. Length frequencies of hatchery (H) and wild (W) adults spawned to create progeny groups. Text in panels designates return year, sex, and mean (SD) fork length (cm). Vertical dashed lines are boundaries between ocean ages (number of winters spent in the ocean or "salt") based on historical length frequencies of known age hatchery adults. Wild adults were aged from otoliths. Three wild females from the 1994 return year (one each at 83, 86, and 90 cm fork length) were 3-salts and the rest of the wild adults were 2-salts. Smolt age composition of the wild adults was 80% age-3 and 20% age-4 for combined return years and sexes.

Figure 3. Mean length after 21 months of rearing in vessels at Clearwater Hatchery plotted against the mean of the 21 monthly ration indices. Each data point represents a cross within a vessel. Mean ration index is equivalent for crosses reared together in a mixed vessel because the ration index applies to the ration delivered to that vessel. Replicate vessels are connected by lines. See text for ration index computational formula.

Figure 4. Length frequencies of yearlings and two-year old at Clearwater Hatchery. Yearlings were sampled after 15 months (1994 year-class) or 12 months (1995 year-class), and two-year-olds after 21 months (both year-classes).

Figure 5. Lengths of age-2 mature males and immature fish at Clearwater Hatchery. Lines within boxes designate medians, box edges 25th and 75th percentiles, whiskers 10th and 90th percentiles, and points 5th and 95th percentiles.

Figure 6. Percentage of mature males versus fish length for two-year olds at Clearwater Hatchery. Each data point represents mean (+SE) maturity within a length class plotted against median length within that class. Length classes are <171, 171-180, 181-190, and >190 mm for the 1994 year-class, and <171, 171-180, 181-190, 191-200, 201-210, and >210 mm for the 1995 year-class. Lines not connecting data points represent relations between maturity and fish length from logistic regressions.

Figure 7. Mean length versus sampling date. Each line represents a rearing vessel.

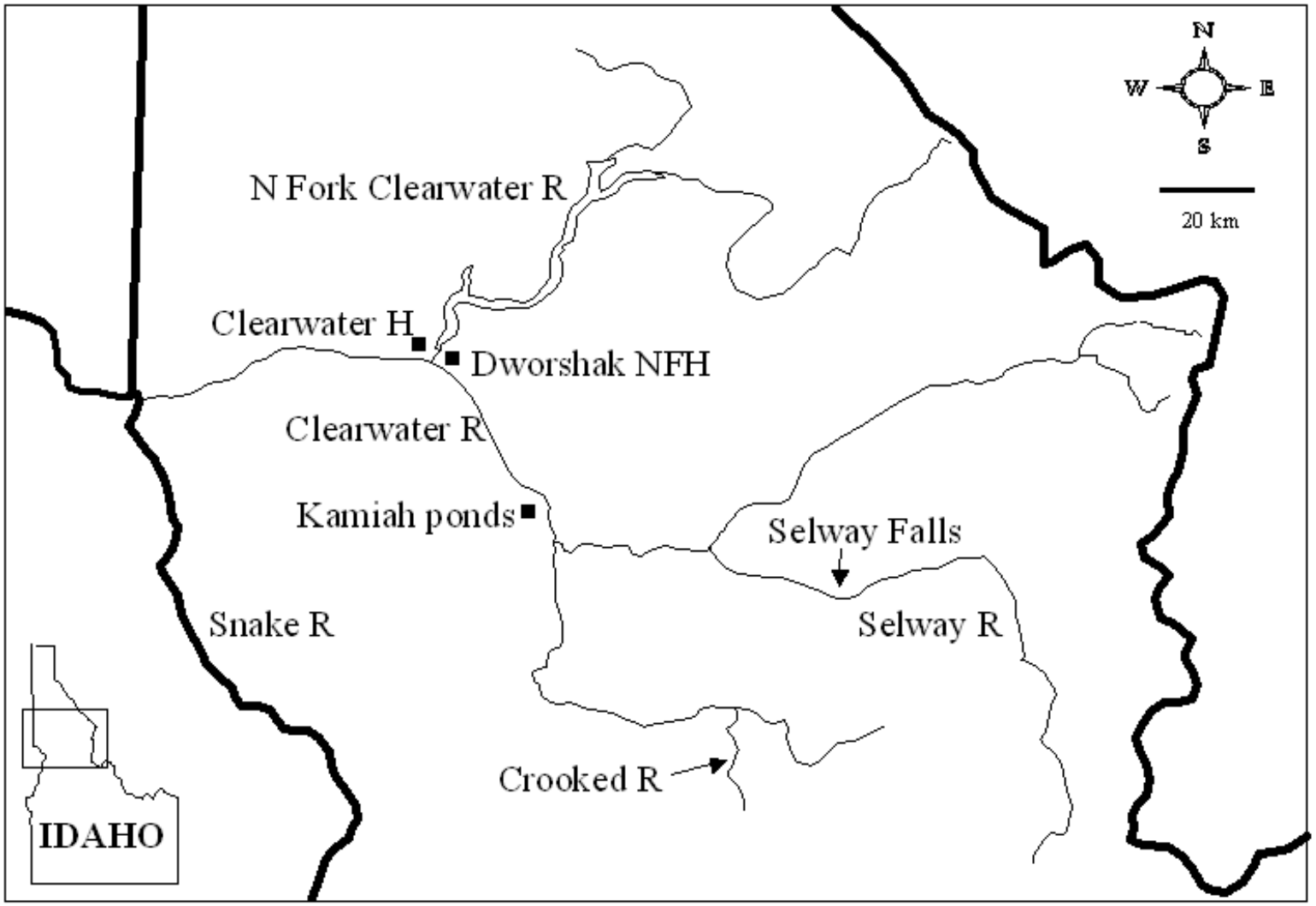
Figure 8. Lengths at tagging of all fish that were released, and of the subset of fish that were detected at the dams. Lines within boxes designate medians, box edges 25th and 75th percentiles, whiskers 10th and 90th percentiles, and points 5th and 95th percentiles.

Figure 9. Detection rate versus fish length for two-year olds at Clearwater Hatchery. Each data point represents mean (+SE) detection rate within a length class plotted against median length within that class. Length classes are <171, 171-180, 181-190, 191-200, and >200 mm for the 1994 year-class, and <181, 181-190, 191-200, 201-210, and >210 mm for the 1995 year-class. Line not connecting data points represent relations between detection rate and fish length from logistic regressions.

Figure 10. Lengths and condition factors for the following groups and times: (1) age-2 fish naturally reared from conception or from the button-up fry stage in Crooked River, captured in Crooked River in August 1996 and measured then; (2) 1994 year-class HxH fish released in Crooked River in April 1996 but not detected at the dams, measured in March 1996 when tagged; (3) residuals from the 1994 year-class HxH release, captured in Crooked River in August 1996 and measured then; (4 and 5) same as 2 and 3 except for WxW fish; and (6 and 7) same as 4 and 5 but for a subset of fish that were tagged at the hatchery and recaptured in Crooked River. Note that group 6 is included in group 4 and group 7 in group 5. An estimated 86% of the fish in group 1 (naturally reared two-year olds) were released in Crooked River in June 1994 as button-up fry and were siblings of fish reared at the hatchery for two years and then released in Crooked River in 1996 (see text). Lines within boxes designate medians, box edges 25th and 75th percentiles, whiskers 10th and 90th percentiles, and points 5th and 95th percentiles. Numbers above boxes indicate sample size.

Figure 11. Density (from snorkel counts) of yearling and older steelhead, and the proportion of those steelhead that were residuals (upper panel); and mean condition factor of residuals and naturally reared two-year olds (lower panel); in relation to distance from the site where the residuals had been released. Fish were counted in July (Idaho Department of Fish and Game; unpublished data) and captured (for proportion residuals and condition factor) in August 1996. Negative distances are upstream and positive distances downstream from the release site. Each data point in the lower panel represents the mean of $\geq$3 fish.

Figure 12. Length frequencies of steelhead early in the Dworshak hatchery program and of our experimental fish. The upper panel shows 1979 and 1980 year-class fish, progeny of adults returning to Dworshak Hatchery, that were reared at Dworshak Hatchery for two years (data from Bjornn and Ringe 1984). The lower two panels show our 1994 and 1995 year-class experimental fish reared in the separate treatment at Clearwater Hatchery for two years. The 1979 and 1980 year-classes were removed by two generations of hatchery rearing from the original North Fork Clearwater River wild population, and our 1994 and 1995 year-class HxH fish were removed by six generations.


N
W
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20 km
N Fork Clearwater R
Clearwater H
Dworshak NFH
Clearwater R
Kamiah ponds
Selway Falls
Snake R
Selway R
Crooked R
IDAHO

Figure 1.

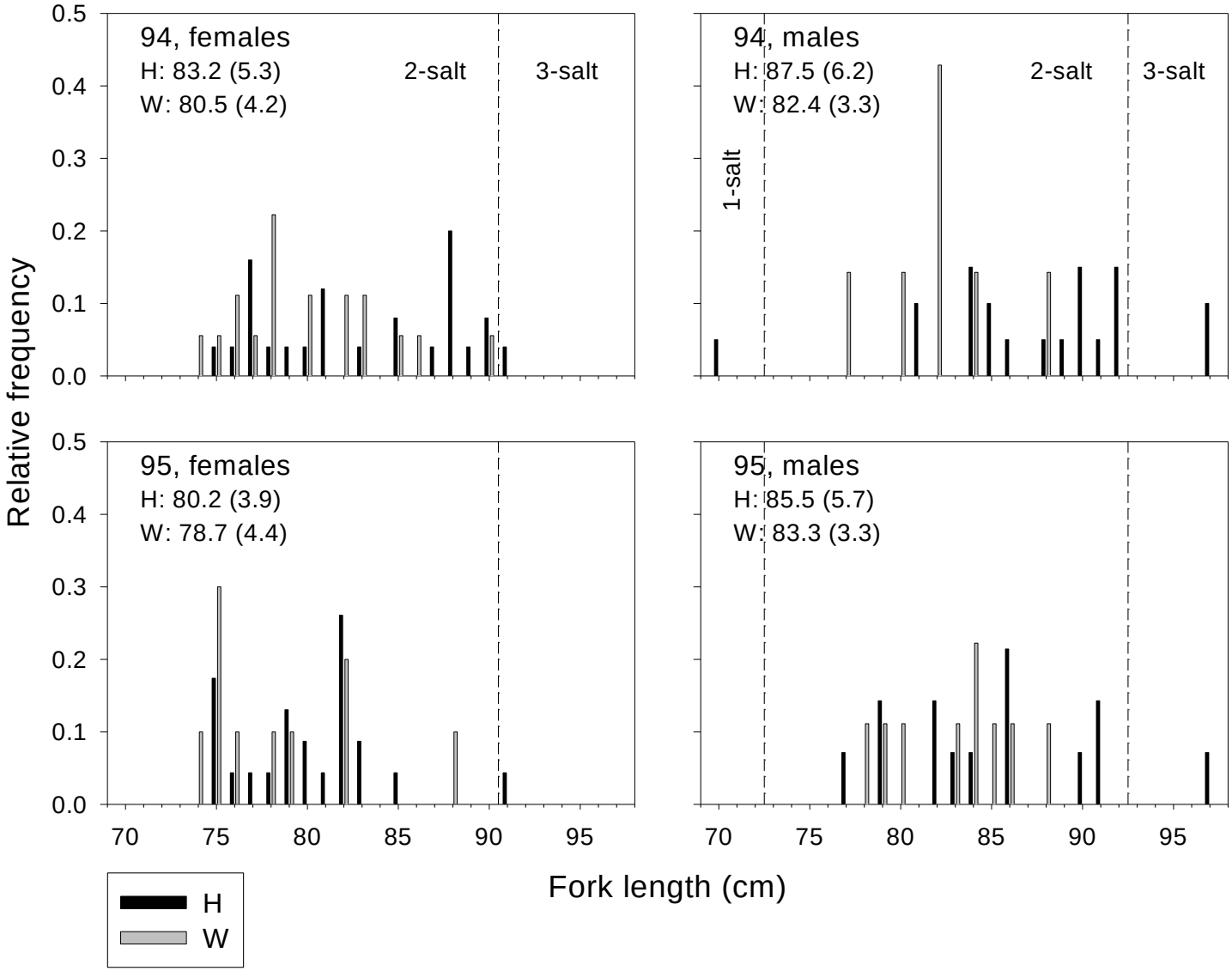
94, females
H: 83.2 (5.3)
W: 80.5 (4.2)
2-salt
3-salt
94, males
H: 87.5 (6.2)
W: 82.4 (3.3)
1-salt
2-salt
3-salt
95, females
H: 80.2 (3.9)
W: 78.7 (4.4)
95, males
H: 85.5 (5.7)
W: 83.3 (3.3)
Relative frequency
0.0
0.1
0.2
0.3
0.4
0.5
70
75
80
85
90
95
Fork length (cm)
H
W

Figure 2.

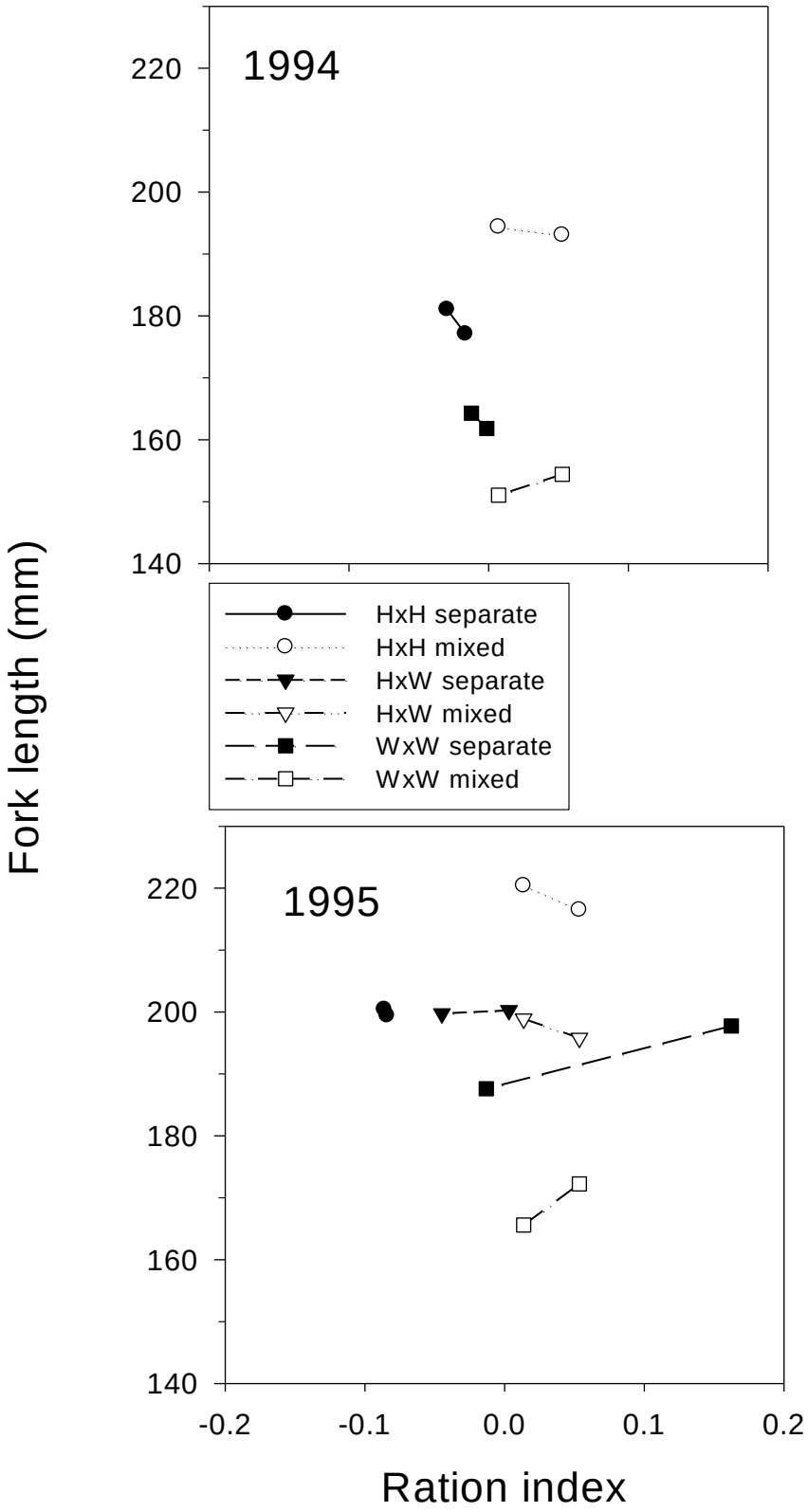
1994
1995
220
200
180
160
140
-0.2
-0.1
0.0
0.1
0.2
Fork length (mm)
Ration index
HxH separate
HxH mixed
HxW separate
HxW mixed
WxW separate
WxW mixed

Figure 3.

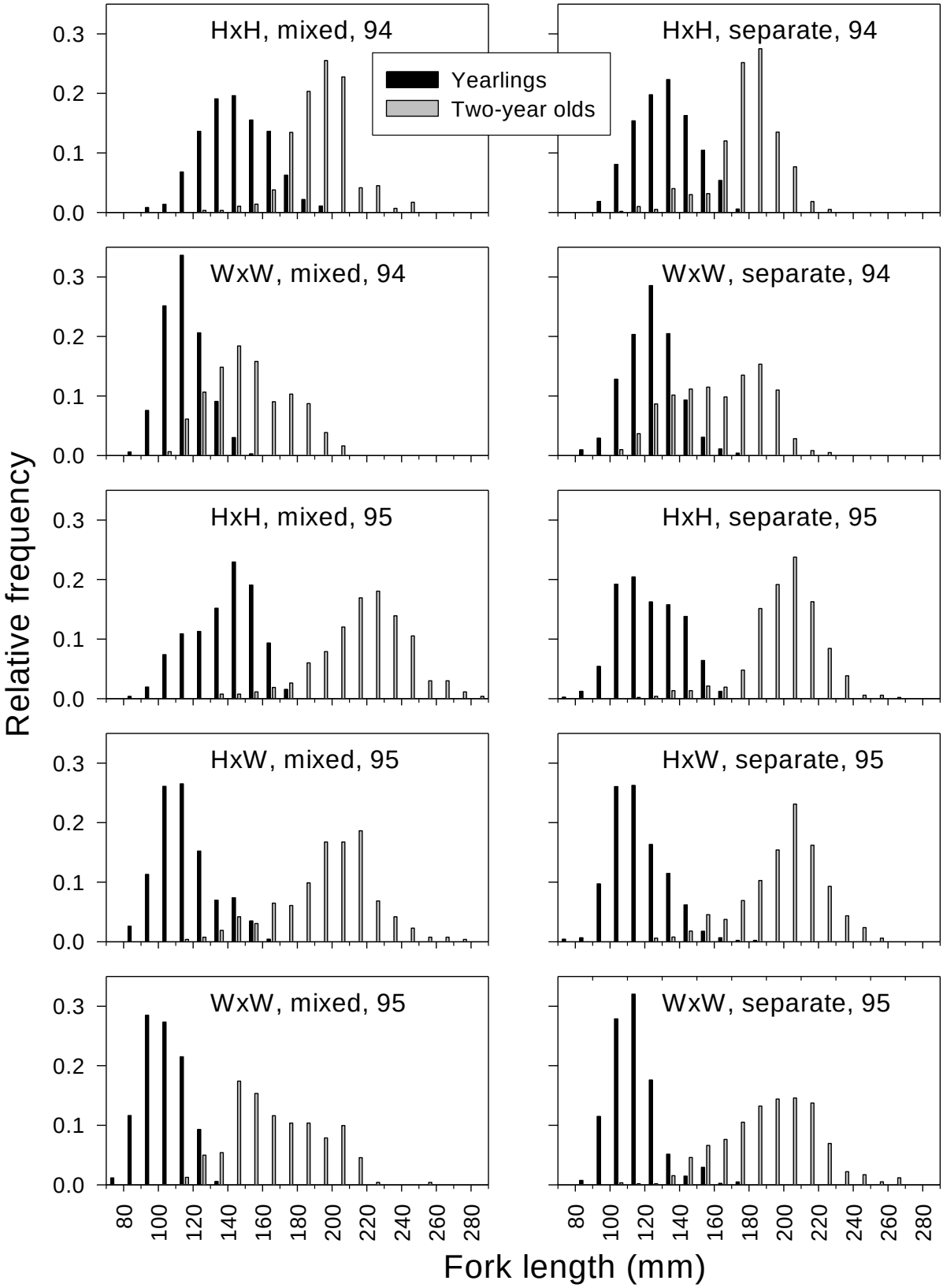
HxH, mixed, 94
HxH, separate, 94
Yearlings
Two-year olds
WxW, mixed, 94
WxW, separate, 94
HxH, mixed, 95
HxH, separate, 95
HxW, mixed, 95
HxW, separate, 95
WxW, mixed, 95
WxW, separate, 95
Relative frequency
0.0
0.1
0.2
0.3
80
100
120
140
160
180
200
220
240
260
280
Fork length (mm)

Figure 4.

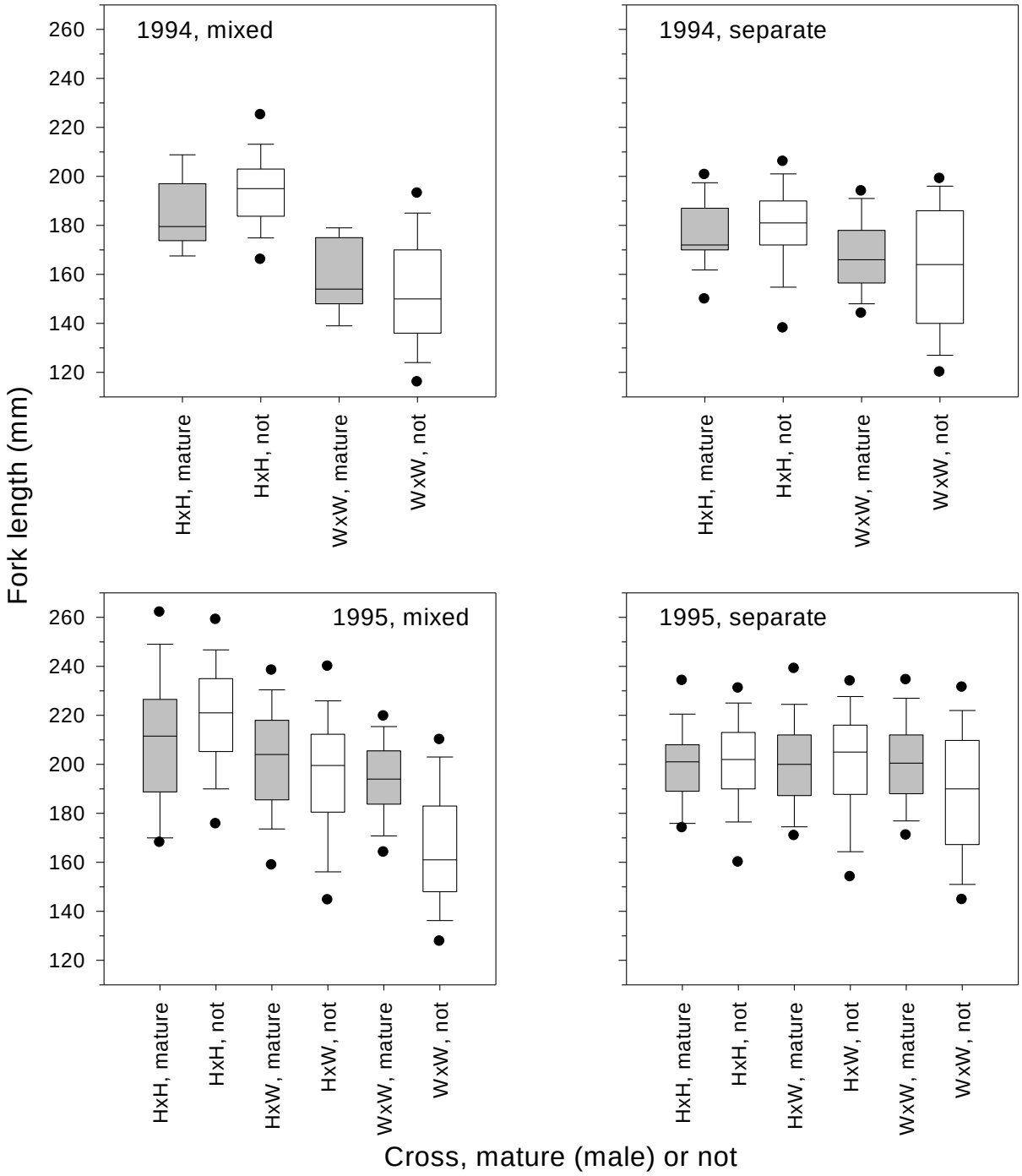
1994, mixed
1994, separate
1995, mixed
1995, separate
Fork length (mm)
260
240
220
200
180
160
140
120
HxH, mature
HxH, not
HxW, mature
HxW, not
WxW, mature
WxW, not
Cross, mature (male) or not

Figure 5.

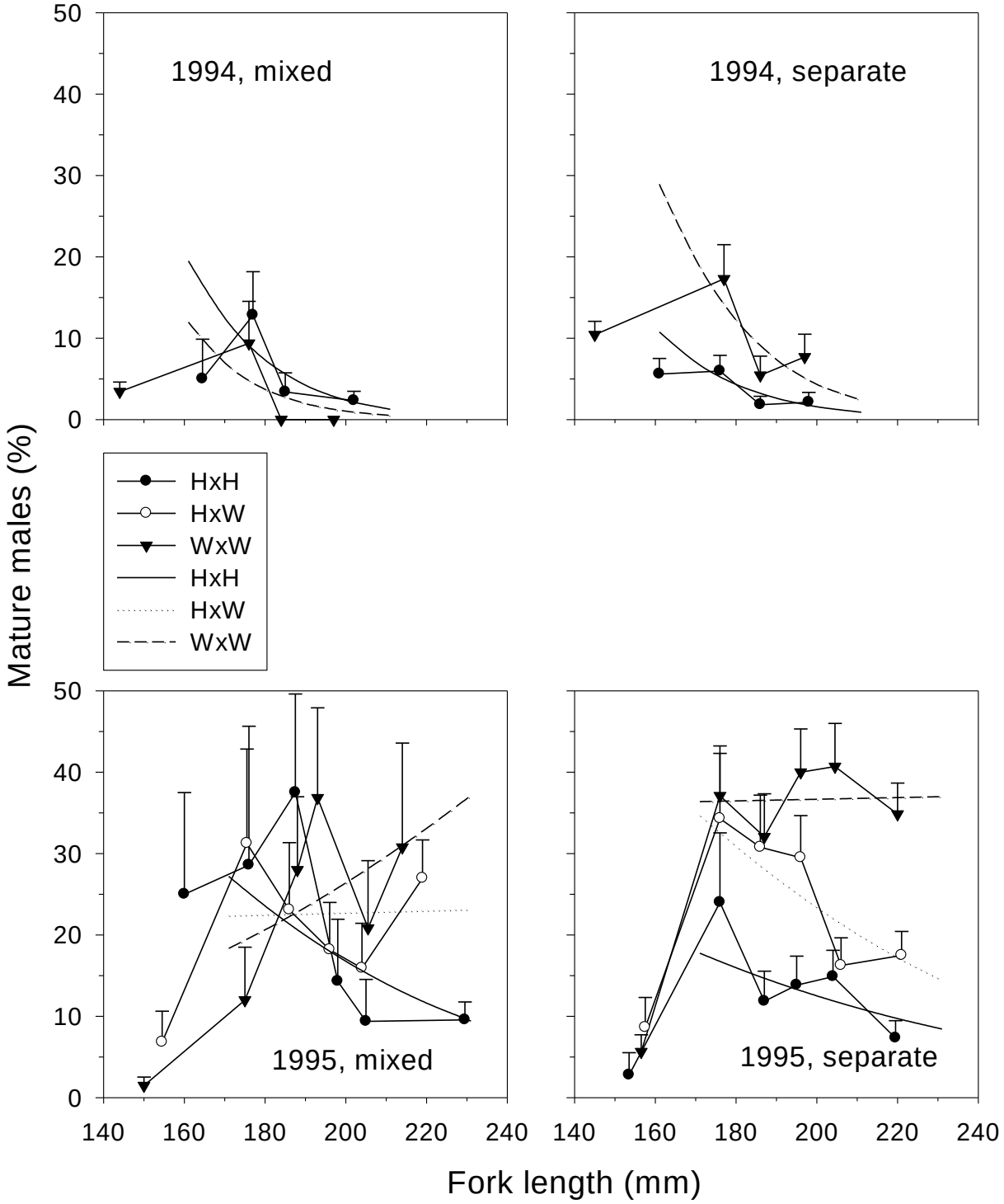
1994, mixed
1994, separate
1995, mixed
1995, separate
HxH
HxW
WxW
HxH
HxW
WxW
Mature males (%)
Fork length (mm)
0
10
20
30
40
50
140
160
180
200
220
240

Figure 6.

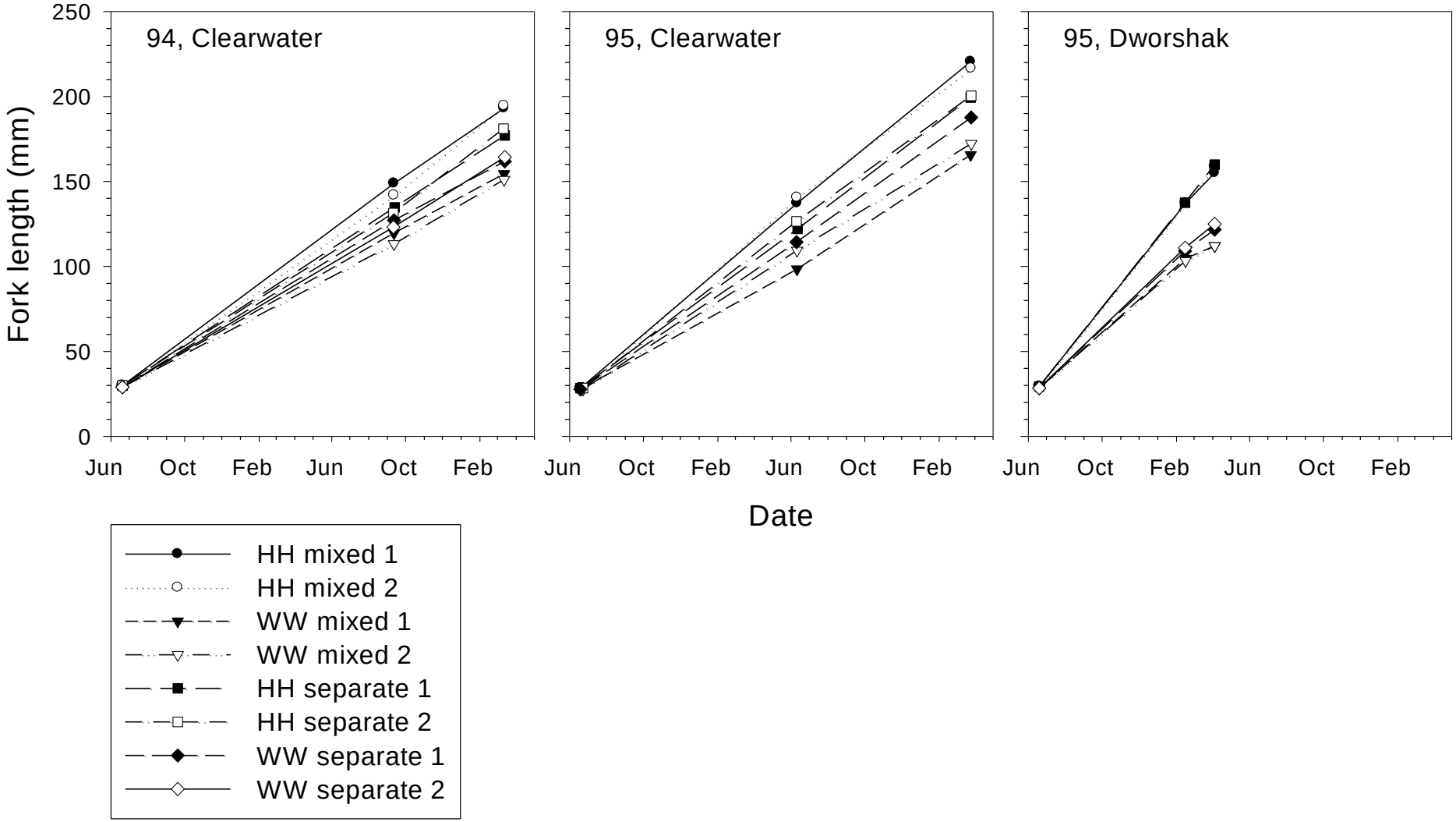
94, Clearwater
95, Clearwater
95, Dworshak
Fork length (mm)
250
200
150
100
50
0
Jun
Oct
Feb
Date
HH mixed 1
HH mixed 2
WW mixed 1
WW mixed 2
HH separate 1
HH separate 2
WW separate 1
WW separate 2

Figure 7.

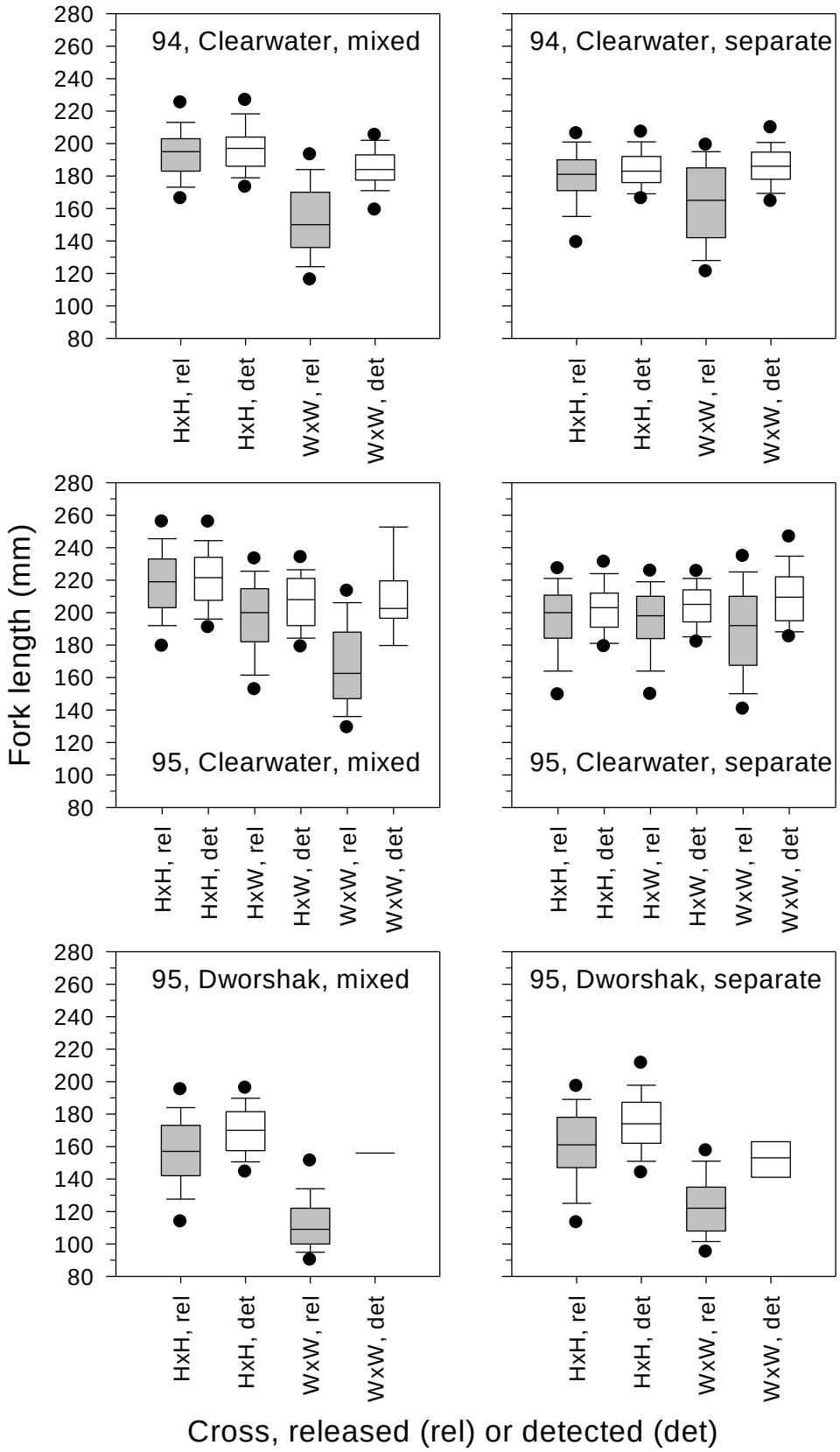
94, Clearwater, mixed
94, Clearwater, separate
95, Clearwater, mixed
95, Clearwater, separate
95, Dworshak, mixed
95, Dworshak, separate
Fork length (mm)
280
260
240
220
200
180
160
140
120
100
80
HxH, rel
HxH, det
HxW, rel
HxW, det
WxW, rel
WxW, det
Cross, released (rel) or detected (det)

Figure 8.

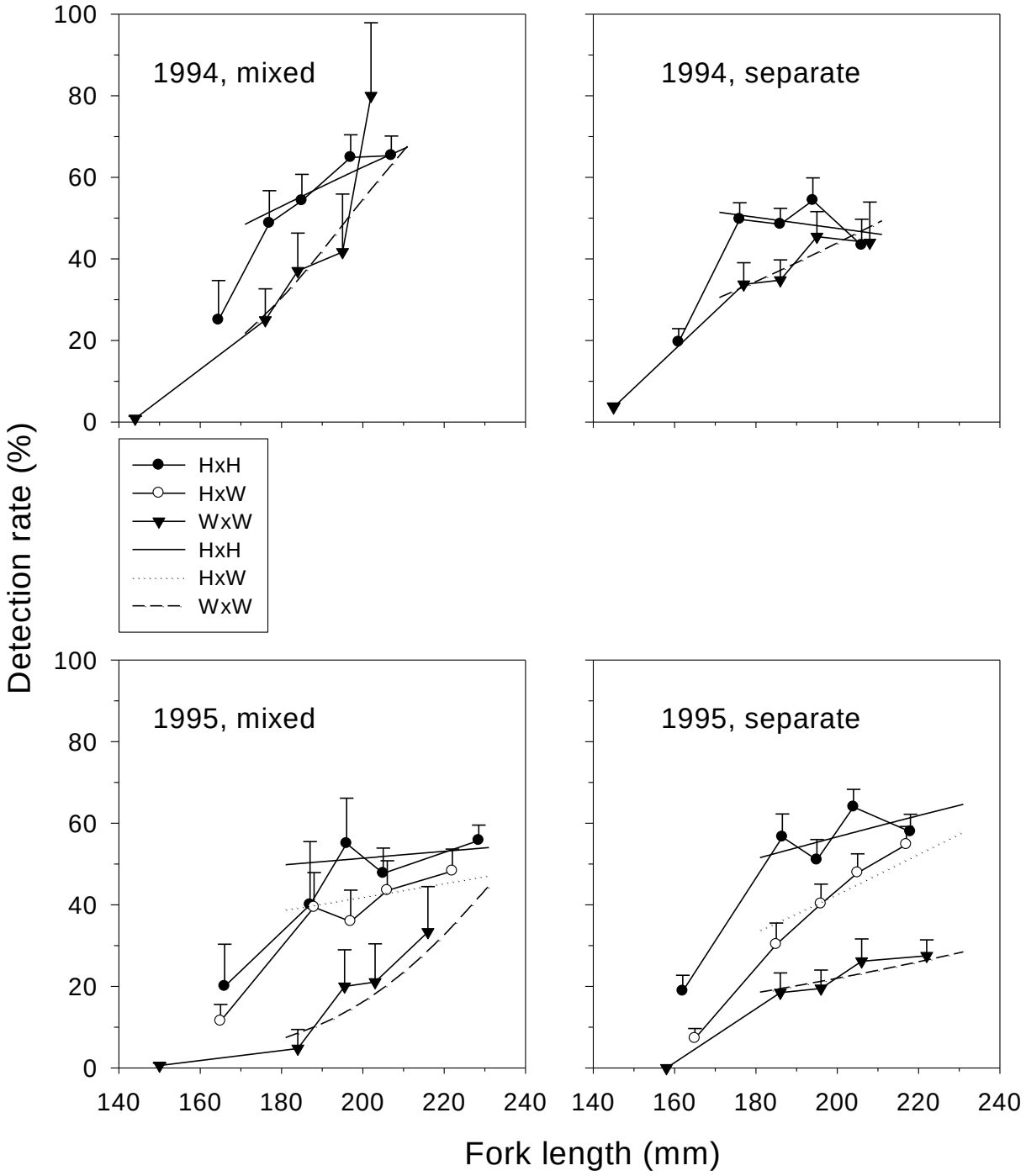
1994, mixed
1994, separate
1995, mixed
1995, separate
HxH
HxW
WxW
HxH
HxW
WxW
Detection rate (%)
Fork length (mm)
0
20
40
60
80
100
140
160
180
200
220
240

Figure 9.

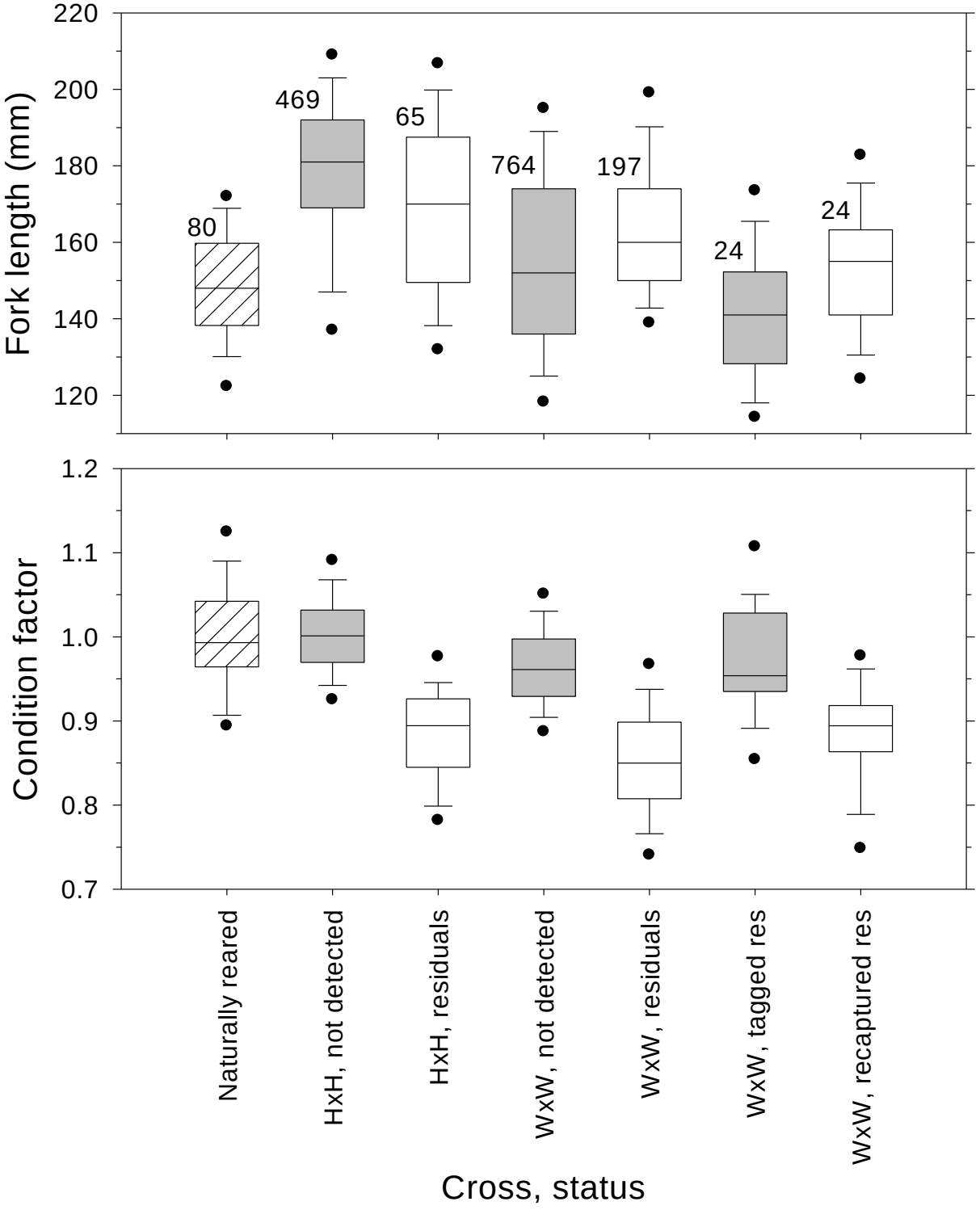
Fork length (mm)
220
200
180
160
140
120
80
469
65
764
197
24
24
Condition factor
1.2
1.1
1.0
0.9
0.8
0.7
Naturally reared
HxH, not detected
HxH, residuals
WxW, not detected
WxW, residuals
WxW, tagged res
WxW, recaptured res
Cross, status

Figure 10.

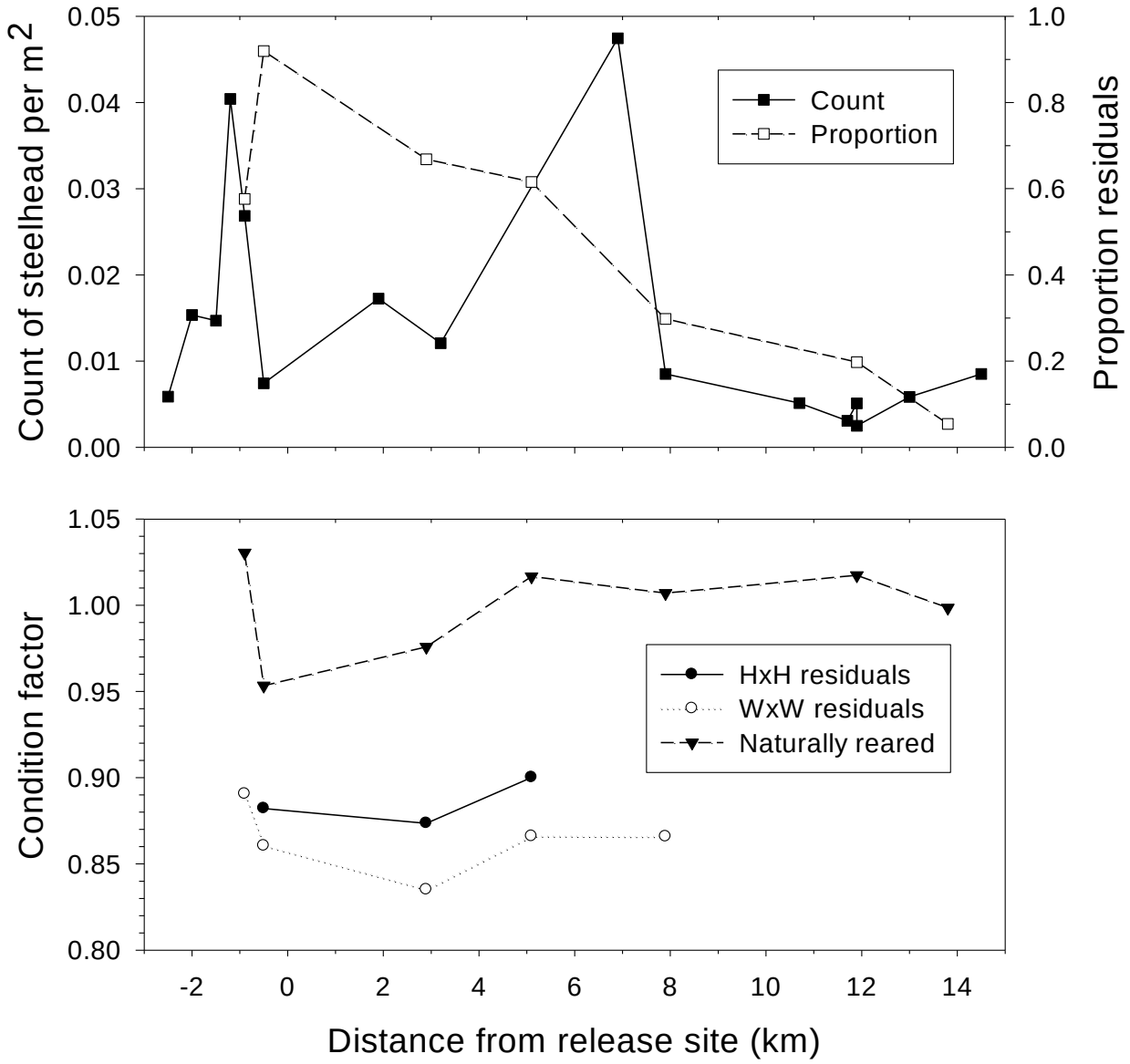
Count of steelhead per m2
Proportion residuals
Count
Proportion
0.05
0.04
0.03
0.02
0.01
0.00
1.0
0.8
0.6
0.4
0.2
0.0
Condition factor
HxH residuals
WxW residuals
Naturally reared
1.05
1.00
0.95
0.90
0.85
0.80
-2
0
2
4
6
8
10
12
14
Distance from release site (km)

Figure 11.

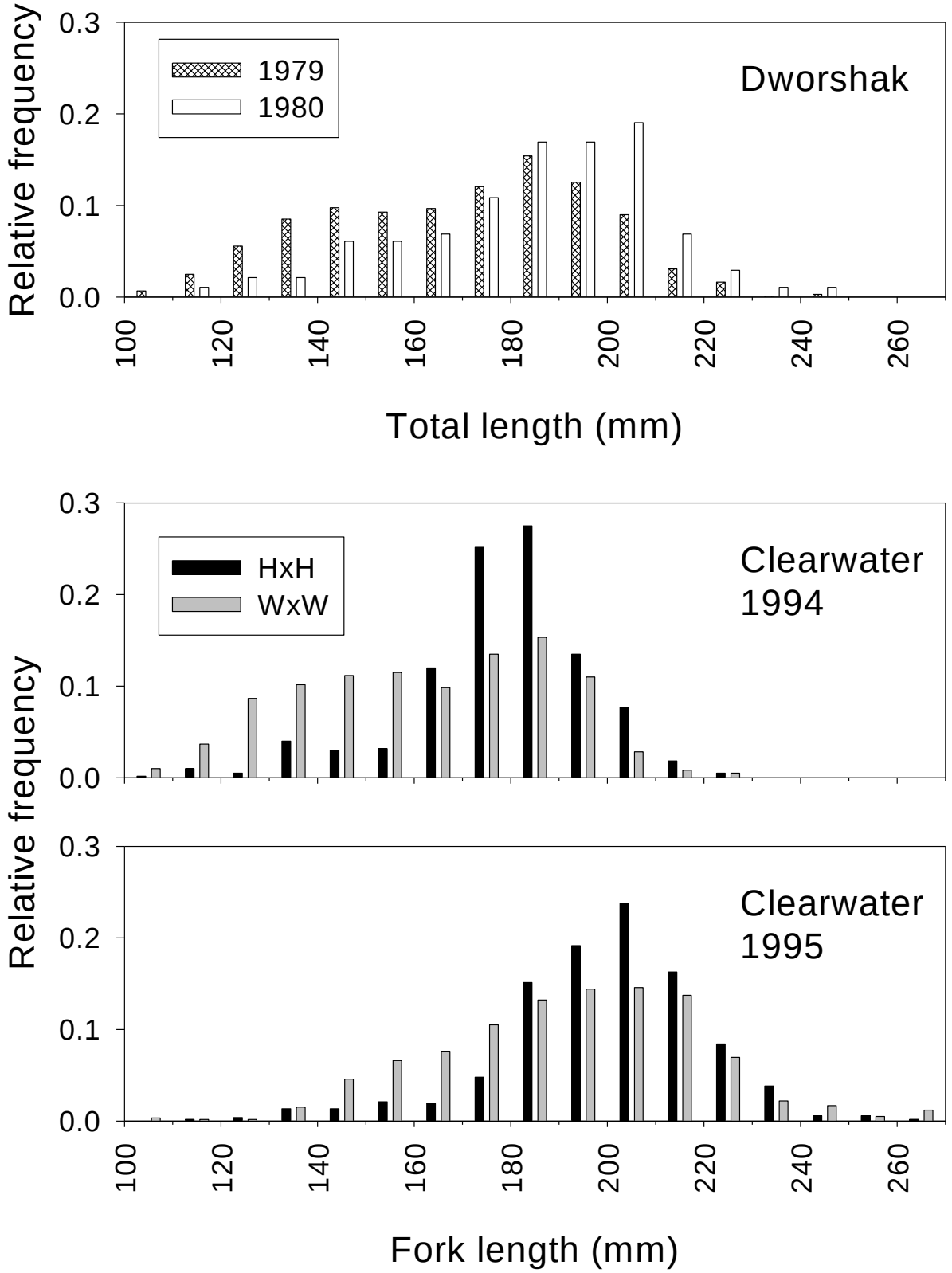
Relative frequency
0.3
0.2
0.1
0.0
1979
1980
Dworshak
100
120
140
160
180
200
220
240
260
Total length (mm)
HxH
WxW
Clearwater
1994
Clearwater
1995
Fork length (mm)

Figure 12.

Chapter 3: Genetic differences between hatchery and wild steelhead for survival, growth, dispersal, and male maturation in a natural stream *(Study site: Twenty-Mile Creek; Stocks: Dworshak hatchery and Selway River wild; Year classes: 1994 and 1995)*

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Abstract

This study was initiated in the early 1990s to provide managers with data comparing genetic fitness for natural rearing, as measured by survival of juveniles in freshwater, between steelhead *Oncorhynchus mykiss* from Dworshak National Fish Hatchery and wild steelhead from the Clearwater River, Idaho. We artificially spawned hatchery steelhead and wild steelhead from the Selway River, a Clearwater River tributary, released the resulting genetically marked (at the *PEPA* allozyme locus) progeny (HxH, HxW from hatchery females and wild males, and WxW) as unfed fry in a second order tributary of the South Fork Clearwater River, and monitored fish residing in the stream or emigrating from it for five years. Barrier falls prevented access to the stream by naturally produced steelhead. Over 90% of the emigrants were one or two years of age and too small to be smolts (mean fork length at age-2 = 103 mm). Per fry released, the HxH cross produced 0.64-0.83 times as many emigrants as the WxW cross ($P<0.05$). The HxH cross produced 0.63 times as many age-4 residuals as the WxW cross for one year-class ($P=0.051$) and 0.68 times as many for the other (ns). Survival from age-1 to age-4 was lower for HxH than for WxW residuals of one year-class ($P<0.05$) and survival from age-2 to age-4 may have been lower for HxH than for WxW residuals of the other ($P=0.062$). Collectively, these results indicate lower survival for HxH than for WxW fish. Size was often greater for HxH than for WxW fish indicating faster growth for the former, and condition factor was also usually greater for HxH than for WxW fish. Dispersal of fry from release sites and emigration of one- and two-year olds from the study stream were greater for WxW than for HxH fish, and apparently neither was from competitive displacement of small by larger fish. Incidence of flowing milt was higher for HxH than for WxW fish at age-2. Peak incidence of flowing milt for older residuals was similar among crosses (about 50%), but the peak occurred at greater size and age for WxW than for HxH residuals. HxW fish were intermediate between HxH and WxW fish, not similar to HxH fish, in survival, growth, condition, dispersal, and maturation, so differences among crosses likely resulted from additive genetic differences between the hatchery and wild populations rather than from maternal differences between hatchery and wild females. During our study, local managers decided against supplementing most wild steelhead populations in the Clearwater basin. Our study indicates that supplementing with Dworshak Hatchery fish is likely to reduce the fitness of wild populations through interbreeding and therefore supports that decision.

Introduction

This study was conceived and initiated in the early 1990's when managers were proposing to use steelhead *Oncorhynchus mykiss* from Dworshak National Fish Hatchery (Figure 1) to supplement wild steelhead throughout the Clearwater River system in Idaho[1]. Previous studies with steelhead in Oregon and Washington (Reisenbichler and McIntyre 1977; Chilcote et al. 1986) suggested that reduced (genetic) fitness for natural rearing should be expected for the hatchery (sea-ranched) population so that

[1] Managers finally settled on a plan to supplement steelhead only in the South Fork Clearwater River and some tributaries downstream from the South Fork. Native populations of salmon and steelhead had been extirpated or severely depleted by an impassable dam in the lower portion of the South Fork during 1927-1962 (Kiefer and Lockhart 1999). Native populations of steelhead upstream from the South Fork are managed entirely for natural production.

interbreeding of hatchery and wild fish would reduce the fitness of the wild population; however, no supporting data were available for steelhead in Idaho. This study was designed to compare the genetic fitness of Dworshak Hatchery steelhead and wild Clearwater River steelhead for natural rearing.

An additional goal of this study and a concurrently conducted companion study (Rubin et al. 2012a) was to test for domestication of the hatchery population. Domestication results from natural selection for traits that are beneficial in the hatchery program and is expected to increase fitness for hatchery rearing but decrease fitness for natural rearing (Reisenbichler and Rubin 1999; Araki et al. 2008). We therefore hypothesized that if the Dworshak Hatchery stock was domesticated, survival would be better for hatchery than for wild progeny reared at the hatchery whereas the opposite would be true under natural rearing. Survival at the hatchery was evaluated in the companion study.

Our primary measure of fitness for natural rearing was survival of juveniles in freshwater. We also evaluated traits that have a bearing on survival and can differ between hatchery and wild fish including growth (Reisenbichler and McIntyre 1977), dispersal (Nagata et al. 1994), and maturation (McGinnity et al. 1997). Steelhead can express resident as well as anadromous life histories (Zimmerman and Reeves 2000). We evaluated maturation of males expressing a resident life history.

We tested for differences in survival and fitness-related traits between progeny of hatchery and wild steelhead reared in a natural stream. Progeny groups were genetically marked and released together in the stream as unfed fry; thus any survival or growth differences resulted from genetic differences between the parental stocks (or maternal differences such as vertically transmitted disease, egg size, or egg quality) rather than environmental differences. Hybrid crosses of hatchery females and wild males were included to assess maternal effects; the hybrids were maternally equivalent to the hatchery cross.

Study Populations and study stream

The Dworshak Hatchery population was initiated with wild steelhead from the North Fork Clearwater River and had been artificially propagated for six generations (average generation time = 4 years; Burge et al. 1994) without importation of other stocks at the onset of our study. Our source of wild adults was the Selway River (Figure 1). A companion study examining performance under hatchery rearing (Rubin et al. 2012a) was conducted with siblings of progeny used in the current study. Descriptions of the parental stocks are given in the companion study. Our study stream was Twentymile Creek, a second order tributary of the South Fork Clearwater River (Figure 1). Native populations of anadromous fish were eliminated from the South Fork by construction of Harpster Dam in 1927 near its mouth (Kiefer and Lockhart 1999). Hatchery origin steelhead and spring Chinook salmon *Oncorhynchus tshawytscha* were reintroduced following dam removal in 1962.

Steelhead fry were released in Twentymile Creek between two road crossings, one at river kilometer (rkm) 3 (elevation 1317 m) and the other at rkm 8.4 (elevation 1540 m; mean gradient = 4.1%). Mean wetted width in this reach was 7.6 m in July 1998. In the lower half of the reach the stream meanders through a meadow, whereas the upper half has a steeper gradient and consists primarily of cascades and plunge pools (Figure 2). Temperature and flow cycles during the study are shown in Figure 3. Mean annual temperature (July 1995-June 1996) at mid-reach was 3.8 ^{o}C.

Falls near the mouth of Twentymile Creek constituted a barrier to upstream migration. The only resident fish we encountered above the falls were an introgressed population of westslope cutthroat trout (*O. clarki lewisi*) and rainbow trout. The hybrid status of this population was confirmed by analysis of several diagnostic allozyme loci (Leary et al. 1987) for a sample of 46 fish collected before we released any steelhead. About 75-80% of the alleles were from cutthroat trout. Most of the fish exhibited the orange throat slashes characteristic of cutthroat trout.

Methods

Experimental crosses

Hatchery (H) adults were obtained from Dworshak Hatchery and wild (W) adults were captured at Selway Falls (Figure 1) in 1994 and 1995. Adults were held until ripe and spawned artificially to create three genetically marked progeny groups: HxH, HxW (1995 only), and WxW. Genotypes at the cytosol non-specific dipeptidase locus (*PEPA**; Shaklee et al. 1990) served as the genetic marks. Adults were screened prior to spawning, and only hatchery adults homozygous for the alternate (**111*) allele and wild adults homozygous for the common (**100*) allele were spawned, thus creating a distinct *PEPA** genotype for each cross.

In 1994 the eggs from each female were divided into two equal portions and each portion was fertilized with milt from a different male. In 1995 the procedure remained the same for WxW crosses; however, HxH and HxW crosses were made by fertilizing half of the eggs from each hatchery female with milt from a hatchery male and the other half with milt from a wild male. Thus maternal contribution was identical between HxW and HxH crosses. Paternal contribution was similar although not identical between HxW and WxW crosses because not every wild male contributed equally to HxW and WxW crosses (Rubin et al. 2012a). Hatchery parents comprised 25 females and 20 males in 1994 and 23 females and 14 males in 1995. Wild parents comprised 18 females and 7 males in 1994 and 10 females and 9 males in 1995. In both years, spawning occurred once or twice per week during April with considerable overlap in time of spawning between hatchery and wild adults (Rubin et al. 2012a). At least 15 unfertilized eggs were sampled from each female, preserved in 10% buffered formalin, and weighed >90 days later.

The fertilized eggs from each female were incubated in a separate tray in vertical incubators. A divider in the center of each tray separated the two half-sib families. Water temperature was adjusted so that embryos from different spawning dates eyed up nearly simultaneously. At eye-up families were sampled proportionately to provide equal numbers of eyed embryos from each cross and ensure that family composition was identical to that for the companion study examining performance under hatchery conditions (Rubin et al. 2012a). The selected embryos were then returned to incubators. Otoliths were thermally marked between hatch and button-up (Volk et al. 1990). This provided a secondary mark that allowed us to distinguish our experimental steelhead from resident trout. Survival between eye-up and button-up was ≥96% for each cross in each year. Sixty fry were measured and ≥300 were collectively weighed from each cross 1-2 days before release. Additional details on adult collection, spawning, and embryo incubation are provided in Rubin et al. (2012a).

Fry release

Nearly equal numbers of button-up (unfed) fry from each cross were released in June (see Table 1 for number of fry released). In 1994, fry were released at five locations (see Figure 4 for the location of the fry release sites). One sixth were released at each of four locations in the lower half of the study reach and two sixths at the upper road. Dispersal from release sites seemed to differ among crosses (see "*Residents*" section of "METHODS" below) so in 1995 we designed release and sampling procedures to test for dispersal differences. Equal numbers of fry were released throughout nine 200 m long sections spaced over the study reach (see Figure 4 for the location of the fry release sections), thus allowing assessment of dispersal by subsequently sampling within and between release sections (see below). In both years, we ensured that cross and family composition of released fry were similar among release sites.

Sampling individuals, identifying resident trout, and aging steelhead

Fish caught in the migrant trap or by electrofishing (see "*Migrants*" and "*Residents*" sections of "Methods" below) were visually identified as steelhead or cutthroat trout, and those classified as

steelhead were measured, weighed, squeezed to test for flowing milt, and sampled for tissue that was frozen and subsequently analyzed for *PEPA** genotype to determine cross (Aebersold et al. 1987). Most putative steelhead captured in 1994 and 1995 were euthanized and their otoliths examined for our thermal mark. Tissue from fish that lacked the mark (i.e., resident trout; *n*=9) was electrophoretically analyzed at *GPI-A** (a locus of glucose-6-phosphate isomerase; Shaklee et al. 1990). All but one of the resident trout exhibited at least one copy of the **92* allele at *GPI-A** which is diagnostic of westslope cutthroat trout (Leary et al. 1987); steelhead carry only the **100* allele. In subsequent years, fin tissue was analyzed at *GPI-A** to screen out resident trout visually misidentified as steelhead and at *PEPA** to determine cross of steelhead, and scales were aged to distinguish the two year-classes of steelhead, thus allowing non-lethal sampling. We continued to examine otoliths from sub-samples of fish to monitor the accuracy of species identification and aging.

Otolith processing followed Volk et al. (1990). Blind tests with marked and unmarked reference specimens collected before fry release indicated 100% accuracy for recognizing marks (*n*=60 each year). A total of 1562 otolith samples were processed from fish visually identified as steelhead from all years and capture methods combined. Of these, 23 were unmarked (i.e., from resident trout), and only one of the unmarked samples was from a fish that would have been classified as a steelhead by allozyme analysis alone (i.e., it did not have the **92* [cutthroat trout] allele at *GPI-A**). The error rate for identifying steelhead by visual identification and allozyme analysis therefore was considered negligible (1 of 1540=0.06%).

Ages of fish are designated herein by incrementing age on 1 January. Error for aging 1- to 3-year old fish from scales ranged from 0-3% (Table 2). Only 5% of known 4-year olds collected in 1999 were incorrectly aged from scales as age-5, but 2 of 6 known 5-year olds were incorrectly aged as age-4 (Table 2). However, only 3% of the fish collected in 1999 and sampled exclusively for scales (otoliths not sampled) were aged as five-year olds, so age-5 fish were rare and error from age-5 fish incorrectly aged as age-4 was negligible. Data from fish collected in 1999 that were aged as five-year olds are presented separately as being from fish of uncertain age, as are data from all fish collected in 2000.

Migrants

We were unable to trap downstream migrants in 1994 for logistical reasons. In 1995-1997, a trap located 100 m above the lower road was operated from April until late July when outmigration slowed (see Figure 5 for periods of trap operation and catch trajectories), then re-deployed in early September and fished through October. In 1998-1999, it was operated from April through October, and in 2000 from April until late July when the study ended. The trap was checked a minimum of three days per week and more typically seven days per week. A floating inclined screen trap (Kray-Meekin type) with a rectangular 0.6 x 0.9 m mouth was used during April-July 1995, and a screw trap (Thedinga et al. 1994) with a 1.5 m diameter cone thereafter. Wings made of plywood and sandbags were constructed to funnel more flow into the screw trap when it was first deployed (September 1995) and were maintained for the duration of the study.

Recapture rate of marked fish released 0.4 km upstream was used to estimate trap efficiency which was in turn used to estimate total emigrants. Prior to release upstream, fish were given a ventral fin clip to provide tissue for allozyme analysis and a caudal fin clip to indicate day of release. Upper and lower caudal clips were alternated daily so that we could tell whether a recapture had been released an odd or an even number of days previously. About 85% of the recaptures had the odd-day clip and 15% the even-day clip suggesting that most fish were recaptured one day after release. In 1995, most migrants were euthanized to provide otoliths (see above). Only 13 fish were released upstream in 1995, too few to generate efficiency estimates. Instead, we used efficiency tests conducted in 1996 to estimate trap efficiency in 1995 (Table 3; see below for further explanation). In 1996, 10 fish were euthanized, 40 were fin-clipped and released upstream, and the remainder were counted and released downstream each

day (if $\leq$10 were caught none were released). In 1997-2000 all trapped fish were clipped and released upstream; otoliths were taken only from recaptured fish.

In all years, efficiency data (upstream releases and recaptures) for the two year-classes and three crosses were pooled to estimate combined efficiency. Fish length was similar between upstream releases and recaptures (P=0.28; n=660; 2-way analysis of covariance with factors status [released or recaptured] and year [1996-1998], and covariate capture date), suggesting that migrant trapping was not size selective and that pooling was valid.

Trap efficiency estimates were temporally stratified with one week as the smallest time increment. DARR software (Bjorkstedt 2000) was used to decide when to enlarge a stratum by pooling over two or more consecutive weeks as well as to generate population estimates for the resulting temporal strata. When assigning recaptures to week of release, we assumed that the odd day clip indicated release one day previously and the even day clip two days previously.

Separate efficiency estimates were not made for September-October because few fish were trapped and therefore few were released or recaptured (Table 3). Fall population estimates were made by applying mean April-July efficiency (Table 3). Efficiency of the Kray-Meekin trap was not estimated from data collected in 1995, but rather from four days of operation (29 June; 1, 11, and 13 July) in 1996 when substantial numbers of marked fish were available for recapture due to high efficiency of the screw trap and large numbers of emigrants. Fish were caught in the screw trap, marked, and released for recapture in the Kray-Meekin. Kray-Meekin efficiency during the four days ranged from 0.03-0.22 (mean = 0.13; SD = 0.08). Kray-Meekin efficiency during April-July 1995 was estimated as the ratio of Kray-Meekin efficiency to screw trap efficiency during late June-mid July 1996 times the mean of screw trap efficiency during April-July 1996 (Table 3). Screw trap efficiency during late June-mid July 1996 (14 days of operation) ranged from 0.28-0.77 (mean = 0.47, SD = 0.13). Thus Kray Meekin efficiency was a relatively constant fraction of screw trap efficiency during late June-mid July 1996, lending some support to our method of estimating Kray-Meekin efficiency in 1995.

Estimates for the proportion of each year-class, and the proportion of each cross within each year-class, were made for each season (spring or fall) of each collection year in two ways. First, proportions were calculated by pooling all fish sampled during that season. Second, proportions were calculated separately for fish sampled in each temporal stratum identified by DARR and then weighted by the estimated total number of migrants in the stratum (from DARR) to correct for any differences in trap efficiency among strata. When a single trap efficiency value was applied to an entire season (all seasons except spring 1996 and spring 1997; Table 3), pooled and weighted estimates were equivalent. Estimates for the total number of migrants from each year-class, and for the variance of such estimates, were also made for each season. Variance estimates for total migrants (both year-classes combined) in a stratum were obtained from DARR, and the variance of the proportion of each year-class in the stratum (from the sample of fish that were aged) was computed as the variance of a proportion (Ott 1984). Variances of cohort-specific population estimates were computed from these using Goodman's (1960) formula for the variance of a product.

We calculated *apparent relative survival* (ARS) for both pooled and weighted estimates of the proportion of each cross to evaluate whether the relative abundances between a pair of crosses changed through time due to differential mortality or emigration. This metric compares the relative abundances observed at some sampling event with the relative abundances when the fry were released:

$ARS_{HH/WW} = (P_{HH2}*P_{WW1})/(P_{HH1}*P_{WW2})$;

P_{HH1} = proportion of HxH fish at fry release, P_{HH2} = proportion of HxH fish at time 2 (e.g., age-1 migrants), P_{WW1} = proportion of WxW fish at fry release, and P_{WW2} = proportion of WxW fish at time 2. $ARS_{HW/WW}$ and $ARS_{HW/HH}$ were computed similarly. Significance was tested only for pooled estimates of ARS (*G*-tests for goodness of fit with the Williams correction; Sokal and Rohlf 1995). Only $ARS_{HH/WW}$ and $ARS_{HW/HH}$ were tested, the former to compare parental populations (hatchery and wild), and the latter to rule out maternal effects. Because HxW and HxH fish were maternal half-siblings, any differences between them must be from genetic rather than maternal differences. We accounted for making two comparisons with sequential Bonferroni adjustment (Sokal and Rohlf 1995). Tests for differences on other metrics (e.g. ARS of residents, size of migrants and residents, see below) were also limited to these two paired comparisons (HxH versus WxW and HxW versus HxH).

In every year except 1996, most of the trapped fish were successfully typed to cross (100, 96, 88, 74, and 85% in 1995 and 1997-2000, respectively). In 1996, we were only able to type 45% of trapped fish to cross, and further, the percentage of successfully typed fish varied over the April-July migration period. Seventy percent of total catch occurred after 23 June. Due to a freezer malfunction only 19% of the fish caught after 23 June were successfully typed to cross compared to 86% before. All whole body samples collected after 23 June, and 86% of fin samples collected during 26 June-2 July, could be resolved for *PEPA** despite the freezer problem and were used in our analyses.

Because sampling efficiency (percentage of trapped fish typed to cross) varied over the migration period in 1996, we computed a third estimate for the proportion of each cross (in addition to pooling samples over the whole migration period and weighting samples by estimated total migrants in each temporal stratum within the period). Using the same temporal strata, samples typed to cross were weighted by the number of fish trapped in each stratum. Because number trapped was a count, not an estimate, we were able to compute the variance of the proportion of each cross (SAS 2000; SURVEYMEANS procedure) and statistically test ARS. The number of fish successfully typed to cross each week after 23 June was $\geq$5 and $\geq$73 for the 1994 and 1995 year-classes, respectively. Thus, at least for the 1995 year-class, sample size after 23 June was sufficiently large to adequately represent this part of the run even though it was underrepresented relative to earlier on (before 23 June).

Residents

Fish residing upstream from the trap (henceforth referred to as residents) were captured with backpack electrofishers each summer from 1994-2000 (capture dates given in Table 4). All captured fish were sampled except in 1995 when 50 sub-yearlings were sampled per site and the remainder were counted and released. Sampling procedures for individual fish (size, tissue for cross determination, hard parts for aging, etc.) were similar to those for migrants. In 1994 and 1995 all sampled fish were euthanized. Thereafter, most were sampled for fin tissue (ventral fin clip) and scales, then released where captured. Yearlings captured in 1995 were frozen and later thawed and sexed. In 1998 and 1999, fish in the sub-sample for otolith analysis were frozen and later thawed, sexed, and their gonads weighed. Gonadosomatic index (GSI) was computed as 100 times thawed gonad weight divided by fresh (in the field) whole body weight.

In 1994, eight sites in the study reach (i.e., between the roads) and one below it (see Figure 4 for site locations) were electrofished with one backpack unit but without blocknets at site boundaries. The sites ranged in length from 13 to 166 m. From 1995 on, site length was set at approximately 100 m. A blocknet was placed at the top of the site (except in 1996, see below), and one (1995) or two (1996 on) backpack units were fished from the bottom to the top of the site with the intent of thoroughly covering the habitat. The site's downstream end was also blocked and two additional passes were conducted in a sub-set of sites each year (see Table 5 for the number of 3-pass sites). From 1995 to 1997 sites were chosen to sample inside and outside of the 200 m-long sections where fry were released in 1995 as well as to cover the study reach (see Figure 4 for site locations). Sampling inside and outside of fry release

sections allowed us to test for dispersal differences among crosses. In 1995, three passes were conducted at both sites associated with the third and ninth (from downstream) release sections, and in 1996 and 1997 at one site from most of the groups of sites clustered around release sections. In 1997, differences between sites inside and outside of fry release sections were no longer apparent, so in 1998 we switched to systematic sampling. The study reach was divided into 54 contiguous 100 m-long sites. In 1998 two thirds of the sites were sampled by skipping every third site, and in 1999 and 2000 half were sampled by skipping every third and fourth site (sites 1 and 2, 5 and 6, etc. were sampled in 1999 and sites 2 and 3, 6 and 7, etc. were sampled in 2000). In all three years, three passes were made at every fourth site sampled. Spot electrofishing in Twentymile Creek above the upper road and in the West Fork (Figure 1) yielded almost no steelhead captures and suggested little upstream movement of experimental fish, probably due to the steep gradient above the upper road (Figure 2) and the small size of the West Fork (about a quarter as much flow in summer as Twentymile Creek upstream from their confluence). In 1997, a 100 m-long site was electrofished starting at the upper road and working upstream, using standard procedures (two backpack units; blocknet at the top). Only one steelhead was captured, compared to an average of 21 and a minimum of 8 steelhead captured at sites downstream from the upper road that year.

In 1996, blocknets were not used at 1- or 3-pass sites. In 1997-1998 we tested whether electrofishing chased fish from sites lacking blocknets. At each of six 100 m-long sites spaced over the length of the study reach, blocknets were placed at the upper boundary and 10 m farther upstream. The 10 m-long section of stream between the nets was thoroughly electrofished and captured fish were placed in buckets, after which the net at the upper boundary of the 100 m site was removed but the net 10 m father upstream was left in place. The 100 m site was then electrofished as usual starting at the downstream end, and we continued electrofishing through the 10 m-long section upstream from the site. The number of fish caught in that 10 m-long section presumably had been chased out of the 100 m site but were prevented from moving farther upstream by the net that was left in place. Only one "chased" fish was caught over the six sites while the number of fish caught within site boundaries averaged 12 (range 3-29). We also stationed an observer at the lower boundary at each site. Visibility was good and we observed only one fish exiting downstream.

Population estimates were made for each 3-pass site with Microfish (Van Deventer and Platts 1985). The mean ratio of the population estimates to the number caught on the first pass (Table 5) was used as an expansion factor to estimate population size in each 1-pass site (Hankin and Reeves 1988). Estimates in 1- and 3-pass sites were then expanded into an estimate of the total population in the study reach (Hankin and Reeves 1988) using N=54 sites as the expansion factor. The variance of the removal estimates (Table 5) was included in the variance of the total estimate (Hankin 1984). In 1995, non-overlapping size distributions between sub-yearlings and yearlings allowed removal and total population estimates to be made separately for each cohort. Thereafter estimates were made for total steelhead and then partitioned into estimates for each year-class as described above for migrants.

Annual estimates were made for the proportion of each cross by pooling samples over sites. Data from sites below the lower road (see Figure 4 for site locations) were not included in such estimates nor were they included in population size estimates because fish below the lower road had already been accounted for by migrant trapping. ARS for residents was computed and its significance tested as described above for migrants. Differences in the proportion of each cross between samples of juveniles collected at different times or places (e.g., between migrants and residents) were tested with contingency table G-tests of independence (Sokal and Rohlf 1995); data were pooled over temporal strata (migrants) or capture sites (residents) for these tests. Trends of increasing or decreasing frequency of one cross relative to another over periods of three or more years were tested by logistic regression of relative frequency against age (Sokal and Rohlf 1995).

Size and condition

Length differences were tested with analysis of variance (AOV) on data pooled over temporal strata (migrants) or sites (residents) unless otherwise noted. Capture date was included as a covariate in migrant analyses if length was correlated with it ($P<0.05$). Condition (weight at length) was tested with analysis of covariance (AOCV) on $\log_{10}$ transformed weight (dependent variable) and $\log_{10}$ transformed length (covariate). Mean condition factor was computed for each cross as $100{,}000*W/L^3$; W was the back-transformed least square mean of $\log_{10}$ weight (g) from the AOCV; L was the geometric mean of lengths (mm) pooled over crosses. This method avoids the problem of correlations between condition factor and length that can occur when the exponent of the weight-length relation differs from 3 and condition factor is computed for individuals and then averaged (Cone 1989). When AOCV indicated that slopes of the weight-length relation differed among crosses ($P<0.05$), condition was not statistically compared among crosses, and mean condition factor was computed with least square mean weights from the full AOCV model (with the cross*length interaction term included) rather than from the "common slope" model with that term excluded.

Results

Egg size, survival to eye-up, and fry size

Mean egg weight was significantly greater for hatchery than for wild females in 1994 (Table 6). In 1995, mean egg weight was greater for hatchery than for wild females, but the difference between means was 70% less than in 1994 and was non-significant. A larger sample size (200) would have been needed to detect a difference of the magnitude we observed in 1995 (0.8 power at a 0.05 significance level). Survival to eye-up was similar among crosses in both years. Mean length was significantly greater for HxH than for WxW fry in 1994 and for HxH and HxW than for WxW fry in 1995.

Migrants

Most migrants were trapped one or two years after release during May-July (Figure 5). According to our population estimates (Table 1), "spring" (April-July) age-1 and age-2 migrants accounted for 52 and 42%, respectively, of total 1994 year-class migrants, and 64 and 32% of total 1995 year-class migrants. Few (<0.1%) of the fry released in June 1995 migrated downstream at age-0 (Table 1). Based on this and similar results from releases of unfed fry for other experiments in Idaho (Rubin et al. 2012d; 2012f), we assume that the number of sub-yearlings leaving the study reach in 1994 also was negligible.

The pooled estimate of $ARS_{HH/WW}$ was significantly <1 for age-1, age-2, and total 1994 year-class migrants and for age-1 and total 1995 year-class migrants (Table 1). In spring 1996, migrants collected after 23 June were sampled at a lower rate than those collected earlier and were therefore underrepresented in the pooled sample (see methods). Furthermore, the relative frequency of 1995 year-class crosses differed after 23 June compared to before (29% HxH, 39% HxW, 32% WxW before; 30% HxH, 28% HxW, 42% WxW after; $P=0.001$; 2x3 *G*-test of independence). Estimates weighted by the number of fish trapped or by estimated total migrants in temporal strata compensated for such temporal differences in sampling rate and therefore should be more accurate than pooled values for 1995 cohort yearlings. Estimates of $ARS_{HH/WW}$ weighted by number trapped or by total migrants (0.77 in each case) were lower than the pooled estimate (0.82), and the estimate weighted by number trapped was significant (Table 1). The pooled estimate of $ARS_{HW/HH}$ was significantly >1 for age-1 and for total migrants; however, weighted estimates deviated from 1 by less than pooled estimates, and the age-1 estimate weighted by number of fish trapped was not significant (Table 1). All estimates (pooled and weighted) indicated intermediate performance by HxW fish (i.e., $ARS_{HH/WW}<ARS_{HW/WW}<1$).

In keeping with the difference in relative frequency of the crosses before and after 23 June, mean capture date of 1995 cohort yearlings differed among crosses (HxH=19 June, HxW=17 June, WxW=20 June; P=0.011; one-way AOV). Mean capture date also differed for 1995 year-class two-year olds (HxH=13 June, HxW=17 June, WxW=19 June; P=0.015). Relative frequency of the crosses for 1995 year-class migrants was similar among ages but differed between seasons; WxW migrants were relatively more frequent in fall than in spring when ages were pooled (Table 1; P=0.015; 2x3 G-test of independence). Nevertheless, the main difference among 1995 year-class crosses was in quantity rather than timing of migrants. No within-year, among ages, or between season differences in timing were apparent for 1994 year-class migrants.

Residents

Relative frequency of the crosses and total density (crosses pooled) of 1995 cohort sub-yearlings differed between sites inside and outside of the fry release sections (Figure 4). Frequency of HxH relative to WxW fish was higher inside than outside (P<0.001), as was frequency of HxH relative to HxW fish (P=0.037; Table 7; 2x2 G-tests of independence). Frequency of HxW fish was intermediate to that of the other crosses both inside and outside (Table 7). Density (fish per m stream length) was on average 3.7 times higher inside (1.52 fish/m) than outside (0.41 fish/m). A similar but less extreme pattern of frequency and density differences was evident for 1995 cohort yearlings (Table 7; P=0.045 [2x3 G-test of independence]; density 1.3 times higher inside [0.83 fish/m] than outside [0.66 fish/m]) but not at age-2 (inside-outside comparisons were not available at age-3 or at age-4 due to a change in sampling design [Figure 4]). Therefore, in addition to pooled estimates, weighted estimates of the proportion of each cross and of ARS were made for 1995 cohort sub-yearlings and yearlings using separate population estimates for the portion of stream inside and outside of the release sections as weighting factors. A similar distribution pattern (HxH relatively less frequent downstream from release sites) was evident for 1994 cohort sub-yearlings below the third, fourth, and fifth (from the bottom) release sites (Figure 4).

The difference in relative frequency of the crosses between inside and outside of fry release sections (1995 cohort sub-yearlings and yearlings) and between release sites and areas downstream from those sites (1994 cohort sub-yearlings) indicates a difference in dispersal rather than differential mortality. Dispersal from release sites was greater for WxW than for HxH fry; dispersal of HxW fry was intermediate.

The pooled estimate of $ARS_{HH/WW}$ was significantly >1 for 1994 year-class two-year olds and 1995 cohort yearlings, and was <1 (P=0.051) for 1995 year-class four-year olds (Table 7). Significance for 1995 cohort yearlings is questionable because the weighted estimate of $ARS_{HH/WW}$ deviated from 1 by less than the pooled estimate and was likely more accurate due to the differences between fry release and non-release strata mentioned above. $ARS_{HW/HH}$ was not significant for any ages (except within release or non-release strata); point estimates indicated intermediate HxW performance at ages 1, 2, and 4. Pooled estimates of ARS for 1995 cohort sub-yearlings were nearly identical to weighted estimates (Table 7) by coincidence. We sampled a higher percentage of the length of the study reach inside (24%) than outside (13%) of the release sections (Figure 4). However, this difference was counterbalanced by sampling a lower percentage of the fish captured at sites inside (52%) than outside (98%) of the release sections. The difference in sampling rate resulted from sampling no more than 50 fish from a site even though many more fish were captured at inside than at outside sites. The percentage of fish sampled was similar between sites inside and outside of the release sections ($\geq$95% at each) for age-1 fish.

Frequency of HxH relative to WxW residents of the 1994 year-class may have decreased from age-2 to age-4 (P=0.06, logistic regression of relative frequency against age; Table 7). Frequency of HxH relative to WxW residents of the 1995 year-class decreased from age-1 to age-4 (P<0.05 for the decrease from age-2 to age-4; the decrease from age-1 [weighted estimate] to age-4 was even greater). Frequency

of HxW relative to WxW residents also decreased from age-1 to age-4 but by less than frequency of HxH residents.

Integrating migrants and residents

Relative frequency of the crosses differed between migrants and residents for 1994 year-class one- and two-year olds and for 1995 cohort yearlings. In all three cases, frequency of HxH relative to WxW fish was significantly lower for migrants (total of fall and spring) than for residents when tests were conducted with pooled samples (Tables 1 and 7; $P \leq 0.03$; 2x2 *G*-tests of independence). Using pooled samples was valid because the differences found were no greater than from weighted estimates of HxH and WxW frequencies (Tables 1 and 7). Frequency of HxW relative to HxH fish was significantly higher for migrants than for residents when pooled samples were used ($P=0.015$); however, the validity of the test is questionable because the difference between migrants and residents was greater than it would have been from weighted estimates.

The difference in relative frequency of the crosses between age-1 migrants and residents, and between age-2 migrants and residents of the 1994 year-class (see preceding paragraph), indicates differential emigration rather than differential mortality. Emigration of one- and two-year olds from the study reach was greater for WxW than for HxH fish. Emigration of HxW fish was intermediate although not statistically different from that of HxH fish.

Frequency and population size estimates for migrants and residents of uncertain age collected in 1999 and 2000 are shown in Table 8. These data indicate that trends seen earlier (few migrants older than age-2; high frequency of WxW relative to HxH four-year olds) continued for five and six-year olds.

Migrant and resident population estimates were incompatible before the age-2 resident samples. For these earlier samples, the estimated number of migrants between successive resident estimates (e.g., age-1 and age-2) was always greater than the difference between the resident estimates (Tables 1 and 7). For example, population estimates for age-1 and age-2 residents of the 1995 year-class were 3624 and 2133, respectively, the difference between them was 1491, yet an estimated 3083 age-2 fish from that year-class emigrated in spring before the age-2 residents were censused. Migrant and resident estimates could be reconciled using upper (age-1 residents) and lower (age-2 residents and spring migrants) limits of 95% confidence intervals ($\pm$2*SE); however, the consistent occurrence of incompatibility between estimates (always present before age-2 residents) indicates a problem with accuracy rather than precision. Either migrant estimates were positively biased, resident estimates were negatively biased (increasing successive resident estimates by the same percentage would increase the difference between them), resident estimates for younger fish were negatively biased relative to those for older fish, or all three. Note that no population estimate was made for 1994 year-class age-0 residents, so compatibility of age-1 migrant and resident estimates of that year-class could not be evaluated. Possible sources and magnitudes of population estimate bias will be addressed below in the discussion section.

Relative survival

Relative frequency of the crosses was similar between age-0 residents in August and fry at release in June (Table 7). No change in relative frequency indicated no difference in survival among crosses; differential emigration could not have been a factor because emigration from the study reach was negligible before August. From age-0 residents to age-4, patterns of $ARS_{HH/WW}$ for migrants and residents indicated lower survival for HxH than for WxW fish (Figure 6). Emigration of 1994 year-class 1- and 2-year olds and of 1995 cohort yearlings was greater for WxW than for HxH fish, leaving a deficit of 1- and 2-year old WxW fish in the study reach. Frequency of HxH relative to WxW residents decreased from age-2 to age-4 (1994 year-class) and from age-1 to age-4 (1995 year-class). This change in relative frequency was due to lower survival for HxH than for WxW residents; it could not have been from

differential emigration because emigration was never greater for HxH than for WxW fish. For the 1995 year-class, significantly fewer total migrants and significantly fewer age-4 residents resulted from the HxH than from the WxW cross. This indicates that for non-migrants (i.e., survivors remaining in the study reach and fish that died in the study reach), survival from release to age-4 was significantly lower for HxH than for WxW fish. The pattern was the same for the 1994 year-class – fewer age-4 residents and fewer total migrants from the HxH than from the WxW cross – but the difference in age-4 residents was not significant. $ARS_{HW/WW}$ indicated that production of migrants and of residents surviving to age-4 was intermediate for HxW fish (Figure 6). Post-emigration survival of migrants was not tracked.

We had planned to estimate annual relative survival. Population estimate bias prevented us from doing so; however, it was still possible to place bounds on annual relative survival. Bounds were calculated by starting and ending year-long intervals with resident samples, except that we started the first year (survival to age-1) at fry release. One bound was obtained by assuming no bias of migrant and resident population estimates as well as no mortality for migrants between emigration and the end of the interval. Population estimates were partitioned by relative frequency of the crosses (e.g., 2133 age-2 residents from the 1995 year-class times 0.355 HxH fish equals 758 age-2 HxH residents). For a given interval (e.g., age-1 to age-2), frequency at the end was computed as the sum of residents at the end (e.g., age-2) and intervening migrants (e.g., age-1 fall and age-2 spring), and frequency at the beginning was that of the initial fry or residents (e.g., age-1 residents). Relative survival computed this way represents a bound because migrant estimates may have been positively biased, resident estimates may have been negatively biased, and some migrants would have died after leaving; in other words, there could not have been more migrants and (or) less residents alive at the end of the interval. The other bound was obtained by assuming extreme positive bias of migrant estimates and (or) negative bias of resident estimates. Relative frequency of the crosses at the end of an interval was computed solely from the residents; intervening migrants were excluded. Relative survival computed this way represents a bound because as migrants become less numerous relative to residents (as they would if bias were extreme), their contribution to frequency of the crosses becomes negligible. True relative survival probably fell between these bounds because population estimates were biased but not infinitely so. These are not bounds in a statistical sense because they were derived from point estimates for relative frequency of the crosses and for population size. Even so, the bounds may be broad enough to include true relative survival given the severe assumptions that the bounds were based on (no estimate bias versus extreme estimate bias).

Bounds on annual relative survival are shown in Table 9. Combined and residents-only estimates both indicated lower survival for HxH than for WxW fish each year from age-2 to age-4 of the 1994 year-class and from age-1 to age-4 of the 1995 year-class. Residents-only estimates, which were conservative (i.e., showed a smaller survival difference between HxH and WxW fish than combined estimates), indicated that survival of HxH fish was 0.36 that of WxW fish from age-2 to age-4 of the 1994 year-class and 0.53 that of WxW fish from age-1 to age-4 of the 1995 year-class. Further, the survival disadvantage of HxH compared to WxW fish appeared to increase with age after age-2 (1994 year-class) or age-1 (1995 year-class) and be greatest from age-3 to age-4. The trend of increasing HxH disadvantage with age was significant for residents-only estimates (Figure 7) but not for combined estimates (P=0.11; data not shown).

Size and condition

Length of age-2 spring migrants and of residents of all ages except age-4 was significantly greater for HxH than for WxW fish of the 1994 year-class (Figure 8). Length of 1995 year-class spring migrants aged 1 and 2 was significantly greater for HxH fish than for WxW or for HxW fish; the latter were intermediate in length (Figure 8). Length of 1995 year-class residents aged 0, 1, and 2 was greater outside than inside of fry release sections, but within each of those strata length was greater for HxH than for WxW fish (Figure 9; 2-way AOCVs; cross and location significant [P<0.05]; cross*location interaction not significant). Length in each release stratum was significantly less for HxW than for HxH

fish at age-1 and at age-2 but not at age-0. When 1995 year-class data were pooled over release strata, length was significantly greater for HxH than for WxW residents at ages 1 and 2 but not at age-0 (Figure 8). The length difference between HxH and WxW age-0 residents was less in the pooled data than within release strata because frequency of WxW relative to HxH fish was higher outside than inside of release sections (Table 7) and mean length of fish (averaged over crosses) was greater outside than inside.

Mean length was greater for HxH than for WxW migrants aged 1 and 2 in most weeks (Figure 10). The relation between length and capture date was relatively flat during spring (Figure 10) although sometimes significant (Figure 8). Slope of the length-date relation never differed among crosses ($P \geq 0.09$). The length-date relation also appeared flat during fall but length increased markedly between the end of the spring and beginning of the fall migration (Figure 10). Mean length was greater for HxH than for WxW residents aged 0, 1, and 2 at nearly all sites (Figure 11). Within ages and sub-populations, length distributions were typically unimodal for all crosses (Figure 12).

Mean condition factor was always greatest for HxH, intermediate for HxW, and least for WxW fish, and these differences among crosses were often significant (Figure 13). Condition of 1995 year-class residents aged 0 and 1 but not 2 was greater outside than inside of release sections; comparisons among crosses were the same (HxH>HxW>WxW) inside and outside (2-way AOCVs; cross and location significant [$P<0.05$]; cross*location interaction not significant).

Sex and maturity

Sex ratio of 1994 year-class age-1 residents was similar between crosses (HxH: 59% males, n=90; WxW: 52% males, n=83; P=0.36). Flowing milt, indicating sexually mature males, was not observed for age-0 or age-1 fish. Among two-year olds, a higher proportion of HxH than of WxW 1994 year-class migrants and 1995 year-class residents produced flowing milt (HxW intermediate in the latter case; Figure 14). Incidence of flowing milt in older fish was higher than for two-year olds and was similar among crosses. Incidence of flowing milt increased with length for all crosses of age-2 1995 year-class residents, but at any given length it was highest for HxH, intermediate for HxW and lowest for WxW fish (Figure 15). For three-year olds the slope of the relation between incidence of flowing milt and length was positive for WxW fish but negative for HxH fish (slope intermediate for HxW fish), and by age-4 the slope was negative for all crosses and there was no difference among crosses in incidence of flowing milt at a given length. Collectively, these relations indicate that peak incidence of flowing milt was similar among crosses (about 50%) but occurred at greater length and age for WxW than for HxH fish (and at intermediate length and age for HxW fish). Due to small sample size the preceding analysis was not conducted for the 1994 year-class, nor were the following analyses concerning sex and maturity in a sub-sample of resident three and four-year olds.

Most males aged 3 or 4 produced flowing milt (Table 10). Whole body weight for males increased from age-3 to age-4 at about the same rate as testes weight such that mean GSI remained unchanged (Table 10). Testes weight was greater for males with flowing milt than not (P=0.048; AOCV with covariate weight; data pooled over ages) as was GSI (P=0.024; unequal variance t-test). Mean weight of ovaries and GSI were very low for age-3 females but increased eight and five fold, respectively, during the next year (Table 10).

Testes weight was greater for HxH than for WxW age-3 males ($P<0.05$; AOCV with covariate length on $\log_{10}$ transformed testes weight pooled across presence or absence of flowing milt; HxW was intermediate but not significantly less than HxH) but was similar among crosses for four-year olds. There was no difference among crosses in sex ratio, GSI of males, ovaries weight, or GSI of females for either age.

Discussion

Potentially confounding factors

Interpretation of our results hinges on whether the *PEPA** genotypes used to mark the crosses were selectively neutral and whether maternal effects influenced performance of progeny. We conducted a separate study to evaluate neutrality of the *PEPA** genotypes (Reisenbichler et al. 2006). In that study, *PEPA** genotype had no effect on "loss" (from mortality or emigration), size, condition, or incidence of precocious males for naturally reared one-, two-, and three-year olds remaining in the study stream (downstream migrants were not trapped), nor did it affect survival, size, or condition of hatchery-reared juveniles; however, *PEPA** genotype did affect smolt-to-adult survival of hatchery reared juveniles. Adult return rate to the hatchery was three times as high for males with the **110/110* genotype (HxH mark in the current study) as for males with the **100/100* genotype (WxW mark) (Reisenbichler et al. 2006). Return rate of males with the **100/110* genotype was intermediate; female return rate was unaffected by *PEPA** genotype. Lack of a mark effect on performance of naturally reared juveniles in the mark evaluation study suggests that the differences among crosses in the current study were not due to the genetic marks. In further support of no mark effect in the current study, WxW fish in the current study carried the *PEPA** genotype that was disfavored in the mark evaluation study for return rate of adult males, yet survival in the current study was higher for WxW than for HxH fish in Twentymile Creek.

In the current study, HxW fish were genetically intermediate to the other crosses but identical to HxH fish in maternity and environment during incubation. Performance of HxW fish generally was intermediate to that of HxH and WxW fish, not similar to that of HxH fish, so differences among crosses likely resulted from additive genetic differences between the hatchery and wild populations rather than from maternal differences between hatchery and wild females or from early environmental differences.

One maternal effect, egg size, may have influenced growth during the first summer after release. HxH and HxW fry were longer and heavier than WxW fry in 1995, presumably because egg size was greater for hatchery than for wild females although the egg size difference was not significant. Two months later in Twentymile Creek, length of age-0 residents was still similar between the HxH and HxW crosses but greater for those crosses than for WxW fish, suggesting a lingering effect of initial egg and fry size. However, condition factor was significantly lower for HxW than for HxH age-0 residents as was weight (P=0.049; 2-way AOV with factors cross and fry release section status [inside or outside]; mean of weights inside and outside of fry release sections=1.44, 1.36, and 1.18 g for HxH, HxW, and WxW fish, respectively). Thus size differences among sub-yearlings appeared to reflect a combination of egg size and genetic effects. Genetic effects predominated for age-1 and older fish and egg size effects were no longer detectable. Length of HxW yearlings (both migrants and residents) was intermediate to that of the other crosses and was significantly less than that of the HxH cross.

Relative survival

For the 1995 year-class, production of migrants and of age-4 residents per fry released was greater for the WxW than for the HxH cross, indicating that survival of non-migrants (i.e., survivors remaining in the study reach and fish that died in the study reach) from release to age-4 was higher for WxW than for HxH fish. The pattern was the same for the 1994 year-class – fewer total migrants and fewer age-4 residents from the HxH than from the WxW cross – however, the difference in production of age-4 residents was not significant. Survival from age-1 to age-4 was higher for WxW than for HxH residents of the 1995 year-class, and survival from age-2 to age-4 may have been higher for WxW than for HxH residents of the 1994 year-class (P=0.06). Collectively, these results indicate higher survival for WxW than for HxH fish. We did not track survival of migrants after they left the study reach, but if post-emigration survival was no lower for WxW than for HxH migrants then survival of the whole population, including migrants, to age-4 was higher for WxW than for HxH fish.

The timing of differential mortality among crosses was interesting. We detected no differential mortality during the two months after fry release. Our population estimate for 1995 year-class age-0 parr at the end of this interval may have been negatively biased but nonetheless suggests that this was a period of high absolute mortality. Reisenbichler and McIntyre (1977) sampled in the fall (September-November) and found survival differences between HxH and WxW steelhead outplanted in streams as eyed eggs or swim-up fry the previous spring. We could not estimate relative survival to age-1 (1995 year-class) or age-2 (1994 year-class) due to differential emigration and population estimate bias. After age-1 (1995 year-class) or age-2 (1994 year-class), survival was higher for WxW than for HxH residents each year, and the survival advantage of WxW over HxH residents appeared to increase with age and be greatest from age-3 to age-4. Leider et al. (1990) found that survival was higher for wild than for hatchery origin steelhead during each of three intervals: egg-to-sub-yearling parr, parr-to-smolt, and smolt-to-returning adult. We are unaware of studies other than ours that report persistent survival differentials between age-1 and older wild and hatchery origin salmonids in streams.

Dispersal

Dispersal of fry from release sites was greater for WxW than for HxH fry. Density was higher inside release sections than outside, apparently because most fish remained where released, and fish size was greater outside than inside, probably because less food was available per individual inside than outside (Rubin et al. 2012f). Thus there appeared to be a growth advantage to dispersal.

Size at release and two months after release was greater for HxH than for WxW fish; however, the difference in dispersal between HxH and WxW fish probably was not from competitive displacement of small by larger fish. A companion study that used only Dworshak Hatchery stock and was designed to test for size-dependent displacement (Rubin et al. 2012f) released swim-up fry of different sizes in discrete sections of Twentymile Creek. Similar to the current study, density was higher inside than outside of fry release sections two months later and initial size differences were still present both inside and outside, yet frequency of initially small, slow growing fish relative to initially large, faster growing fish was the same inside and outside. This indicates that fry dispersal was independent of fish size in the companion study. The key point for interpretation of results of the current study is that slower growth for WxW fish than for HxH fish probably did not lead to competitive displacement of WxW by HxH fish. Dispersal was greater for WxW than for HxH fish for some other reason.

Most emigration from the study reach occurred within two years of release when fish were too small to be smolts migrating to the ocean. Mean fork length of naturally produced smolts in the Clearwater River drainage during 1993-1999 ranged from 155-189 mm among seven tributaries (Byrne 2001). Based on these data, most migrants in our study did not attain smolt size until age-3 (fall) or age-4 (spring). Thus the majority emigrants from the study reach were dispersing downstream but not continuing on to the ocean, at least not until later years. Downstream movement of yearling and older pre-smolts during May-July is common for steelhead in the Clearwater River basin. Large numbers of pre-smolt migrants were observed from fry released for our other studies (Rubin et al. 2012d; 2012f) and from natural populations in Running Creek (Holubetz and Leth 1997) and Fish Creek (Byrne 2005).

Emigration of one- and two-year olds was greater for WxW than for HxH fish, and size of age-1 and age-2 migrants and residents was greater for HxH than for WxW fish. A companion study (Rubin et al. 2012f) found emigration at age-1 to be size dependent; however, emigration rate was higher rather than lower for large than for small individuals. Thus not only was there no competitive displacement of small by larger fish but in fact the opposite occurred – greater dispersal by large than by small fish. Results from the current study also suggest that larger than average individuals from each population (WxW and HxH) emigrated, and further that the trend of greater emigration by large compared to small individuals was more pronounced for WxW than for HxH fish. The length gain from residents of an age

to migrants one year older was often greater for WxW than for HxH fish (false only for age-0 to age-1 fish of the 1995 year-class) whereas the length gain from residents of an age to residents one year older was always less for WxW than for HxH fish (Figure 16). The pattern of greater length gain for WxW than for HxH fish from residents to migrants but less length gain for WxW compared to HxH fish from residents to residents is consistent with emigration of a higher proportion of large fish from the WxW population than from the HxH population and a resulting lower proportion of large WxW fish remaining upstream. This is important to interpretation of our finding that more migrants were produced by the WxW than by the HxH cross. These WxW migrants probably comprised large, fast growing individuals from the WxW population.

Growth and condition

Mean length was always greater for HxH than for WxW migrants and residents aged 1-2, and the difference was significant for at least one sub-population (migrants or residents) from each of those ages for each year-class. As noted above, these length differences did not result from maternal differences in egg or fry size, with the possible exception of the length differences for sub-yearlings. Size selective mortality probably was not responsible for the differences either since it seems unlikely that mortality targeted small HxH fish but was not size selective for WxW fish or only targeted large WxW fish. Size dependent emigration (i.e., emigration by larger than average individuals) may have affected size distributions of fish remaining in the study reach; however, the trend of greater size for HxH than for WxW fish was apparent for both migrants and residents. We conclude that the size differences among crosses represented growth differences.

Condition factor showed remarkably consistent differences among crosses (HxH>HxW>WxW) over all sub-populations and ages. A companion study (Rubin et al. 2012f), which used only Dworshak Hatchery stock, found growth differences among experimental groups of steelhead released as unfed fry in Twentymile Creek; however, unlike in the current study, no accompanying differences in condition factor were found except for age-0 residents. Companion study findings suggest that the condition factor differences among crosses in the current study were not simply a bi-product of the growth differences among crosses. In other words, the difference in condition factor among crosses in the current study likely represented a genetic difference in regulation of growth in weight relative to length, in addition to and independently from the previously mentioned genetic difference in growth.

Maturity

Schmidt and House (1979) examined hatchery-reared yearling steelhead for presence of flowing milt during the spawning season (April), held mature males separately from immature fish, and thereafter sampled mature males and immature fish monthly (June-December) for testes weight and GSI. Mean testes weight and GSI for age-3 resident males in the current study, even those without flowing milt, were at least as high as for mature males sampled monthly during summer (July-September) from the Schmidt and House (1979) study. Mean testes weight and GSI for age-3 resident males from the current study were also an order of magnitude higher than for immature males (i.e., males that were not flowing milt the previous April) sampled during summer by Schmidt and House (1979). This suggests that nearly all age-3 males in the current study were mature and capable of spawning in the spring of their third year. The low ovaries weight and GSI of age-3 resident females in the current study, and the marked increase in both from age-3 to age-4, indicates that most age-3 females were immature and incapable of spawning in the spring of their fourth year, whereas most age-4 females were maturing and likely capable of spawning the following spring at age-5 (Tyler et al. 1990). That resident females first spawned at age-5 was consistent with the first appearance of naturally produced *O. mykiss* yearlings in the study reach in 2000 (USGS, unpublished data). These yearlings were probably the offspring of 1994 year-class age-5 females.

Age-2 residents were not sexed or sampled for gonad weight, but the lower incidence of flowing milt for them compared to older residents probably indicated that only a fraction of the age-2 males were mature. Furthermore, we think it likely that incidence of flowing milt was correlated with the proportion of age-2 males that were mature. If so, then a higher proportion of HxH than of WxW males matured at age-2, and maturation at that age occurred at a smaller size for HxH compared to WxW males. The difference among crosses in the slope of the relation between flowing milt presence and fish size at age-3 also indicated a difference between HxH and WxW males, as did greater testes weight for HxH than for WxW age-3 males, but the meaning of these differences is unclear since nearly all age-3 males were mature regardless of cross.

Survival was lower for HxH than for WxW residents from age-2 to age-4 and maturation may have been earlier for HxH than for WxW males (higher proportion of HxH than of WxW males matured at age-2); however, the survival difference between crosses probably was not due to lower survival of mature males compared to immature fish for two reasons. First, sex ratio of the 1995 year-class was 52% males at age-3 (*n* males+females=54) and 70% males at age-4 (*n*=38), suggesting that survival from age-3 to age-4 was no worse for males than for females. Second, the survival difference between crosses was greater between ages 3 and 4 than between ages 2 and 3, yet nearly all males were mature by age-3.

Population estimate bias

Migrants.—Positive bias of migrant estimates would result from marked fish exhibiting greater trap avoidance than naïve fish or from some marked fish not resuming their downstream migration after being released above the trap. The former seems unlikely because the trap was located at the head of a pool which minimizes trap avoidance (Roper and Scarnecchia 1996), and wings funneled fish into the trap. There was evidence of the latter in 1996. When sampling residents in August, we checked for marked fish (from trap efficiency tests) and found that about 8% of the fish marked and released the previous spring were still above the trap. Eight percent retention of marked fish above the trap would have positively biased the estimate of spring migrants by 9% (i.e., estimate=1.09*true number). Marked fish above the trap could also have resulted from spring migrants moving back upstream before the August census; however, because of the barrier near the mouth of Twentymile Creek migrants could not get back upstream unless they stopped moving downstream within 3 km of the trap. Further, only 6% of the marked fish above the trap in August were farther upstream than the trap efficiency test release site. The percentage of marked fish remaining above the trap in August could not be determined for years after 1996 because marks were similar among years so marked fish could not be assigned to the year in which they were released.

Our assumption that recaptures with the odd day clip had been released one day previously and those with the even day clip two days previously resulted in assigning some recaptures to the wrong week of release. We evaluated whether this may have biased migrant population estimates by examining recapture data from wild steelhead caught in a screw trap in Fish Creek (tributary to the Lochsa River, a tributary of the Middle Fork Clearwater River) in 2004, tagged with individually coded passive integrated transponder tags, and released above the trap (Byrne 2005). The data were obtained by querying the PTAGIS data base (Martinson et al. 1997). We restricted our analysis to fish <120 mm long that were captured during June-July and recaptured before August because these fish most closely resembled age-1 and age-2 migrants in Twentymile Creek. We chose 2004 because there were more emigrants from Fish Creek within these ranges than in other years for which data were available.

Our method of assigning odd and even day recaptures would have resulted in 88% of Fish Creek recaptures being correctly assigned to week of release and 12% incorrectly assigned. An assignment error of this magnitude would have positively biased the Twentymile Creek population estimate by 2% in 1996 and by 4% in 1997 (1996 and 1997 were the only two years with sufficient data to estimate weekly efficiencies; Table 3; Figure 5). Thus positive bias of migrant estimates from marked fish remaining

upstream and from incorrect assignment of recaptures to week of release may have been about 12% (i.e., 1.09*1.03=1.12). Counteracting this was the fact that an unknown proportion of total spring migrants were excluded from estimates for 1995-1997 because we stopped operating the trap before the end of spring migration (Figure 5).

Residents.—Negative bias of resident estimates could have resulted from sampling at sites with lower than average fish densities; however, it does not appear that we "missed" high density portions of the study reach in any year (Figure 4). Negative bias could also have resulted from fish living outside of the study reach, either upstream or in the West Fork (Figure 1); however, as noted above few fish were captured during spot electrofishing in these areas.

A more likely source of bias was the 3-pass removal estimates used to expand 1-pass catch. Two studies comparing removal estimates to known population size in small Idaho streams reported substantial negative bias of 3-pass estimates. Average negative bias was 33% (i.e., estimate=0.67*true number) for rainbow trout whose median lengths ranged from 72-136 mm among sites (Rosenberger and Dunham 2005), and was 34% and 57% for westslope cutthroat trout and bull trout *Salvelinus confluentus*, respectively, in the 100-199 mm length class (Peterson et al. 2004). Rosenberger and Dunham (2005) found that bias increased with stream size (cross sectional area). Peterson et al. (2004) found the same for bull trout although not for cutthroat trout. Twentymile Creek was larger than the average sized stream surveyed in either study. Three-pass removal effectiveness was similar between our study and the two cited above as evidenced by the similarity of standard statistics generated by the removal estimate software (e.g., probability of capture) between our study (Table 5) and theirs. Thus negative bias of our three-pass removal estimates was probably at least as great as it was in those two studies.

Peterson et al. (2004) found that negative bias increased substantially with decreasing size of bull trout (81% for the 70-99 mm length class); an equivalent test could not be conducted for cutthroat trout due to small sample size in the shorter length class. In Twentymile Creek, negative bias may have been greater for younger, smaller fish compared to later on. It seems likely that the age-0 population estimate for the 1995 year-class, made for fish that were only 49 mm long on average, was a gross underestimate compared to that for yearlings of the same cohort, since the age-1 estimate was almost as large as the age-0 estimate despite much emigration in between (Tables 1 and 7).

Domestication versus ancestry

We found differences among crosses in survival, dispersal, growth, condition, and male maturation, and all of these differences were caused by genetic differences between the hatchery and wild parental stocks. Genetic differences between these two populations could have resulted from divergence of the hatchery population from its wild founder population due to aspects of the hatchery program, or alternatively from ancestral differences between the wild founder population and the wild population used in our study which originated from a different tributary of the Clearwater River (Figure 1).

Domestication selection is natural selection for traits that are beneficial in the hatchery program (Reisenbichler and Rubin 1999). It is expected to increase fitness for hatchery rearing but decrease fitness for natural rearing, and it is considered the most likely mechanism by which hatchery culture can rapidly, in a few generations, result in decreased fitness for natural rearing (Reisenbichler et al. 2004; Araki et al. 2008). Relative survivals from the current study, and from the companion study where siblings of the fish released in Twentymile Creek fish for the current study were instead reared in hatcheries (Rubin et al. 2012a), are consistent with domestication of the hatchery population. The current study documented lower survival for HxH than for WxW fish in Twentymile Creek, and the companion study showed higher survival to Lower Snake and Columbia River dams for HxH than for WxW fish reared for the standard one year period at Dworshak Hatchery or for two years at neighboring Clearwater Hatchery prior to release. Thus progeny of the hatchery population exhibited greater fitness in the

hatchery program but reduced fitness for natural rearing relative to wild origin fish, consistent with the expectations of domestication.

Analysis of genetic differentiation among Idaho steelhead populations based on presumably neutral DNA microsatellite markers showed that the Dworshak Hatchery population had ancestral affinities with other Clearwater River populations but that it was also distinct from those populations (Nielsen et al. 2009). At the watershed scale (10 watersheds and 3 hatchery complexes), the Dworshak population clustered with other upper Clearwater River watersheds, including the Lochsa, Selway, and South Fork Clearwater rivers (98% bootstrap support). However, within the upper Clearwater River cluster the Lochsa and Selway rivers clustered together as distinct from Dworshak steelhead and the South Fork (99% bootstrap support). At the population scale (74 natural populations and 5 hatcheries), North Fork Clearwater River populations (steelhead from Dworshak Hatchery and *O. mykiss* from Collins Creek that were landlocked by Dworshak Dam) clustered together as distinct from other Idaho populations (80% bootstrap support), indicating that the difference between North Fork Clearwater River populations and other Idaho populations predated construction of Dworshak Dam and Hatchery. At the population scale, Clearwater River tributaries including the North Fork clustered together, but only the North Fork branch received >50% bootstrap support. The existence of genetic differentiation based on neutral markers is neither necessary nor sufficient evidence that differences in local adaptation exist; however, they suggest the opportunity.

The North Fork Clearwater and Selway rivers are geographically proximate, share the same geology, and are much more similar to each other than to a hatchery (Reisenbichler and Rubin 1999). Consequently we believe that the Selway population was genetically similar to the original North Fork population for fitness-related traits; however, the degree of similarity is unknown. The North Fork stock may have been favored at the hatcheries because the hatcheries obtained their water from the North Fork; however, temperatures differed from those experienced by the ancestral North Fork population because water supplied to Clearwater Hatchery came from the reservoir behind Dworshak Dam, and Dworshak Hatchery employed a water reuse system to provide heated water during the winter. The Selway River population may have been favored in Twentymile Creek because that stream is a tributary of the South Fork Clearwater River which is closer to the Selway River than to the North Fork (Figure 1) and therefore perhaps more similar in environment (Garcia de Leaniz 2007). We cannot rule out ancestral differences as the cause of all or part of the survival differences we observed; however, our finding that survival was higher for hatchery than for wild origin fish in the hatchery program but lower in the wild is consistent with results from most other studies that tested for such differences (Reisenbichler and Rubin 1999, Araki et al. 2008) and with expected consequences of domestication.

Management implications

This study was undertaken to provide managers with data comparing the genetic fitness of Dworshak Hatchery fish and wild fish for natural rearing in the Clearwater River drainage. Relative survivals in Twentymile Creek indicate lower fitness of Dworshak Hatchery fish than of Selway River wild fish for natural rearing. Furthermore, genetic differences were found between these populations in several other traits that may be related to fitness. This study suggests that supplementing wild populations in the Clearwater River drainage with Dworshak Hatchery fish will reduce the fitness of the wild populations if interbreeding occurs. Araki et al. (2006) documented interbreeding in the wild between hatchery and wild summer-run steelhead; wild fish interbred with hatchery fish from a traditional hatchery program (non-local origin, multiple hatchery generations) as well as hatchery fish from a supplementation hatchery (local origin, single hatchery generation). There was also strong evidence of interbreeding between resident *O. Mykiss* and anadromous adults (Araki et al. 2006). Based on this, we think it likely that hatchery supplementation programs for steelhead will result in some level of interbreeding between hatchery and wild fish. During our study, local managers decided against

supplementing most steelhead populations in the Clearwater River basin to avoid any risk of reducing fitness. Our study supports that decision.

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References

Aebersold, P. B., G. A. Winans, D. J. Teel, G. B. Milner, and F. M. Utter. 1987. Manual for starch gel electrophoresis – a method for the detection of genetic variation. NOAA Technical Report NMFS 61.

Araki, H., Ardren, W. R., Olsen, E., Cooper, B. and Blouin, M. S. 2007. Reproductive success of captive-bred steelhead trout in the wild: evaluation of three hatchery programs in the Hood River. Conservation Biology 21:181–190.

Araki, H., Berejikian, B. A., Ford, M. J. & Blouin, M. S. 2008. Fitness of hatchery-reared salmonids in the wild. Evol. Appl. 1, 342–355.

Bjorkstedt, E. P. 2000. DARR (Darroch Analysis with Rank-Reduction): A method for analysis of stratified mark-recapture data from small populations, with application to estimating abundance of smolts from outmigrant trap data. National Marine Fisheries Service, Administrative Report SC-00-02.

Burge, H. L., Faler, M., Roseberg, R. B., Jones, R. N., and Olson, J. M. 2004. Adult steelhead returns to Dworshak NFH in 2002-2003 and prognosis for 2003-2004. Appendix B *in* Annual Report, Fiscal Year 2003, U.S. Fish and Wildlife Service, Idaho Fishery Resource Office, Ahsahka, Idaho.

Byrne, A. 2001. Idaho Fish and Game, fishery research - steelhead supplementation in Idaho rivers summary report 1 January 1993 - 31 December 1999. Bonneville Power Administration Report DOE/BP-01466-6. Portland, OR.

Byrne, A. 2005. Steelhead supplementation studies: Steelhead supplementation in Idaho Rivers, 2004 annual report. Project No. 199005500, 92 electronic pages, BPA Report DOE/BP-00020874-1.

Chilcote, M. W., S. A. Leider, and J. J. Loch. 1986. Differential reproductive success of hatchery and wild summer-run steelhead under natural conditions. Transactions of the American Fisheries Society 115:726-735.

Cone, R. S. 1989. The need to reconsider the use of condition indices in fisheries science. Transactions of the American Fisheries Society 118:510-514.

Garcia de Leaniz, C., Fleming, I. A., Einum, S., Verspoor, E., Jordan, W. C., Consuegra, S., Aubin-Horth, N., Lajus, D., Letcher, B. H., Youngson, A. F., Webb, J. H., Vøllestad, L. A., Villanueva, B., Ferguson, A. and Quinn, T. P. 2007. A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. Biological Reviews 82:173–211.

Goodman, L. A. 1960. On the exact variance of products. Journal of the American Statistical Association 42:708-713.

Hankin, D. G. 1984. Multistage sampling designs in fisheries research: applications in small streams. Canadian Journal of Fisheries and Aquatic Sciences 41:1575-1591.

Hankin, D. G., and G. H. Reeves. 1988. Estimating total fish abundance and total habitat area in small streams based on visual estimation methods. Canadian Journal of Fisheries and Aquatic Sciences 45:834-844.

Holubetz, T. B., and B. D. Leth. 1997. Evaluation and monitoring of wild/natural steelhead trout production, annual progress report. Period covered: January 1, 1995-December 31, 1995. Report to Bonneville Power Administration, Department of energy, Portland, OR; contract number DE-BI79-91BP21182.

Kiefer, R. B., and J. N. Lockhart. 1999. Intensive evaluation and monitoring of chinook salmon and steelhead trout production, Crooked River and upper Salmon River sites Annual Progress Report January 1, 1995-December 31, 1995, Report to Bonneville Power Administration, Contract No. 1991BP21182, Project No. 199107300, 65 electronic pages (BPA Report DOE/BP-21182-11).

Leider, S. A., P. L. Hulett, J. J. Loch, and M. W. Chilcote. 1990. Electrophoretic comparison of the reproductive success of naturally spawning transplanted and wild steelhead trout through the returning adult stage. Aquaculture 88:239-252.

Leary, R. F., F. W. Allendorf, S. R. Phelps, and K. L. Knudsen. 1987. Genetic divergence and identification of seven cutthroat trout subspecies and rainbow trout. Transactions of the American Fisheries Society 116:580-587.

Martinson, R.D., R.J. Graves, R.B. Mills, & J.W. Kamps. 1997. Monitoring of downstream salmon and steelhead at federal hydroelectric facilities - 1996. Annual report. U.S. Dept. of Energy, Bonneville Power Administration, Portland, OR (U.S.A.).

P. McGinnity, C. Stone, J. B. Taggart, D. Cooke, D. Cotter, R. Hynes, C. McCamley, T. Cross, and A. Ferguson. 1997. Genetic impact of escaped farmed Atlantic salmon (*Salmo salar* L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farmed, and hybrid progeny in a natural river environment. ICES Journal of Marine Science 54:998-1008.

Nagata, M., M. Nakajima, and M. Fujiwara. 1994. Dispersal of wild and domestic masu salmon fry (*Oncorhynchus masou*) in an artificial channel. Journal of Fish Biology 45:99-109.

Nielsen, J. L., A. Byrne, S. L. Graziano, and C. C. Kozfkay. 2009. Steelhead genetic diversity at multiple spatial scales in a managed basin: Snake River, Idaho. North American Journal of Fisheries Management 29:680-701.

Ott, L. 1984. An introduction to statistical methods and data analysis, second edition. Duxbury Press, Boston.

Peterson, J. T., R. F. Thurow, and J. W. Guzevich. 2004. An evaluation of multipass electrofishing for estimating the abundance of stream-dwelling salmonids. Transactions of the American Fisheries Society 133:462-475.

Reisenbichler, R. R., M. C. Hayes, S. P. Rubin, L.A. Wetzel, and B. M. Baker. 2006. *PEPA** genotype affects return rate for hatchery steelhead. Transactions of the American Fisheries Society 135:28-39.

Reisenbichler, R.R., & J.D. McIntyre. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, Salmo gairdneri. J. Fish. Res. Board Can. 34: 123-128.

Reisenbichler, R. R., and S. P. Rubin. 1999. Genetic changes from artificial propagation of Pacific salmon affect the productivity and viability of supplemented populations. ICES Journal of Marine Science 56:459-466.

Reisenbichler, R. R., S. P. Rubin, L.A. Wetzel, and S. R. Phelps. 2004. Natural selection after release from a hatchery leads to domestication in steelhead, *Oncorhynchus mykiss*. Pages 371-383 In K.M. Leber, H.L. Blankenship, S. Kitada, and T. Svåsand [editors] Stock Enhancement and Sea Ranching: developments, pitfalls, and opportunities. 2nd edition. Blackwell Science Ltd, Oxford.

Roper, B. B., and D. L. Scarnecchia. 1996. A comparison of trap efficiencies for wild and hatchery age-0 chinook salmon. North American Journal of Fisheries Management 16:214-217.

Rosenberger, A. E., and J. B. Dunham. 2005. Validation of abundance estimates from mark recapture and removal techniques for rainbow trout captured by electrofishing in small streams. North American Journal of Fisheries Management 25:1395-1410.

Rubin, S. P., R. R. Reisenbichler, J. E. Hensleigh, L. A. Wetzel, and B. M. Baker. 2012a. Genetic differences between hatchery and wild steelhead for growth and survival in the hatchery and seaward migration after release. Chapter 2 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, J. E. Hensleigh, F. E. Leonetti, B. M. Baker, S. L. Slatton, and K. D. Stenberg. 2012d. Differences in survival and growth in hatchery and stream environments, and in maturation of residuals in a stream, between progeny of hatchery and wild steelhead. Chapter 1 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, and K. D. Stenberg. 2012f. Effect of size of unfed fry at release on survival and growth of juvenile steelhead in streams and a hatchery. Chapter 7 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

SAS Institute Inc. 2000. SAS Online DOC^{R}, Version 8. SAS Institute, Cary, North Carolina.

Schmidt, S. P., and E. W. House. 1979. Precocious sexual development in hatchery-reared and laboratory-maintained male steelhead trout (*Salmo gairdneri*). Journal of the Fisheries Research Board of Canada 36:90-93.

Shaklee, J. B. F. W. Allendorf, D. C. Morizot, and G. S. Whitt. 1990. Gene nomenclature for protein-coding loci in fish. Transactions of the American Fisheries Society 119:2-15.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry, 3rd edition. W. H. Freeman and Company, New York.

Thedinga, J. F., M. L. Murphy, S. W. Johnson, J. M. Lorenz, and K. V. Koski. 1994. Determination of salmonid smolt yield with rotary-screw traps in the Situk River, Alaska, to predict effects of glacial flooding. North American Journal of Fisheries Management 14:837-851.

Tyler, C. R., J. P. Sumpter, and P. R. Witthames. 1990. The dynamics of oocyte growth during vitellogenesis in rainbow trout (*Oncorhynchus mykiss*). Biology of Reproduction 43:202-209.

Van Deventer, J. S., and W. S. Platts. 1985. Computer software system for entering, managing, and analyzing fish capture data from streams. Research Note, Intermountain Research Station, USDA Forest Service, Ogden, Utah.

Volk, E. C., S. L. Schroder, and K. L. Fresh. 1990. Inducement of unique banding patterns as a practical means to mass-mark juvenile pacific salmon. American Fisheries Society Symposium 7:203-215.

Zimmerman, C. E., and G. H. Reeves. 2000. Population structure of sympatric anadromous and nonanadromous *Oncorhynchus mykiss*: evidence from spawning surveys and otolith microchemistry. Canadian Journal of Fisheries and Aquatic Sciences 57:2152-2162.

Tables

Table 1. Pooled and weighted estimates of frequency and adjusted relative frequency (ARS) of migrants. Weighted estimates are shown only when they differ from pooled estimates (i.e., when trap efficiency data were not pooled over an entire season). * indicates a significant difference of ARS from 1.0 ($P<0.05$); $^{+}$ indicates a marginally significant difference ($P=0.062$). *n*=number of fry released or number of trapped fish that were typed to cross; *N*hat=estimated total number of migrants reaching the trap site; *N* trapped=total number of migrants that were trapped. Spring=April-July; Fall=September-October.

				Pooled							Weighted					
				Proportion			ARS				Proportion			ARS		
Year-class	Age	Season	*n*	HxH	HxW	WxW	HxH/WxW	HxW/WxW	HxW/HxH	*N*hat (SE) or *N* trapped	HxH	HxW	WxW	HxH/WxW	HxW/WxW	HxW/HxH
1994	Fry	16-17 June[a]	26,739[b]	0.495	-	0.505	-	-	-	-	-	-	-	-	-	-
	1	Spring	181	0.425	-	0.575	0.756 $^{+}$	-	-	1685 (366)	-	-	-	-	-	-
		Fall	42	0.381	-	0.619	0.629	-	-	108 (5)	-	-	-	-	-	-
		Total	223	0.417	-	0.583	0.731 *	-	-	1793 (366)	0.423	-	0.577	0.748	-	-
	2	Spring	311	0.350	-	0.650	0.551 *	-	-	1360 (121)	0.338	-	0.662	0.522	-	-
			-	-	-	-	-	-	-	458[c]	0.329[d]	-	0.671[d]	0.502*[d]	-	-
		Fall	0	-	-	-	-	-	-	0 (0)	-	-	-	-	-	-
		Total	311	0.350	-	0.650	0.551 *	-	-	1360 (121)	0.338	-	0.662	0.522	-	-
			-	-	-	-	-	-	-	458[c]	0.329[d]	-	0.671[d]	0.502*[d]	-	-
	3	Spring	10	0.300	-	0.700	0.438	-	-	50 (20)	0.398	-	0.602	0.674	-	-
		Fall	1	1.000	-	0.000	-	-	-	4 (0)	-	-	-	-	-	-
		Total	11	0.364	-	0.636	0.584	-	-	54 (20)	0.444	-	0.556	0.815	-	-
	4	Spring	3	0.000	-	1.000	0.000	-	-	14 (4)	-	-	-	-	-	-
		Fall	0	-	-	-	-	-	-	0 (0)	-	-	-	-	-	-
		Total	3	0.000	-	1.000	0.000	-	-	14 (4)	-	-	-	-	-	-
	Total	Spring	505	0.374	-	0.626	0.611 *	-	-	3108 (386)	0.385	-	0.615	0.639	-	-
		Fall	43	0.395	-	0.605	0.668	-	-	112 (5)	0.404	-	0.596	0.691	-	-
		Total	548	0.376	-	0.624	0.615 *	-	-	3220 (387)	0.386	-	0.614	0.641	-	-

[a]Dates fry were released.
[b]Total number of fry released.
[c]Number of fish trapped; note that not all trapped fish were typed to cross.
[d]Weighted average of the proportion of the cross in each temporal stratum (from samples that were typed to cross); weights were the total number of migrants trapped in the stratum rather than the estimated total number of migrants in the stratum (see text).

Table 1, continued.

				Pooled							Weighted					
				Proportion			ARS				Proportion			ARS		
Year-class	Age	Season	*n*	HxH	HxW	WxW	HxH/WxW	HxW/WxW	HxW/HxH	*N*hat (SE) or *N* trapped	HxH	HxW	WxW	HxH/WxW	HxW/WxW	HxW/HxH
1995	Fry	20-21 June[a]	36,084[b]	0.332	0.330	0.338	-	-	-	-	-	-	-	-	-	-
	0	Spring	0	-	-	-	-	-	-	0 (0)	-	-	-	-	-	-
		Fall	9	0.000	0.333	0.667	0.000	0.511	-	23 (1)	-	-	-	-	-	-
		Total	9	0.000	0.333	0.667	0.000	0.511	-	23 (1)	-	-	-	-	-	-
	1	Spring	955	0.292	0.347	0.361	0.824 *	0.981	1.191 *	6219 (313)	0.291	0.325	0.384	0.772	0.864	1.119
			-	-	-	-	-	-	-	2343[c]	0.295[d]	0.316[d]	0.389[d]	0.771*[d]	0.831[d]	1.078[d]
		Fall	0	-	-	-	-	-	-	0 (0)	-	-	-	-	-	-
		Total	955	0.292	0.347	0.361	0.824 *	0.981	1.191 *	6219 (313)	0.291	0.325	0.384	0.772	0.864	1.119
			-	-	-	-	-	-	-	2343[c]	0.295[d]	0.316[d]	0.389[d]	0.771*[d]	0.831[d]	1.078[d]
	2	Spring	642	0.315	0.354	0.332	0.966	1.090	1.128	3083 (427)	0.324	0.354	0.322	1.023	1.124	1.099
		Fall	10	0.200	0.100	0.700	0.291	0.146	0.502	41 (3)	0.200	0.100	0.700	0.291	0.146	0.502
		Total	652	0.313	0.350	0.337	0.944	1.060	1.122	3124 (427)	0.322	0.351	0.327	1.002	1.097	1.094
	3	Spring	53	0.245	0.358	0.396	0.630	0.925	1.467	277 (67)	-	-	-	-	-	-
		Fall	10	0.400	0.200	0.400	1.018	0.511	0.502	61 (15)	-	-	-	-	-	-
		Total	63	0.270	0.333	0.397	0.693	0.859	1.240	338 (68)	0.273	0.330	0.397	0.701	0.850	1.212
	4	Spring	11	0.091	0.455	0.455	0.204	1.022	5.020	45 (18)	-	-	-	-	-	-
		Fall	2	0.000	0.000	1.000	0.000	0.000	-	10 (4)	-	-	-	-	-	-
		Total	13	0.077	0.385	0.538	0.145	0.730	5.020	55 (18)	0.075	0.374	0.552	0.138	0.692	5.020
	Total	Spring	1661	0.298	0.350	0.352	0.863 *	1.019	1.180 *	9623 (534)	0.299	0.336	0.365	0.835	0.940	1.126
		Fall	31	0.194	0.194	0.613	0.322 *	0.323	1.004	135 (16)	0.242	0.178	0.580	0.424	0.314	0.739
		Total	1692	0.296	0.348	0.356	0.846 *	0.997	1.178 *	9758 (534)	0.299	0.334	0.368	0.826	0.927	1.121

[a]Dates fry were released.
[b]Total number of fry released.
[c]Number of fish trapped; note that not all trapped fish were typed to cross.
[d]Weighted average of the proportion of the cross in each temporal stratum (from samples that were typed to cross); weights were the total number of migrants trapped in the stratum rather than the estimated total number of migrants in the stratum (see text).

Table 2. Rate at which fish of known age were incorrectly aged from scales. Known age was determined from otolith marks thermally induced during embryo incubation.

		Known 1994 year-class			Known 1995 year-class		
Collection year	Sub-population	Correctly aged	Incorrectly aged	Error rate (%)	Correctly aged	Incorrectly aged	Error rate (%)
1996	Migrants	33	1	2.9	28	1	3.4
1997	Residents	3	0	0.0	37	0	0.0
1999	Residents	4	2	33.3	38	2	5.0

Table 3. Number of migrants trapped, marked, and recaptured; and estimated trap efficiency. Spring = April-July; fall = September-October (1995-1997) or August-October (1998-1999); in 2000 the trap was only operated during spring.

			Number of fish				
Year	Season	Trap	Trapped	Marked and released (M)	Recaptured (R)	R/M	Trap efficiency (SE)
1995	Spring	Kray Meekin	181	13	2	0.154	0.107[a] (0.023)
	Fall	Screw	51	0	-	-	0.389[b] (0.016)
1996	Spring	Screw	2,702[c]	996	435	0.437	0.389[d] (0.016)
		Kray Meekin	99[c]	153[e]	20[e]	0.131	0.107[a] (0.023)
	Fall	Screw	0	0	-	-	
1997	Spring	Screw	682	678	192	0.283	0.244[d] (0.018)
	Fall	Screw	11	10	0	0.000	0.244[f] (0.018)
1998	Spring	Screw	62	61	13	0.213	0.213[g] (0.052)
	Fall	Screw	13	13	6	0.462	0.213[f] (0.052)
1999	Spring	Screw	15	13	4	0.308	0.308[g] (0.128)
	Fall	Screw	4	4	1	0.250	0.308[f] (0.128)
2000	Spring	Screw	20	18	5	0.278	0.278[g] (0.106)

[a]Equals (mean efficiency of screw trap in spring 1996 (0.389))*(ratio of Kray Meekin efficiency to screw trap efficiency during late June-mid July 1996 (0.276)); see text.
[b]Equals mean efficiency during spring 1996; based on assumption that efficiency was equivalent between fall 1995 and spring 1996.
[c]We estimate that 3,061 fish would have been caught if only the screw trap had been operated (equals (number caught in screw trap)+(number caught in Kray Meekin)/(ratio of Kray Meekin efficiency to screw trap efficiency during late June-mid July 1996 (0.276))).
[d]Mean of weekly estimates shown in Figure 5.
[e]Number of fish recaptured in the Kray Meekin trap on 29 June and 1, 11, and 13 July of fish marked and released on preceding days.
[f]Assumed equal to mean spring efficiency.
[g]Single whole-season estimate; release-recapture data pooled across all weeks.

Table 4. Times and locations of electrofishing in Twentymile Creek.

Year	Dates	Portion of stream (m above lower road)
1994	24-27 Aug	-60-5400
1995	9-15 Aug	-370-5140
	26 Aug	5260-5340
1996	6-13 Aug	150-5400
1997	22-29 Jul	150-5400
1998	4-11 Aug	0-3070
	18-23 Aug	3070-5290
1999	18-24 Aug	0-2210
	31 Aug-6 Sep	2400-5290
2000	22-28 Aug	60-3350
	7-8 Sep	3690-5400

Table 5. Statistics for precision and accuracy of 3-pass removal estimates, and the expansion factor used to estimate population size in sites where only one pass was made. *N*hat=removal estimate.

Statistic	Collection year, age(s) of fish collected						
	1995, 0	1995, 1	1996, 1-2	1997, 2-3	1998, 3-4	1999, 4-5	2000, 5-6
Number of 3-pass sites	4	4	4	4	9	7	7
Mean probability of capture[a]	0.60	0.59	0.54	0.45	0.74	0.66	0.63
Mean ratio of total 3-pass catch to *N*hat	0.94	0.94	0.89	0.81	1.00	0.99	1.00
Mean ratio of *N*hat to first pass catch	1.80	1.91	2.03	2.39	1.40	1.49	1.50
SE(ratio of *N*hat to first pass catch)	0.13	0.28	0.30	0.47	0.04	0.16	0.14

[a]Computed by the removal estimate software (Van Deventer and Platts 1985).

Table 6. Mean (SD) [*n*] egg size; survival, temperature, and density during incubation from fertilization to the eyed stage; and final size of fry at release. Egg weight was the same for HxH and HxW crosses because the same hatchery females were used in both. Likewise, incubation temperature and number of eggs per tray were the same for HxH and HxW crosses because each incubation tray contained both crosses from a single hatchery female, separated by a divider. Letters (x, y, z) indicate significance of comparisons between pairs of crosses; within a year-class, means without a letter in common are different ($P<0.05$); na=not applicable (test conducted between H and W females).

	1994		1995		
Variable	HxH	WxW	HxH	HxW	WxW
Egg weight (g)[a]	0.119 (0.014) [25] x	0.102 (0.008) [18] y	0.107 (0.012) [23] x	0.107 (0.012) [23] na	0.102 (0.011) [10] x
Survival to eye-up (%)[b]	84.2 (17.5) [50] x	73.6 (31.6) [36] x	95.3 (7.2) [23] x	93.4 (9.8) [23] x	97.3 (3.0) [20] x
Incubation temperature	8.5	9.0	8.5	8.5	8.3
Number of eggs per tray[a]	6761 (1292) [25] x	4846 (831) [18] y	6893 (1549) [23] x	6893 (1549) [23] na	4778 (707) [10] y
Fry length (mm)[c]	29.9 (1.0) [60] x	29.0 (0.8) [60] y	29.3 (1.0) [60] x	29.2 (0.9) [60] x	28.4 (0.9) [60] y
Fry weight (g)[d]	0.194	0.163	0.196	0.185	0.168

[a]Standard deviation among females.
[b]Standard deviation among families.
[c]Standard deviation among individuals.
[d]No SD or test statistics because fry were weighed collectively rather than individually.

Table 7. Pooled and weighted estimates of frequency and adjusted relative frequency (ARS) of residents. Weighting factors were the estimated number of fish inside and outside of sections where fry were released in 1995 (Figure 4). The relative frequency of crosses differed between inside and outside strata only for 1995 cohort sub-yearlings and yearlings (see text); thus weighted estimates were made only for those groups. Asterisks indicate a significant difference of ARS from 1.0. *n*=number of captured fish typed to cross; *N*hat=estimated total number of residents.

				Pooled							Weighted					
				Proportion			ARS				Proportion			ARS		
Year-class	Age	Strata	*n*	HxH	HxW	WxW	HxH/WxW	HxW/WxW	HxW/HxH	*N*hat (SE)	HxH	HxW	WxW	HxH/WxW	HxW/WxW	HxW/HxH
1994	0	Combined	288	0.462	-	0.538	0.877	-	-	-	-	-	-	-	-	-
	1	Combined	181	0.530	-	0.470	1.154	-	-	1321 (326)	-	-	-	-	-	-
	2	Combined	115	0.652	-	0.348	1.916 *	-	-	625 (75)	-	-	-	-	-	-
	3	Combined	26	0.577	-	0.423	1.393	-	-	208 (45)	-	-	-	-	-	-
	4	Combined	15	0.400	-	0.600	0.681	-	-	28 (7)	-	-	-	-	-	-
1995	0	Inside	229	0.393	0.341	0.266	1.503 *	1.307	0.870	2413 (470)	-	-	-	-	-	-
		Outside	134	0.216	0.343	0.440	0.501 *	0.797	1.592 *	1417 (333)	-	-	-	-	-	-
		Total	363	0.328	0.342	0.331	1.010	1.057	1.046	3830 (576)	0.328	0.342	0.331	1.009	1.056	1.047
	1	Inside	364	0.415	0.319	0.266	1.585 *	1.223	0.771 *	1369 (164)	-	-	-	-	-	-
		Outside	332	0.328	0.340	0.331	1.009	1.050	1.041	2255 (328)	-	-	-	-	-	-
		Total	696	0.374	0.329	0.297	1.279 *	1.131	0.884	3624 (367)	0.361	0.332	0.307	1.198	1.107	0.924
	2	Combined	256	0.355	0.324	0.320	1.130	1.035	0.916	2133 (227)	-	-	-	-	-	-
	3	Combined	431	0.336	0.299	0.364	0.941	0.840	0.893	812 (35)	-	-	-	-	-	-
	4	Combined	144	0.264	0.313	0.424	0.634 *[a]	0.754	1.189	380 (29)	-	-	-	-	-	-

[a] *P*=0.051 after Bonferroni adjustment for two paired comparisons.

Table 8. Proportion of each cross and population size for steelhead of uncertain age collected in 1999 and 2000. Note that HxW fish collected in 2000 could only have been from the 1995 year-class and therefore were all five-year olds.

Collection year	Possible ages	Sub-population	*n*	Proportion HxH	HxW	WxW	*N*hat (SE)
1999	4, 5	Migrants	1	0.000	0.000	1.000	4 (2)
		Residents	18	0.333	0.000	0.667	38 (10)
2000	5, 6	Migrants	17	0.353	0.235	0.412	72 (27)
		Residents	66	0.227	0.288	0.485	188 (17)

Table 9. Annual relative survival (RS) in Twentymile Creek computed from combined migrants and residents or from residents alone (using weighted estimates from Tables 1 and 7, or pooled estimates if equivalent to weighted). True RS probably was between combined and residents-only values (see text). RS is shown for intervals $\geq$2 years (computed as the product of annual relative survivals) when combined and residents-only estimates both show the same direction of survival differences between HxH and WxW fish. Residents-only estimates of $RS_{HH/WW}$ from age-2 to age-4 were tested with logistic regression of relative frequency against age. * indicates significance (P<0.05); $^{+}$ indicates marginal significance (P=0.062).

Year-class	Interval	RS HH/WW Combined	HH/WW Residents only	HW/WW Combined	HW/WW Residents only	HW/HH Combined	HW/HH Residents only
1994	Fry-1	0.91	1.15	-	-	-	-
	1-2	0.68	1.66	-	-	-	-
	2-3	0.63	0.73	-	-	-	-
	3-4	0.36	0.49	-	-	-	-
	2-4	0.23	0.36^{+}				
1995	Fry-1	0.91	1.20	0.94	1.11	1.04	0.92
	1-2	0.89	0.94	0.98	0.93	1.10	0.99
	2-3	0.72	0.83	0.79	0.81	1.09	0.98
	3-4	0.68	0.67	0.90	0.90	1.32	1.33
	1-4	0.44	0.53[a]	0.69	0.68	1.59	1.29
	2-4	0.49	0.56*	0.71	0.73	1.44	1.30

[a]Could not be tested statistically because such a test required pooled estimates of $ARF_{HH/WW}$ for each age, but the estimate for age-1 fish, unlike those for fish aged 2-4, was a weighted estimate (Table 7).

Table 10. Means (SDs) for variables associated with sex and maturity from a sub-sample of 1995 year-class residents. GSI=gonadosomatic index.

Age	Sex	*n*	Proportion within age	Fork length (mm)	Weight (g)	Weight of gonads (g)	GSI (%)
3	Females	26	0.48	139 (15)	28.9 (8.7)	0.09 (0.07)	0.3 (0.2)
	Males						
	Flowing milt	23	0.43	140 (10)	32.6 (7.4)	2.90 (1.03)	8.9 (2.3)
	Not	5	0.09	139 (14)	30.4 (8.1)	2.48 (0.87)	8.1 (1.5)
4	Females	15	0.30	161 (12)	46.1 (11.9)	0.74 (0.45)	1.6 (1.0)
	Males						
	Flowing milt	12	0.48[a]	160 (17)	51.1 (9.4)	4.35 (0.92)	8.7 (1.6)
	Not	11	0.22	163 (15)	52.2 (12.0)	3.69 (0.79)	7.3 (1.5)

[a]In collection year 1999, we euthanized one quarter of the ripe males (i.e., with flowing milt) that we caught and one half of the other fish (females and males without milt). We euthanized 12 ripe males and 26 other fish, of which 15 turned out to be females and the other 11 males. Therefore, the numbers we used to compute "proportion within age" were 15 females, 24 males with flowing milt (double the number euthanized), and 11 males without flowing milt.

Figures

Figure legends

Figure 1. Study area.

Figure 2. Characteristics of the study reach. Wetted width was measured at 20 m intervals, and depth and substrate at 0.25, 0.5, and 0.75 of width at each transect. Gradient between each pair of transects was measured with a clinometer by people standing in the stream and was occasionally negative after accounting for water depth due to measurement error. Substrate particle size in a 1 m^2 area was indexed as 1 (sand, <2 mm), 2 (gravel, 2-64 mm), 3 (cobble 64-256 mm), or 4 (boulders, >256 mm) by which size class covered the most area. Means were computed for each of 54 approximately 100 m long contiguous sites and plotted against the midpoint of the site. Also plotted are counts of boulders (>0.5 m diameter) and pieces of large woody debris (LWD; length>1 m and diameter>0.1 m) at least partially submerged in each site. All measurements were made in July 1998.

Figure 3. Daily mean water temperature and staff gage height during 1995-1999. The staff gage and a temperature logger were located at the lower road and a second logger at the upper road.

Figure 4. Density of residents at each sample site. Text in each panel designates year-class and age. In all cases except 1994 cohort sub-yearlings, the total number of fish in a site was determined by removal estimates (3-pass sites) or expansion factors based on removal estimates (1-pass sites), and then partitioned by proportion of each cross from captured fish (see text for details). For 1994 cohort sub-yearlings, the number of fish was the un-expanded number caught in one pass. Vertical dashed lines designate locations where fry were point-released in 1994 or sections where they were scatter-released in 1995 (short dashes delineate the bottom and long dashes the top of a section). These boundaries are not shown for age-3 and age-4 fish because their distribution was no longer related to release sites. Data from sites below the lower road (94 and 95 collection years) are shown for interest but were not included in estimates for residents (ARS, mean length, etc.).

Figure 5. Number of migrants trapped each week. Text in each panel designates year-class and age. Two adjustments were made to increase data comparability. First, April-July 1994 cohort yearlings, which were caught in the Kray Meekin trap, were multiplied by 3.6, the ratio of the efficiency of the screw trap, which was used at all other times, and the Kray Meekin. Second, during April-July 1996 (1994 cohort two-year olds and 1995 cohort yearlings), fish typed to cross were expanded (divided) by weekly sampling efficiency. Sampling efficiency was computed as the number of fish assayed for cross divided by the total number of fish trapped and is shown by the dotted line. At all other times nearly every trapped fish was assayed for cross. Screw trap efficiency (proportion of total migrants trapped) is shown by the solid lines for periods when data were sufficient to make weekly estimates.

Figure 6. Apparent survival of HxH and of HxW relative to WxW fish ($ARS_{HH/WW}$ and $ARS_{HW/WW}$, respectively) for the 1994 (top) and 1995 (bottom) year-class at successive ages. ARS values are weighted estimates from Tables 1 and 7 (or pooled estimates if they equivalent to weighted estimates). An asterisk indicates that ARS differs significantly from 1 as determined by comparing weighted estimates to results of statistical tests conducted with pooled estimates (see text).

Figure 7. Residents-only estimates of annual $RS_{HH/WW}$ from age-2 (1994 year-class) or age-1 (1995 year-class) to age-4 (Table 9) versus ending age (e.g., 2 represents the interval age-1 to age-2). The regression line was fit to all five data points.

Figure 8. Mean length. Text in upper left of each panel designates year-class and sub-population. Error bars are 1 SD. Letters above groups of bars indicate significance of paired comparisons (a: HxH and WxW differ; b: HxW and HxH differ). A pound sign indicates that capture date had a significant effect on length ($P<0.05$) and was included as a covariate; means are least square means from the analysis. The slope of the length-date relation never differed among crosses ($P\geq0.09$). Spr=spring (April-July); fal=fall (September-October).

Figure 9. Mean length of 1995 year-class residents inside and outside of fry release sections. Error bars are 1 SD. Letters above mean values indicate significance of paired comparisons (a: HxH and WxW differ; b: HxW and HxH differ).

Figure 10. Mean length of migrants trapped each week. Text in each panel designates year-class and age.

Figure 11. Mean length of residents at each site. Text in each panel designates year-class and age. Vertical dashed lines designate locations where fry were point-released in 1994 or sections where they were scatter-released in 1995 (short dashes delineate the bottom and long dashes the top of a section).

Figure 12. Length distributions of 1995 year-class HxH and WxW fish. Text in each panel designates cross and sub-population; migrants include only those trapped during April-July. Within an age relative frequencies sum to 1.

Figure 13. Mean condition factor. Text in upper left of each panel designates year-class and sub-population; fall migrants were excluded. Letters above groups of bars indicate significance of paired comparisons (a: HxH and WxW differ; b: HxW and HxH differ). An asterisk indicates that slopes of the weight-length relation differed among crosses ($P<0.05$) in the AOCV used to test for a difference in weight at common length. When slopes differed, paired comparisons were not made.

Figure 14. Proportion of age-2 and older fish with flowing milt. Text in upper left of each panel designates year-class and sub-population; migrant data are pooled across spring and fall. Error bars are 1 SD. Letters above groups of bars indicate significance of paired comparisons (a: HxH and WxW differ; b: HxW and HxH differ).

Figure 15. Percentage of 1995 year-class residents expressing milt in relation to length. Within an age, separate lines were fit for each cross by logistic regression (SAS 2000). An asterisk indicates that regression slopes differed. Letters indicate that intercepts at a common slope differed (a: HxH and WxW differ; b: HxW and HxH differ). Lines were fit for fish of all lengths of a given age but are plotted with the tails of the length distributions omitted.

Figure 16. Change in mean length (i.e., length gain) from residents of an age to migrants or residents one year older, for WxW relative to HxH fish:

Relative length gain = $(L_{WW2}-L_{WW1})/(L_{HH2}-L_{HH1})$;

L_{WW1} = mean length of WxW fish at time 1 (e.g., age-0 residents); L_{WW2} = mean length of WxW fish at time 2 (e.g., age-1 migrants); L_{HH1} and L_{HH2} = the same for HxH fish.

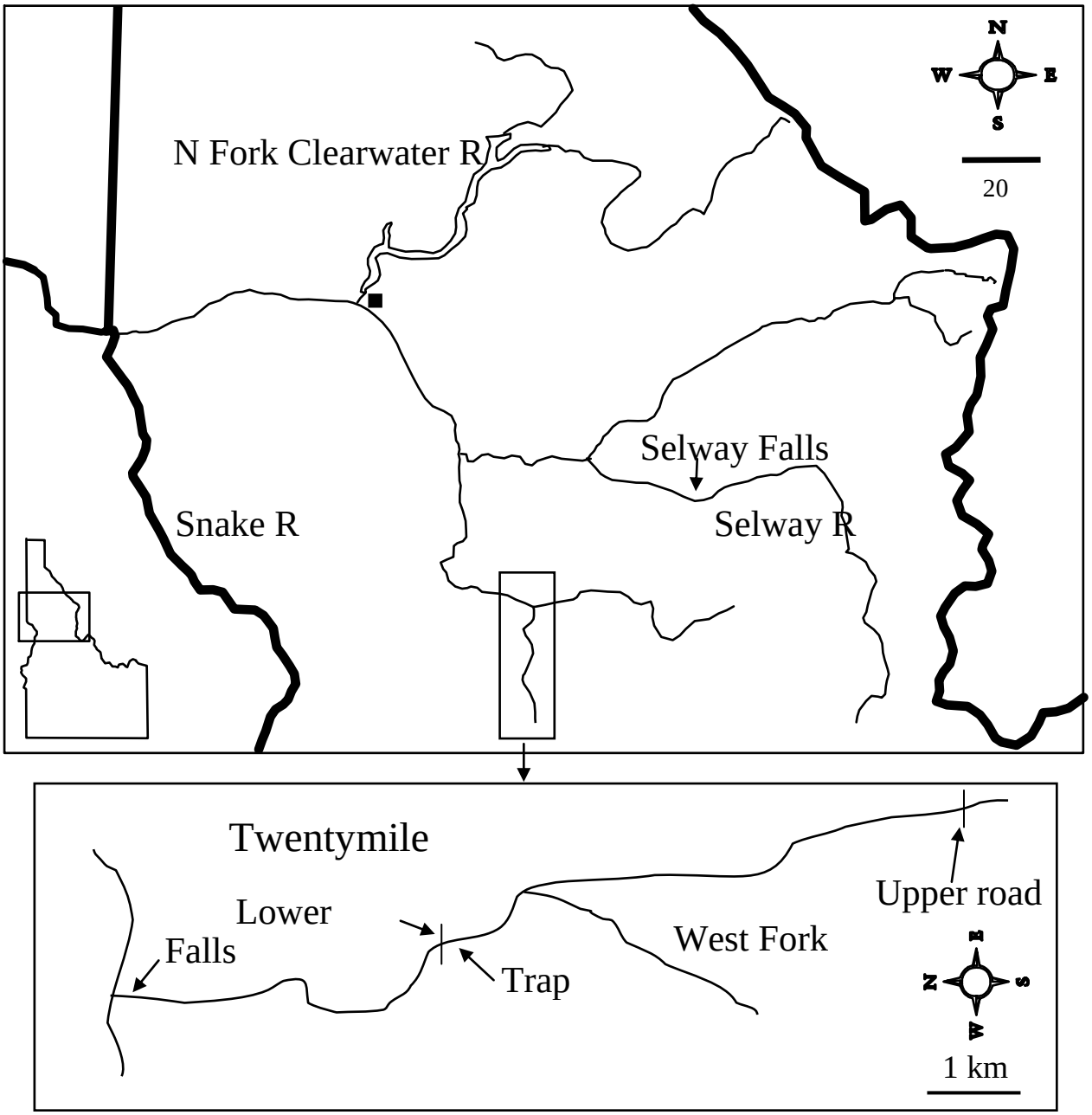
N
W
E
S
20
N Fork Clearwater R
Selway Falls
Snake R
Selway R
Twentymile
Lower
Falls
Trap
West Fork
Upper road
1 km

Figure 1.

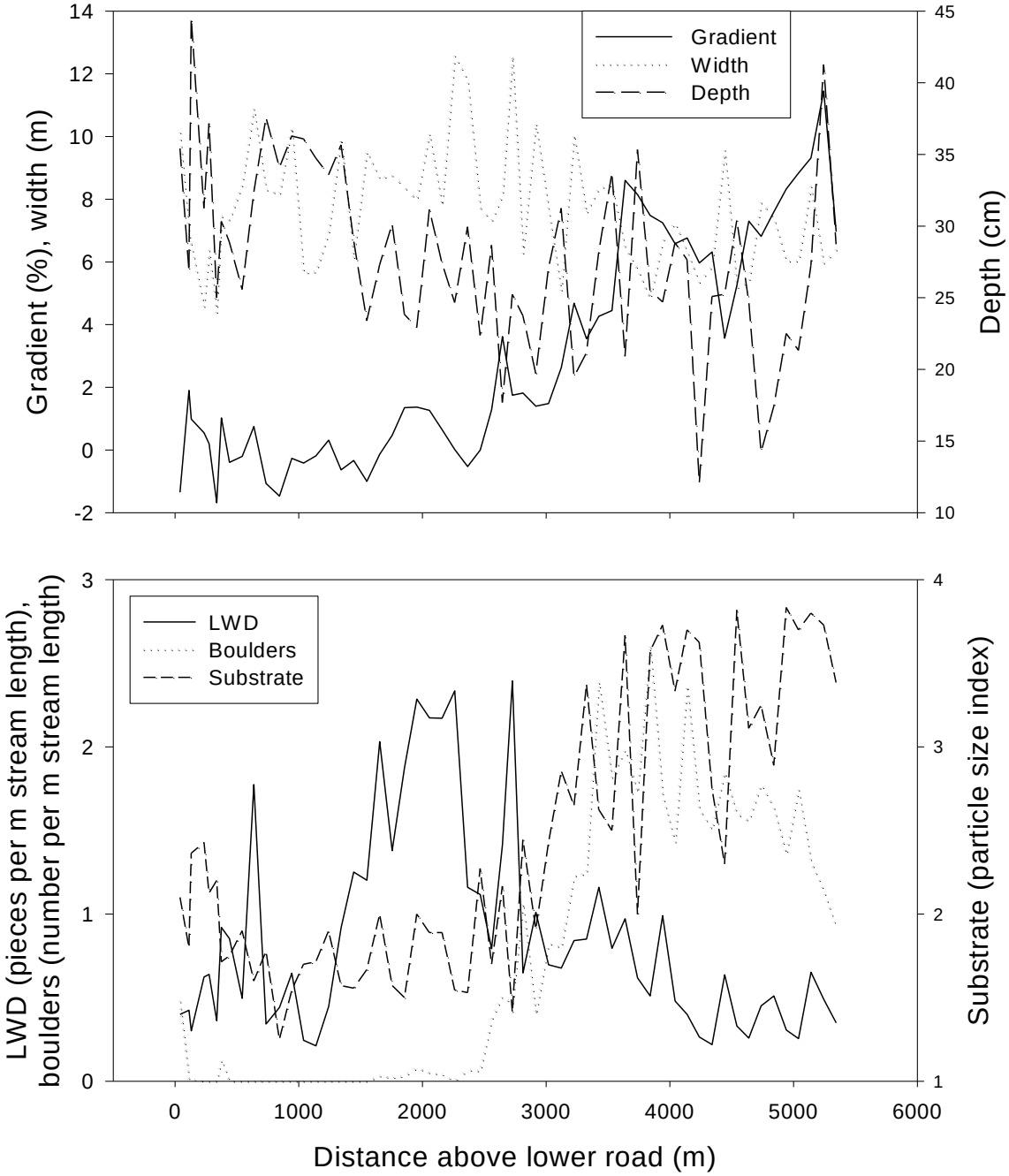
Gradient
Width
Depth
Gradient (%), width (m)
Depth (cm)
LWD
Boulders
Substrate
LWD (pieces per m stream length), boulders (number per m stream length)
Substrate (particle size index)
Distance above lower road (m)

Figure 2.

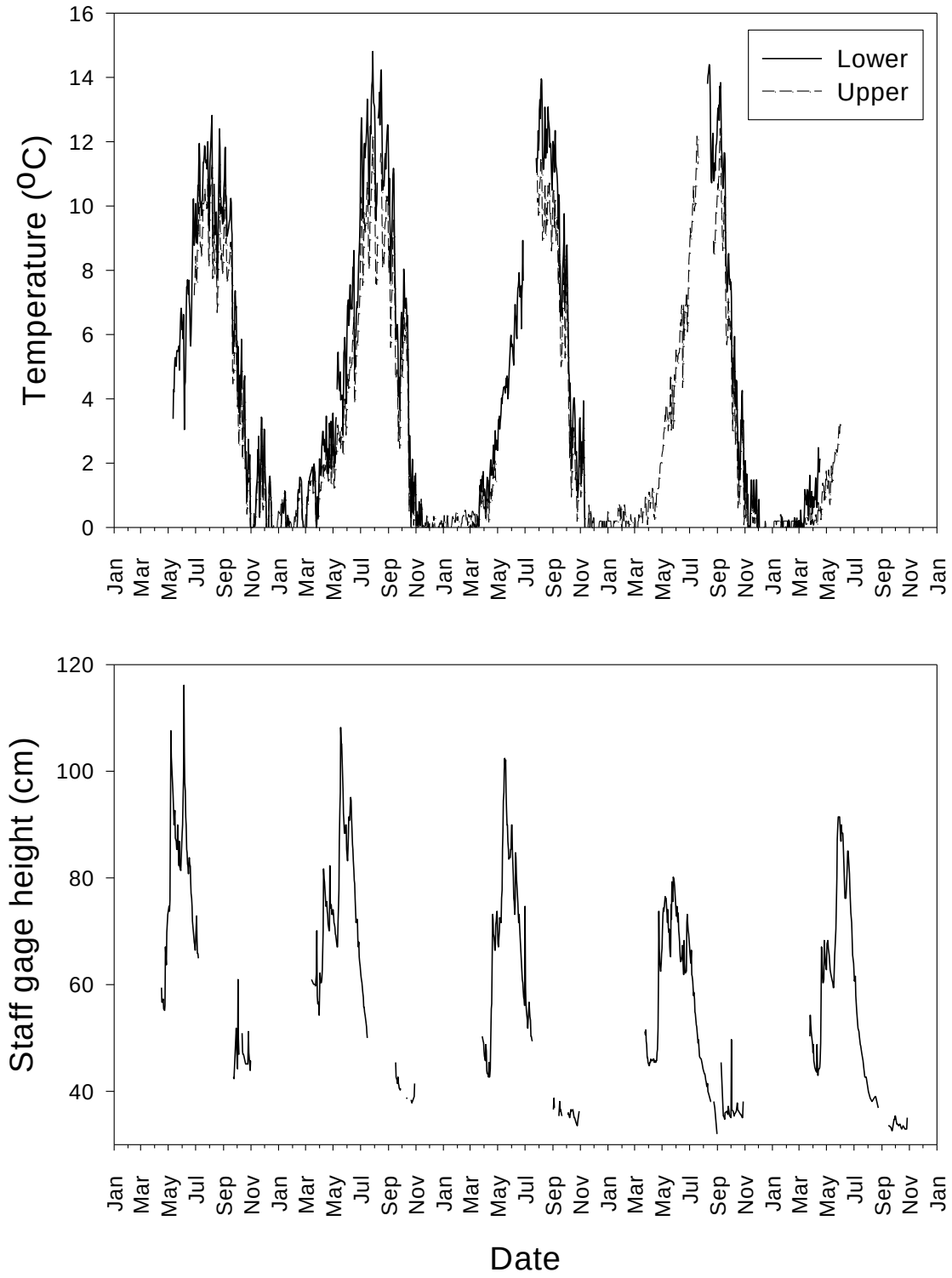

Figure 3.

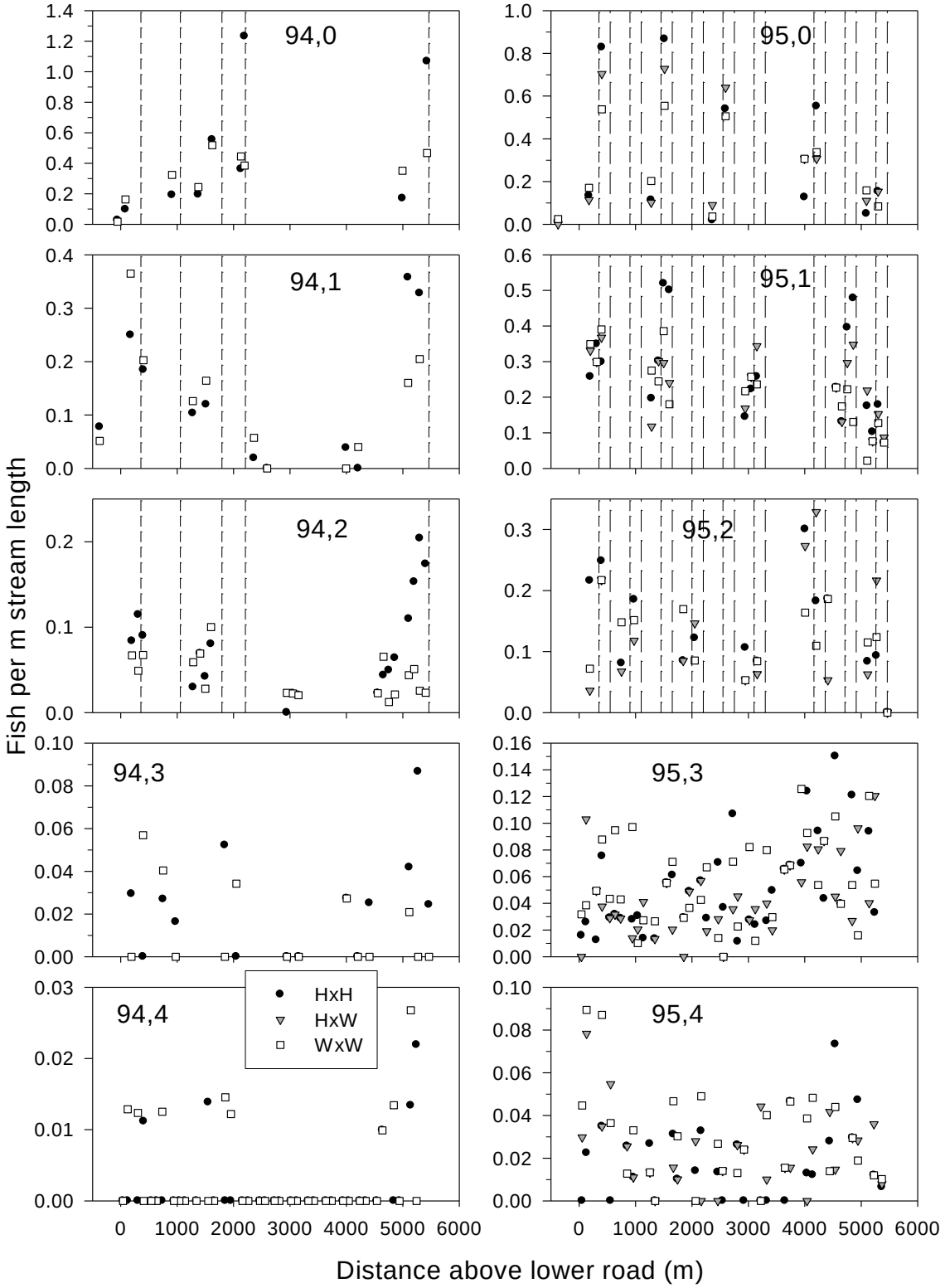
94,0
95,0
94,1
95,1
94,2
95,2
94,3
95,3
94,4
95,4
HxH
HxW
WxW
Fish per m stream length
Distance above lower road (m)

Figure 4.

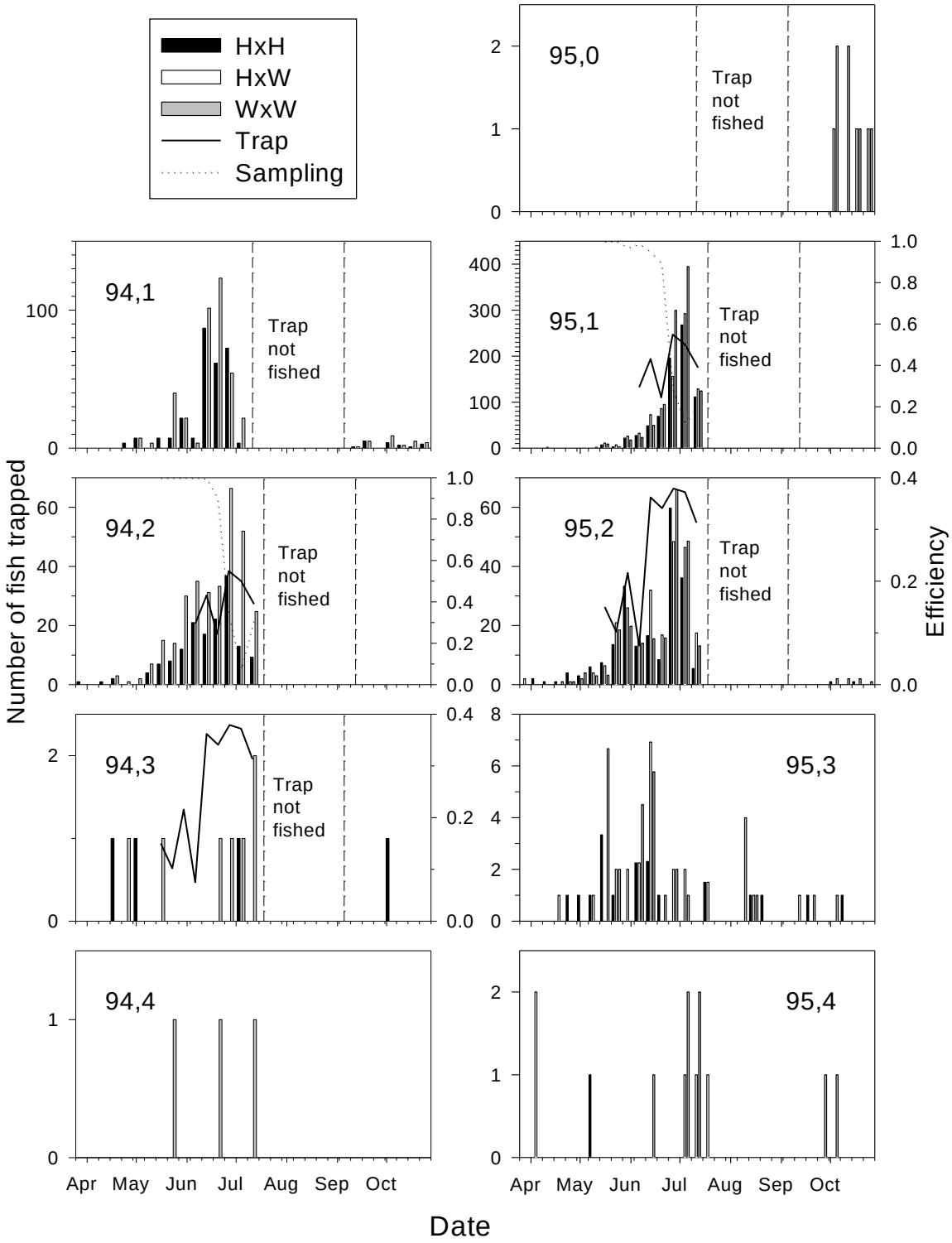
HxH
HxW
WxW
Trap
Sampling
95,0
94,1
95,1
94,2
95,2
94,3
95,3
94,4
95,4
Trap not fished
Number of fish trapped
Efficiency
Apr
May
Jun
Jul
Aug
Sep
Oct
Date

Figure 5.

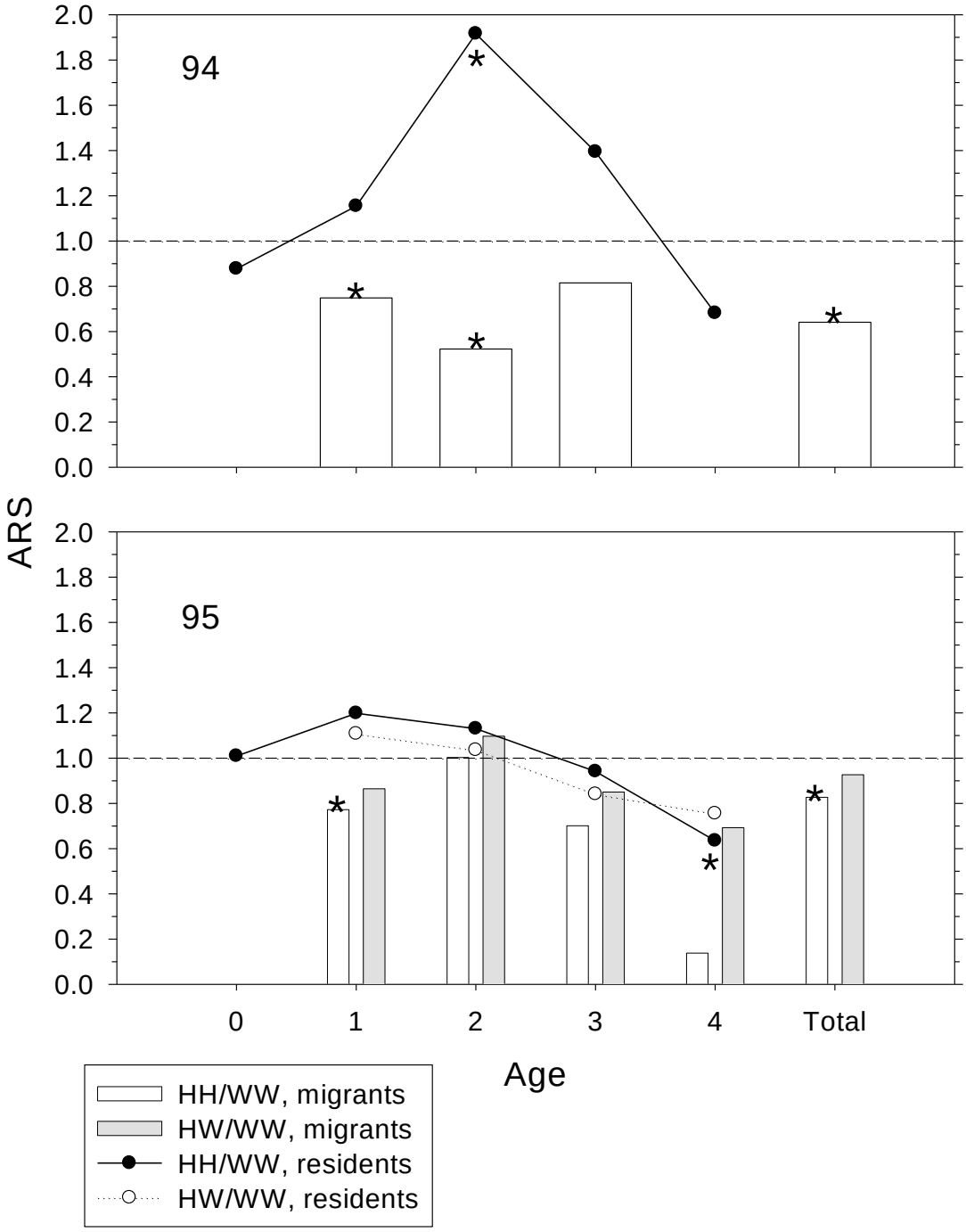
94
95
ARS
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
Total
Age
HH/WW, migrants
HW/WW, migrants
HH/WW, residents
HW/WW, residents

Figure 6.

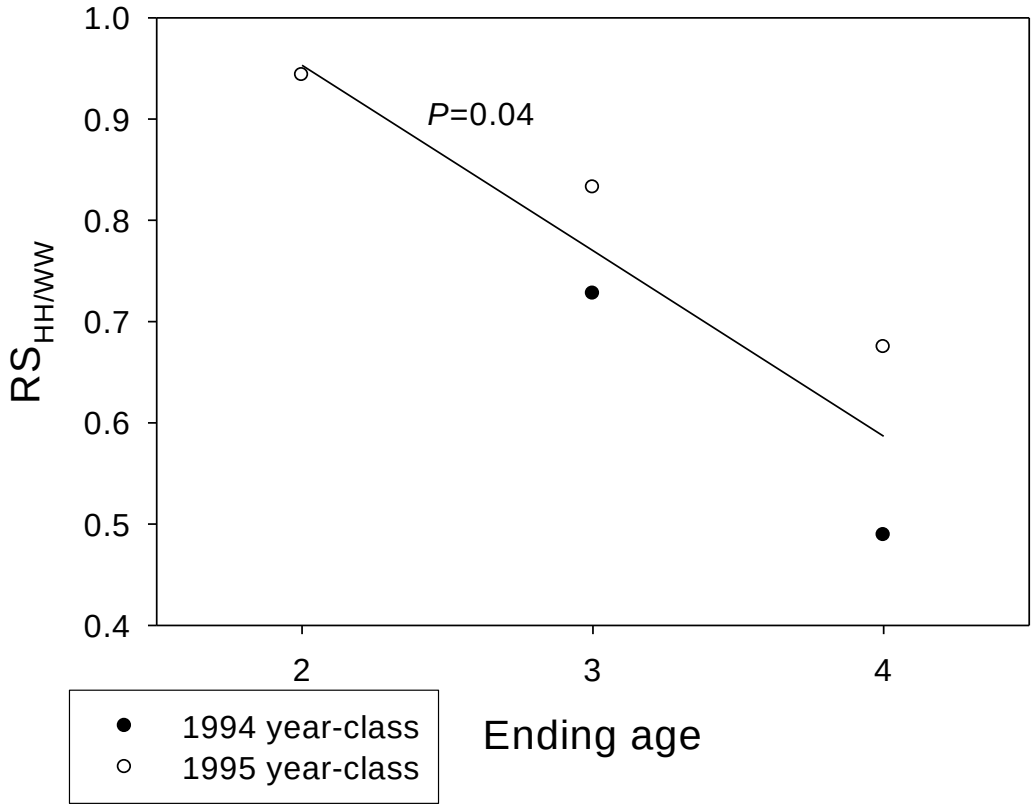
1.0
0.9
0.8
0.7
0.6
0.5
0.4
RS HH/WW
P=0.04
2
3
4
Ending age
1994 year-class
1995 year-class

Figure 7.

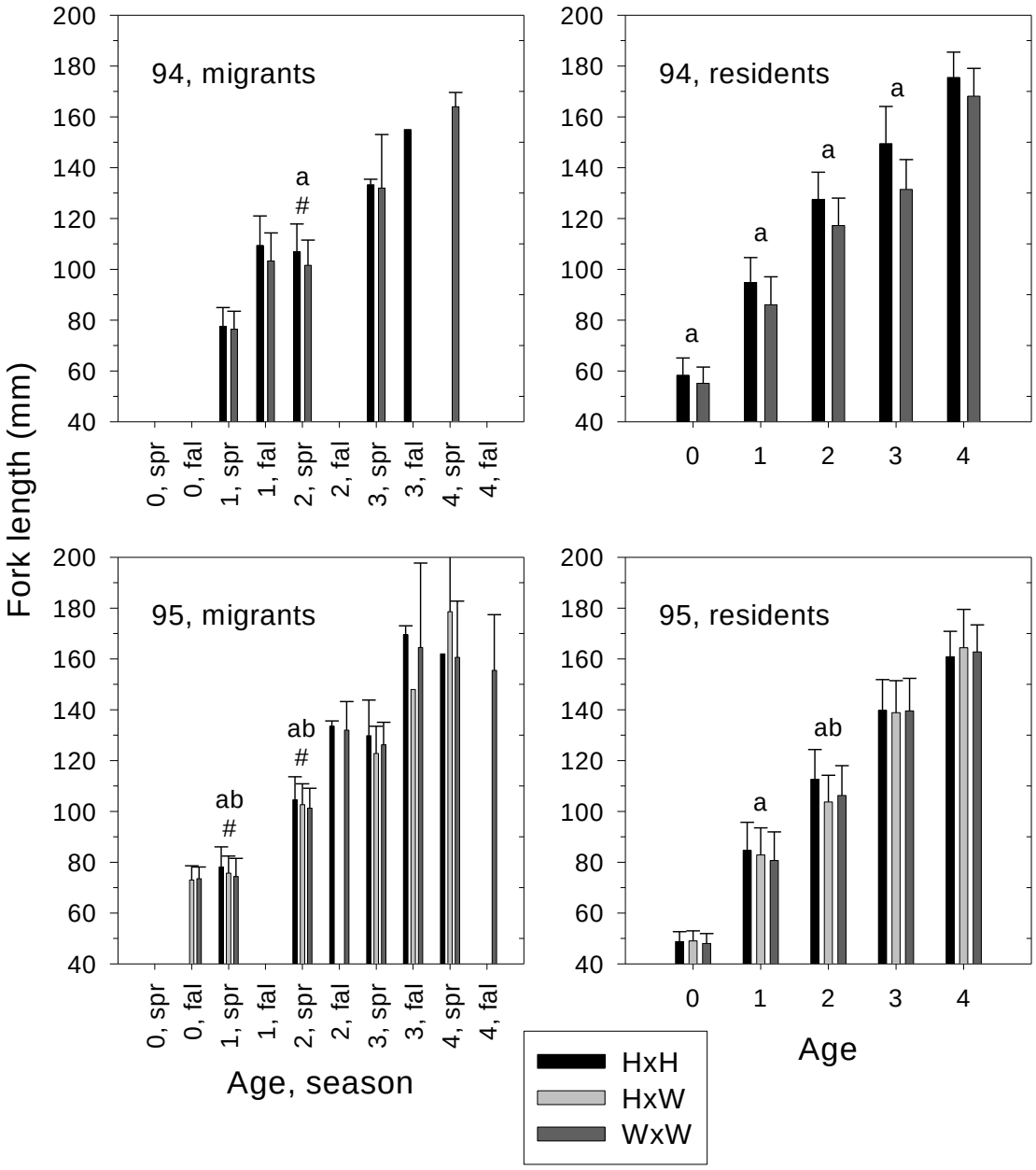
94, migrants
94, residents
95, migrants
95, residents
Fork length (mm)
Age, season
Age
HxH
HxW
WxW
0, spr
0, fal
1, spr
1, fal
2, spr
2, fal
3, spr
3, fal
4, spr
4, fal
0
1
2
3
4
a
ab
#

Figure 8.

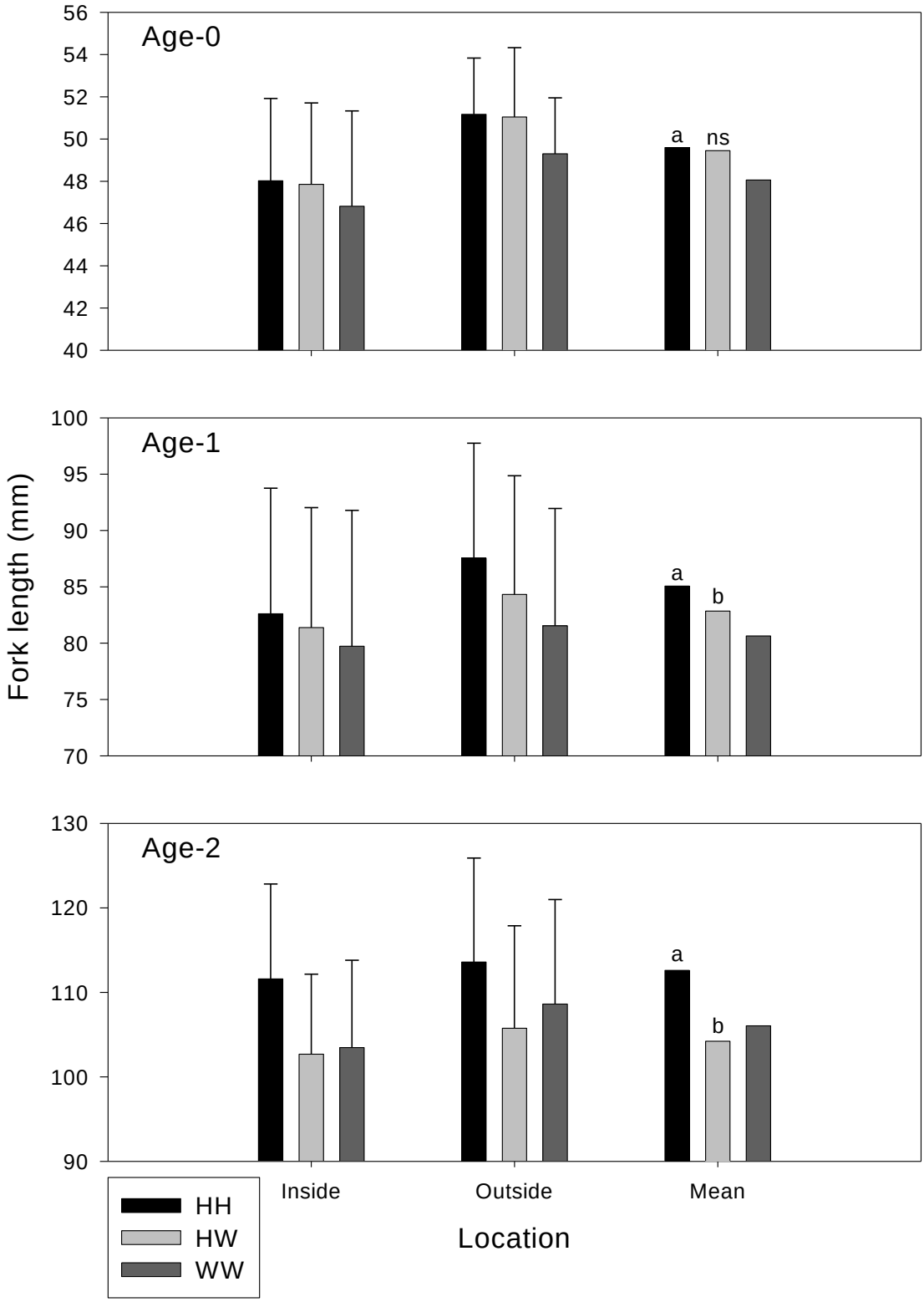
Age-0
Age-1
Age-2
Fork length (mm)
56
54
52
50
48
46
44
42
40
100
95
90
85
80
75
70
130
120
110
100
90
a
ns
a
b
a
b
Inside
Outside
Mean
Location
HH
HW
WW

Figure 9.

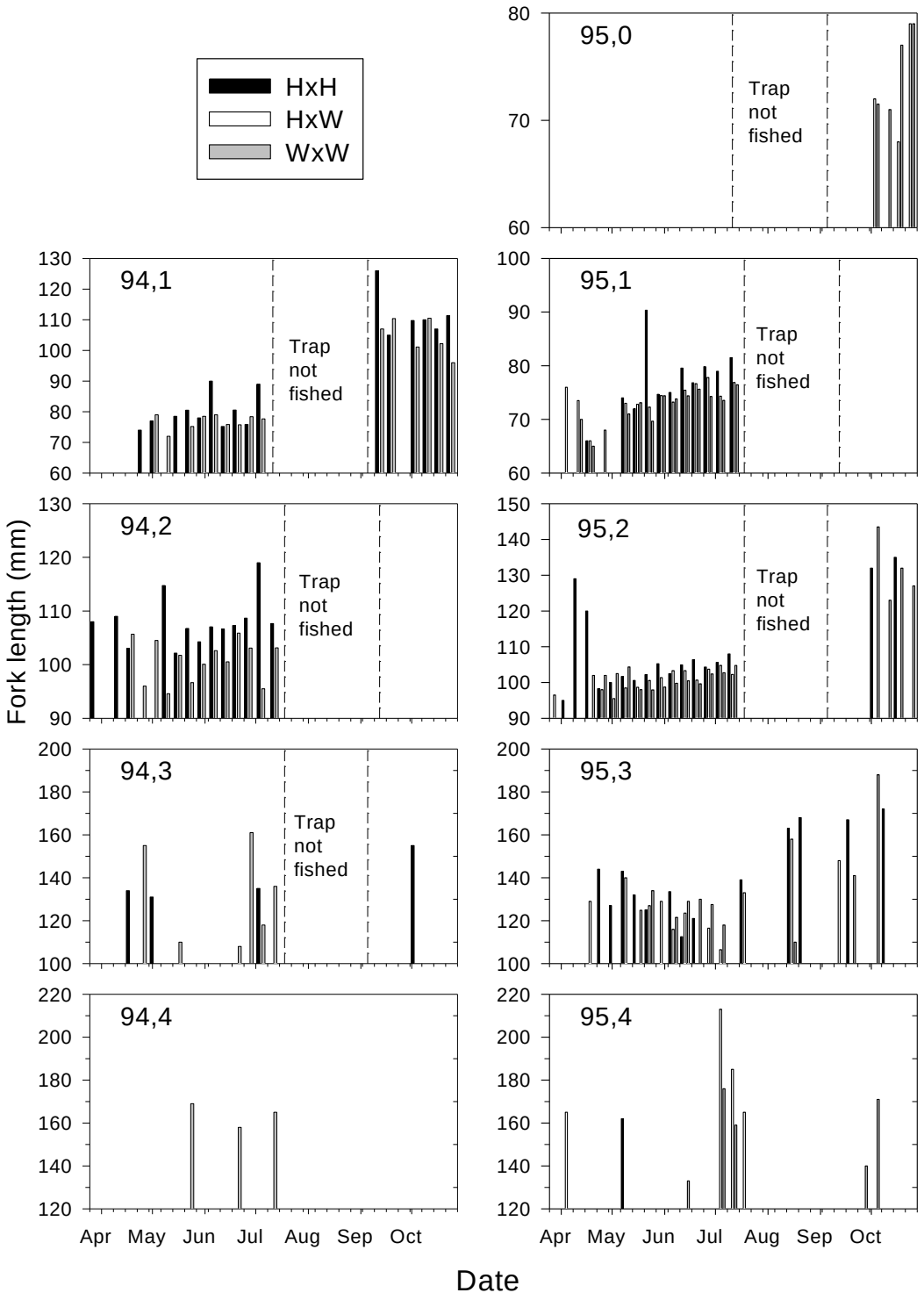
HxH
HxW
WxW
95,0
94,1
95,1
94,2
95,2
94,3
95,3
94,4
95,4
Trap not fished
Fork length (mm)
Date
Apr
May
Jun
Jul
Aug
Sep
Oct

Figure 10.

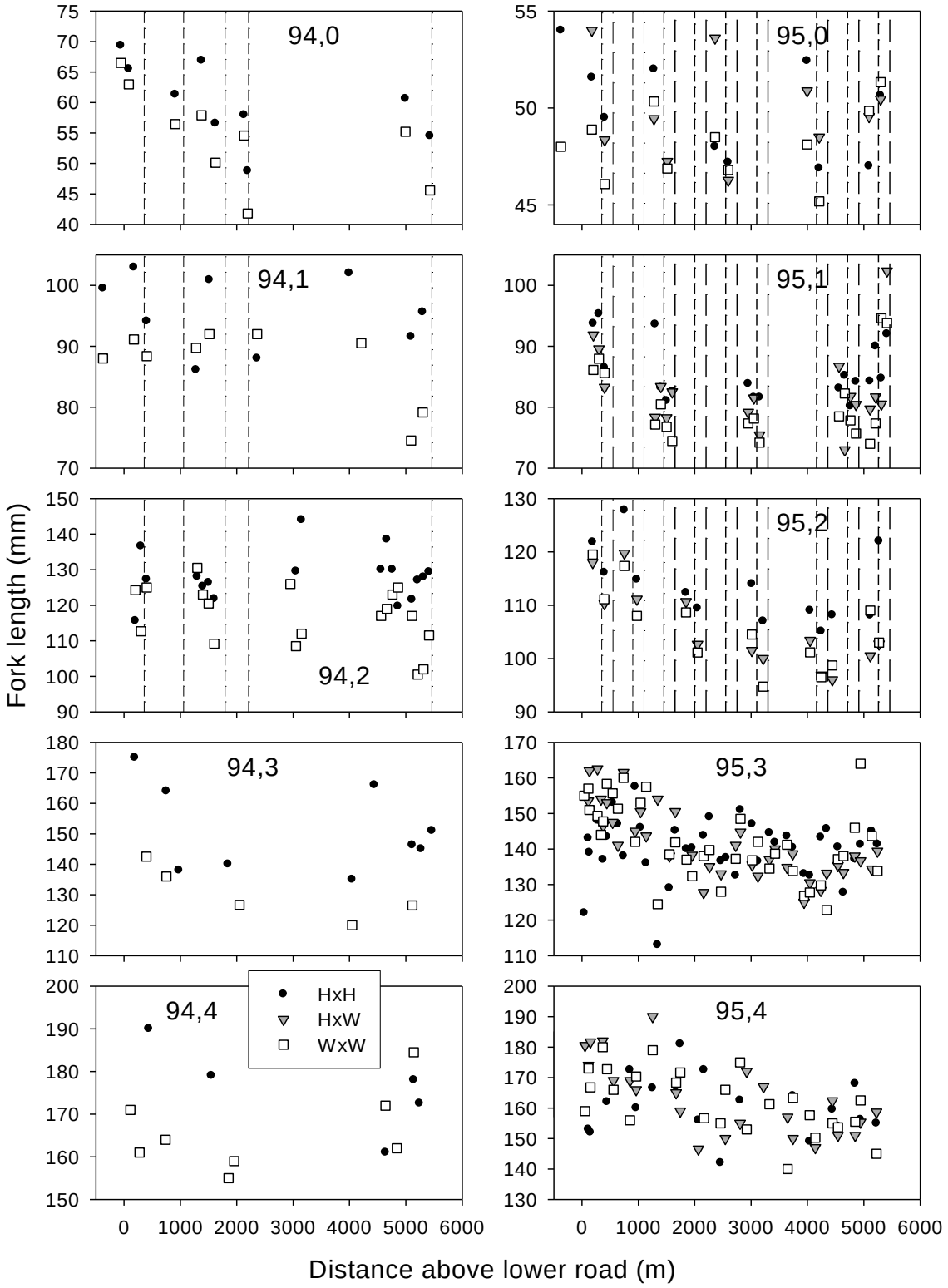
94,0
95,0
94,1
95,1
94,2
95,2
94,3
95,3
94,4
95,4
HxH
HxW
WxW
Fork length (mm)
Distance above lower road (m)

Figure 11.

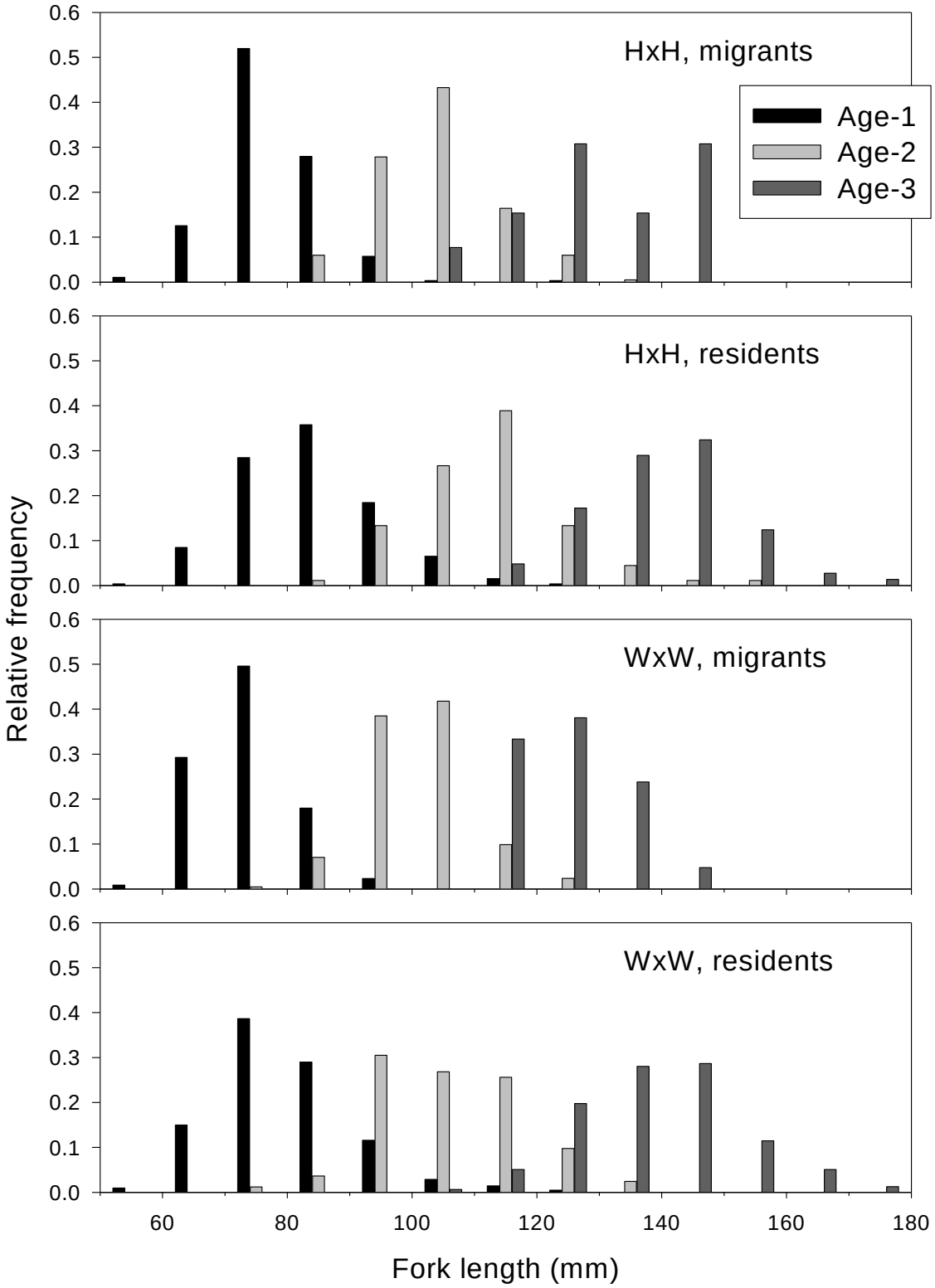
HxH, migrants
Age-1
Age-2
Age-3
HxH, residents
WxW, migrants
WxW, residents
Relative frequency
Fork length (mm)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
60
80
100
120
140
160
180

Figure 12.

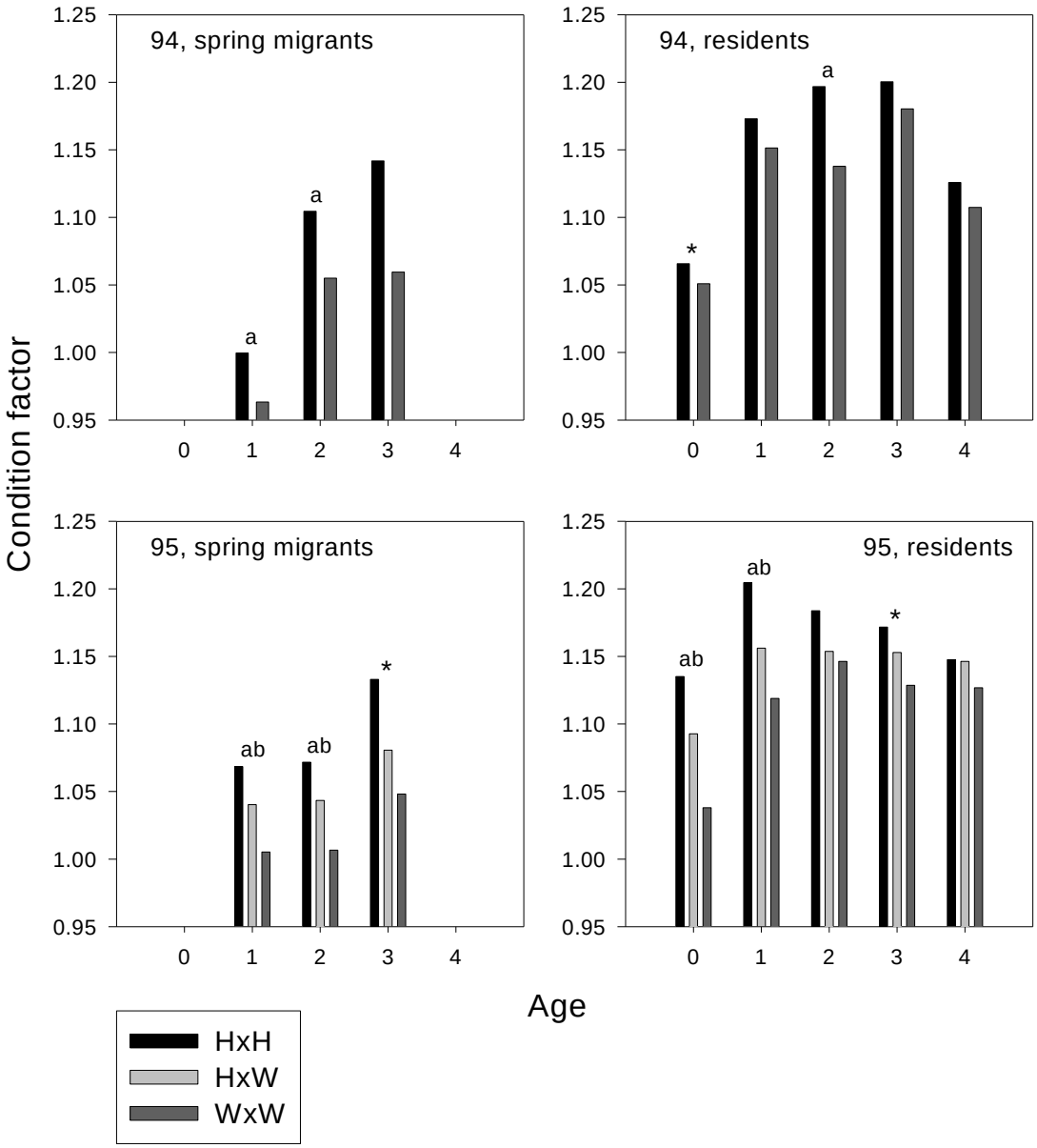
94, spring migrants
94, residents
95, spring migrants
95, residents
Condition factor
Age
1.25
1.20
1.15
1.10
1.05
1.00
0.95
0
1
2
3
4
a
ab
*
HxH
HxW
WxW

Figure 13.

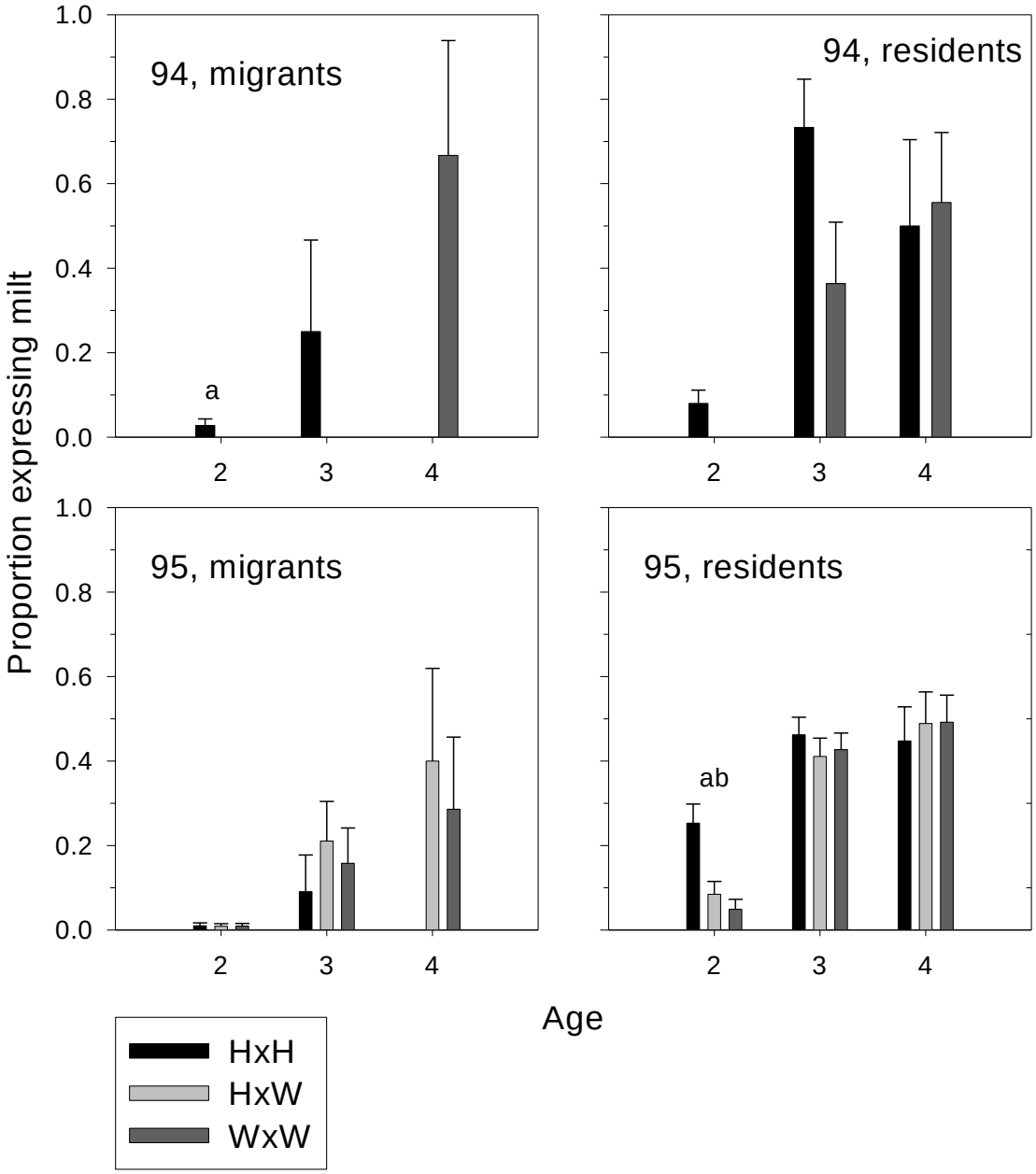
94, migrants
94, residents
95, migrants
95, residents
a
ab
Proportion expressing milt
Age
1.0
0.8
0.6
0.4
0.2
0.0
2
3
4
HxH
HxW
WxW

Figure 14.

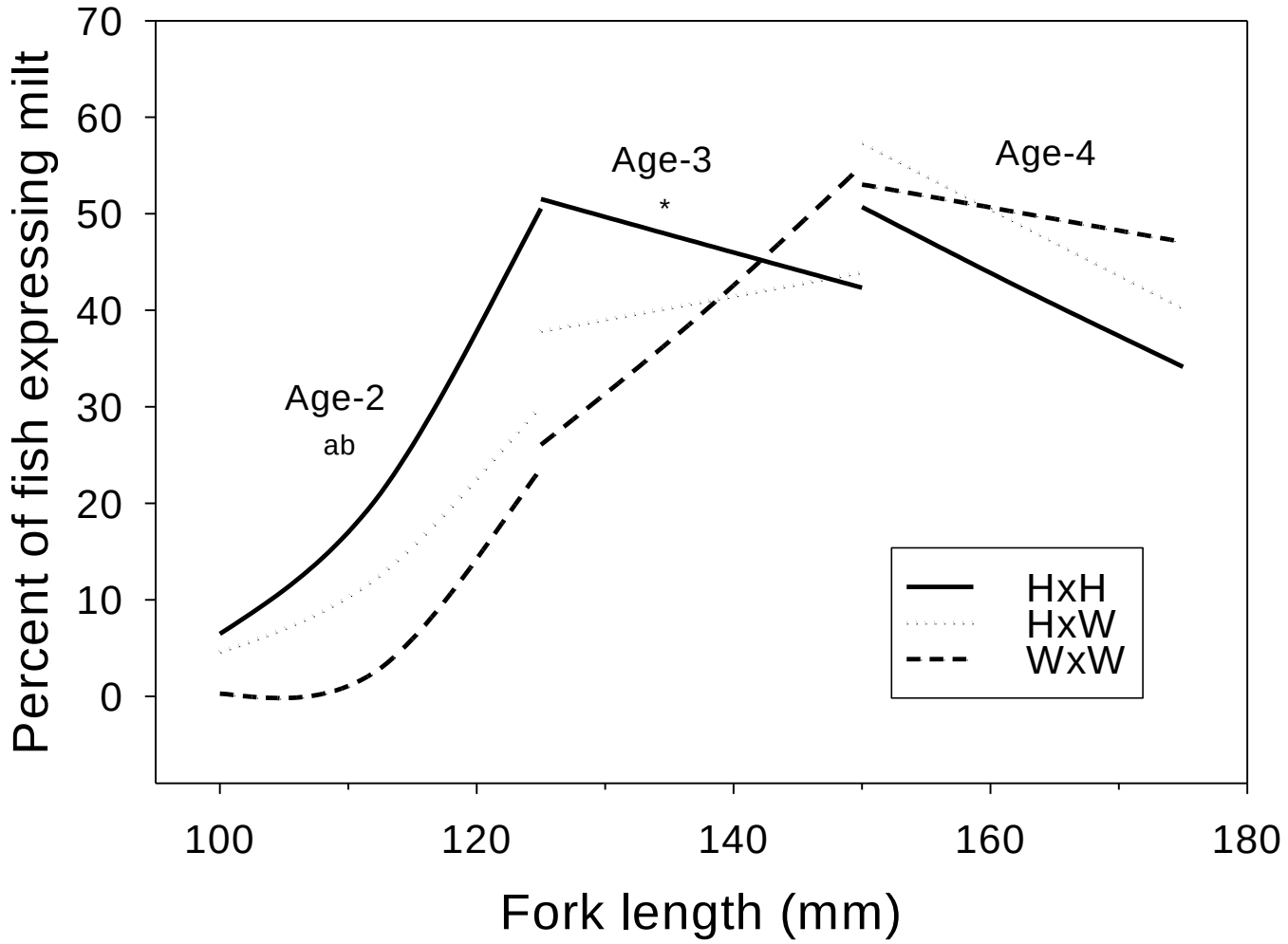
Percent of fish expressing milt
70
60
50
40
30
20
10
0
Age-2
ab
Age-3
*
Age-4
HxH
HxW
WxW
100
120
140
160
180
Fork length (mm)

Figure 15.

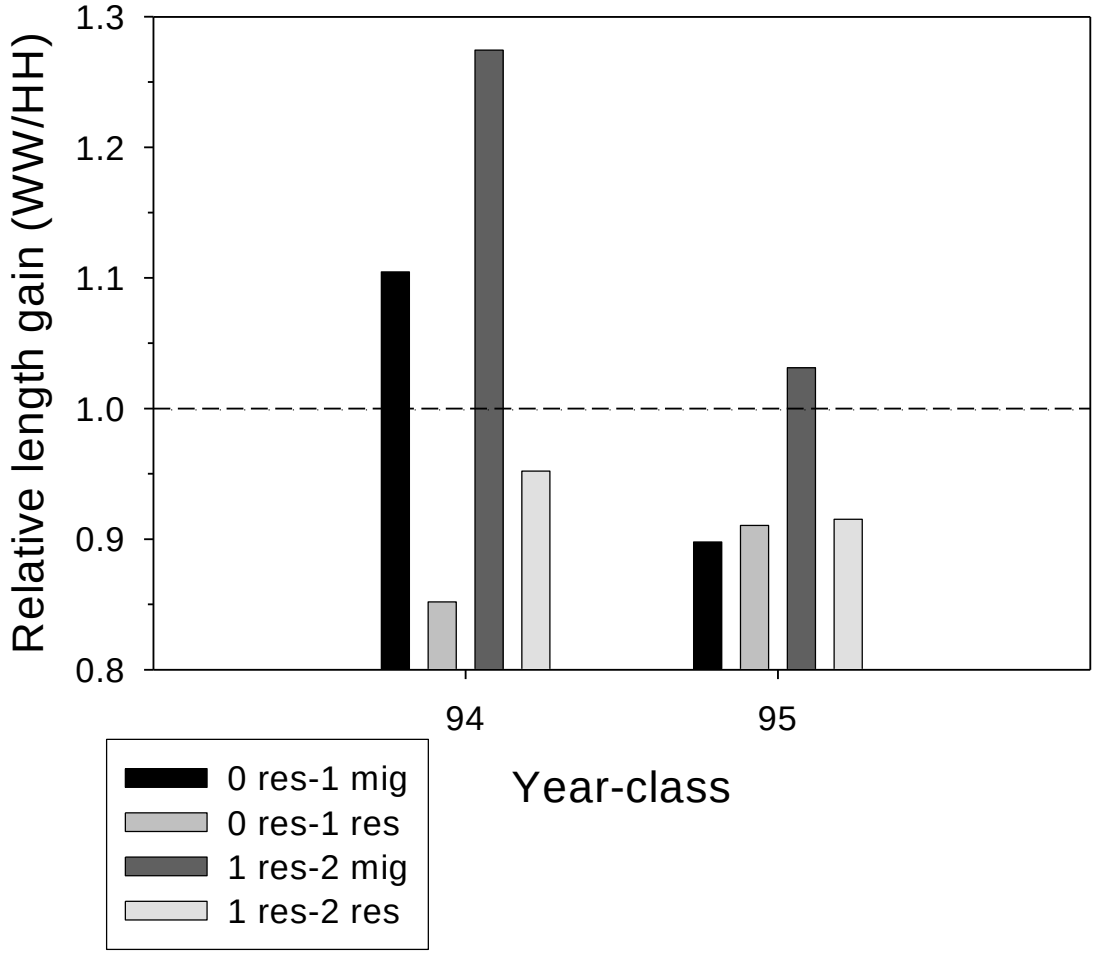
1.3
1.2
1.1
1.0
0.9
0.8
Relative length gain (WW/HH)
94
95
Year-class
0 res-1 mig
0 res-1 res
1 res-2 mig
1 res-2 res

Figure 16.

Chapter 4: Predicted time from fertilization to maximum wet weight for steelhead alevins based on incubation temperature and egg size *(Study site: Western Fishery Research Center, Seattle; Stock: Dworshak hatchery; Year class: 1996)*

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Abstract

The accuracy of a model that predicts time between fertilization and maximum alevin wet weight (MAWW) from incubation temperature was tested for steelhead *Oncorhynchus mykiss* from Dworshak National Fish Hatchery on the Clearwater River, Idaho. MAWW corresponds to the button-up fry stage of development. Embryos were incubated at warm (mean=11.6°C) or cold (mean=7.3°C) temperatures and time between fertilization and MAWW was measured for each temperature. Model predictions of time to MAWW were within 1% of measured time to MAWW. Mean egg weight ranged from 0.101-0.136 g among females (mean = 0.116). Time to MAWW was positively related to egg size for each temperature, but the increase in time to MAWW with increasing egg size was greater for embryos reared at the warm than at the cold temperature. We developed equations accounting for the effect of egg size on time to MAWW for each temperature, and also for the mean of those temperatures (9.3°C).

Introduction

We conducted this study because some of our other studies required manipulation of incubation temperatures to synchronize developmental stage among steelhead *Oncorhynchus mykiss* embryos from different spawning dates. A model developed by Jensen (1988) predicts time to the button-up fry stage of development from incubation temperature for several species of Pacific salmon including steelhead. One objective of the current study was to test the accuracy of this model for steelhead from Dworshak National Fish Hatchery on the Clearwater River in Idaho. Because the water content of tissue is greater than that of yolk, wet weight of salmonid alevins increases as yolk is converted to tissue and then decreases after complete yolk absorption if feeding is not initiated (Bams 1970). Maximum alevin wet weight (MAWW) is used in the temperature model as a proxy for the button-up stage and is easily measured. We tested model accuracy by incubating embryos at two different temperatures, measuring time to MAWW for each temperature, and comparing those measurements to model predictions of time to MAWW. The temperatures approximated the minimum and maximum temperatures experienced by embryos in our other studies (Rubin et al. 2012d, 2012g).

At any given temperature, time to MAWW is less for embryos from small eggs than from large eggs (Rombough 1985). A second objective of the current study was to derive a quantitative relation between time to MAWW and egg size that could be used in conjunction with the temperature model to predict time to MAWW for embryos from eggs of various sizes.

Methods

Twelve pairs of adults were spawned (one-to-one matings) on 26 March 1996. Samples of unfertilized eggs from each female were preserved in 10% buffered formalin, and $\geq$90 days later (after egg weight stabilized) 60 eggs from each female were weighed collectively to estimate mean egg weight. Samples of fertilized eggs were transported to the Western Fisheries Research Center, Seattle, Washington later on the spawning day. Embryos were incubated in two vertical-tray incubators, one supplied with warm water (mean=11.6°C from 27 March to the end of sample collection; SD daily mean=0.5°C) and the other with cold water (mean=7.3°C; SD=0.5°C). Embryos from individual families were held separately in compartments within the incubators and each family was represented in each

incubator. Density was equalized among families when embryos were first placed in the incubators and again at the eyed stage after dead embryos were removed.

Samples of alevins from each family were collected from the warm incubator daily during 6-13 May and from the cold incubator every other day during 8-18 June. The sampling dates were selected to span the period during which alevins completed yolk absorption and began to lose weight. Samples were preserved in 10% buffered formalin, and $\geq$90 days later 10-15 individuals from each sample were measured for length, weight, and yolk weight. Time to MAWW was estimated for each temperature treatment by fitting quadratic regressions of alevin weight versus days post-fertilization and solving the resulting equations for the number of days that gave the maximum weight. Regressions were fit to data pooled over families to estimate time to MAWW for average sized eggs. Proportion yolk (yolk weight divided by total weight) at estimated time of MAWW was linearly interpolated from mean proportion yolk on preceding and succeeding sampling dates. Quadratic regressions of weight versus days post-fertilization were also fit to data grouped by egg size. Resulting estimates of time to MAWW were then linearly regressed against egg size to assess the effect of egg size on time to MAWW for each temperature regime (warm or cold).

Jensen (1988) presented a microcomputer program that predicts time to various stages of steelhead embryonic development as a function of constant incubation temperature. We modified the program to accept inputs of daily mean temperature, rather than a single temperature for the entire incubation period. The original program predicts days to eye-up at different temperatures (e.g., 25.7 days at 7°C; 22.3 days at 8°C). At any given temperature, each day accounts for a percentage of the time between fertilization and eye-up (e.g., 3.9% [=100%/25.7] at 7°C and 4.5% at 8°C). We entered mean temperature for day 1 into the original program, obtained the prediction for days to eye-up, and calculated percentage of total time to eye-up accounted for by that day. This was repeated for subsequent days, and the percentages calculated for each day were summed. We assumed that eye-up occurred on the date when the sum reached 100%. The same procedure was repeated to obtain time between eye-up and hatch and between hatch and MAWW. Tested against data from steelhead incubated at Dworshak National Fish Hatchery (USGS, unpublished data), the modified program gave accurate predictions of time to eye-up and time between eye-up and hatch, but actual time between hatch and MAWW was consistently 30% less than predicted. We therefore made one final modification: percent of time between hatch and MAWW predicted by the original model for one day at a given temperature (e.g., 2.3% at 7°C) was divided by 0.7 (e.g., 2.3%/0.7=3.3%). The modified program was used to predict time to MAWW for embryos incubated at the warm and cold temperature regimes.

Results

Alevin weight increased and then decreased within the sampling periods of both temperature treatments (Figure 1). After an initial increase, weight decreased moderately from days 43 to 45 and then increased moderately from days 45 to 47 of the warm treatment before showing a final decrease (Figure 1). The pattern of two weight peaks under the warm treatment was apparent for most families when families were examined separately. Only a single weight peak was observed for the cold treatment (Figure 1). Length increased continuously and proportion yolk decreased continuously over the sampling period but both measures slowed their rate of change as yolk absorption neared completion and weight loss commenced (true for both temperature treatments; Figure 1). Quadratic regression lines fit to weight versus time are shown in Figure 2, and estimates of the regression coefficients as well as other statistics associated with the regressions are shown in Table 1.

Estimates of time of MAWW, MAWW, and proportion yolk at MAWW are given in Table 2. MAWW was less for the warm than for the cold treatment suggesting that conversion of yolk to tissue was less efficient at warm than at cold temperatures, a finding in agreement with other studies (e.g.,

Heming 1982). Temperature model predictions of days between fertilization and MAWW differed from regression estimates of days to MAWW by less than 1% for both warm and cold temperatures (Table 2).

The range of mean egg weights among female parents was 0.101-0.136 g (mean = 0.116; SD = 0.010). Quadratic regressions could not be fit separately for each family because alevin sampling did not extend far enough past MAWW to clearly define time of MAWW for some families. Instead, families were ranked by the mean egg size of their female parent, and regressions were fit to three-family moving averages (i.e., first to pooled data for families with the first, second, and third smallest eggs; next to data for families with the second, third, and fourth smallest eggs; etc.). Time to MAWW appeared positively related to egg size for each temperature, and the increase in time to MAWW with increasing egg size (i.e., the slope of the regression line) appeared greater for the warm than for the cold temperature (Figure 3). We did not statistically test whether the slopes of the regressions differed from zero or differed between temperatures because the moving averages were not statistically independent. Nevertheless, the regressions provide the best attainable representation of egg size effects given the limitations of our data. The regression line for each temperature ran through or close to the data point representing time to MAWW and mean egg size from data pooled over all families (Figure 3), lending credence to the regressions.

The regressions of time to MAWW versus egg size were standardized to enable their use in conjunction with temperature model-predicted time to MAWW:

$y = 1/(a/c + (b/c)*x)$;

y = (model-predicted time to MAWW)/(egg size-specific time to MAWW); a = the intercept of the time to MAWW versus egg size regression for the warm (or cold) temperature treatment (Figure 3); b = the slope of that regression, and c = temperature model-predicted time to MAWW at the warm or cold temperature. The ratio between model-predicted time to MAWW and egg size-specific time to MAWW (i.e., y in the equation above) serves as an index of relative development. The units of the index are percentage of time to MAWW (e.g., 0.9 means 90% of egg size-specific time to MAWW, 1.1 means 110% of egg size-specific time to MAWW). Equations for each temperature are shown in Figure 4. Alevins from small eggs reached MAWW early compared to the model-predicted MAWW date (overdeveloped on the predicted MAWW date; $y>1.0$) whereas alevins from the largest eggs reached MAWW late (underdeveloped on the predicted MAWW date; $y<1.0$), especially when reared in warm water. The mean of the regressions for warm and cold treatments is also provided as an intermediate relation (Figure 4) which may represent egg size effects at the mean of the warm and cold temperatures (9.3^{o}C); however, it is unknown whether egg size effects change regularly with temperature such that interpolation would be justified.

Discussion

Temperature model predictions of time between fertilization and MAWW were within 1% of measured time to MAWW. The temperature model resulted from two modifications to Jensen's (1988) model, one of which allowed inputs of daily mean temperatures rather than a single temperature for the whole incubation period. However, because the warm and cold incubation temperatures that we used were nearly constant, entering daily mean temperatures differed little from entering a single temperature for the entire incubation period. The model accurately predicted time to MAWW when temperatures were nearly constant; we did not test model accuracy under variable temperature regimes.

We modified the model to allow input of daily temperatures because we used it for other studies employing variable temperatures, typically 7°C for part of the incubation period and 12°C for the rest. We measured proportion yolk for some of these studies (Rubin et al. 2012e, 2012f, 2012g) and found it to be inversely related to percent of model-predicted time to MAWW (Figure 5). Further, the two data

points from the current study fell approximately where they should along this relation (Figure 5). The correlation between proportion yolk and predicted time to MAWW suggests at least some level of model-prediction accuracy under variable temperature regimes; however, formal testing with variable temperatures is needed for validation. We note that only a rough correlation between proportion yolk and predicted time to MAWW was expected for our other studies since they represented a range of incubation temperature regimes and egg sizes (0.089-0.124 g for the latter), and the current study suggests that both of these factors can affect proportion yolk (yolk conversion efficiency greater at lower temperatures; development rate faster for smaller eggs).

The other modification we made to Jensen's model reduced the predicted time between hatch and MAWW by 30%. This modification was necessary for accurate prediction of time to MAWW, implying that at any given temperature, rate of development between hatch and MAWW is faster for Dworshak Hatchery steelhead than for the steelhead stock which provided data for the original model (Jensen 1988).

One anomaly in our data was the presence of two peaks of alevin weight, rather than just one, under the warm treatment (Figure 1). The cause of this anomaly is unknown. The quadratic term of the quadratic regression was significant for the warm treatment despite the anomaly (Table 1). Time to MAWW estimated by the regression, 46.4 days, was closer to the second peak which was the higher of the two (Figure 1).

Our results suggest that time to MAWW increases with egg size, in agreement with other studies (e.g., Rombough 1985), and that the increase in time to MAWW with increasing egg size is greater at warm than at cold temperatures, which as far as we know has not been shown by other studies. However, our findings on egg size effects were based on moving averages and therefore could not be statistically tested. Further work is needed to validate effects of egg size on time to MAWW at different temperatures.

Acknowledgements

We thank the Bonneville Power Administration (Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052) for providing funding and our contracting officers Tom Vogel and Jeff Gislason for administrative oversight. We thank Howard Burge and Mike Hayes for reviewing this chapter. We also thank the many persons that provided help or support including Gayle Brown, Howard Burge, Jay Hensleigh, Elain Lee, Bill Miller, Jill Olson, Ralph Roseburg, Karl Stenberg, and Lisa Wetzel.

References

Bams, R. A. 1970. Evaluation of a revised hatchery method tested on pink and chum salmon fry. Journal of the Fisheries Research Board of Canada 27:1429-1452.

Heming, T.A. 1982. Effects of Temperature on Utilization of Yolk by Chinook Salmon (*Oncorhynchus tshawytscha*) Eggs and Alevins. Canadian Journal of Fisheries and Aquatic Sciences 39:184-190.

Jensen, J. O. T. 1988. A microcomputer program for predicting embryonic development in Pacific salmon and steelhead trout. World Aquaculture 19:80-81.

Rombough, P. J. 1985. Initial egg weight, time to maximum alevin wet weight, and optimal ponding times for chinook salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences 42:287-291.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, J. E. Hensleigh, F. E. Leonetti, B. M. Baker, S. L. Slatton, and K. D. Stenberg. 2012a. Differences in survival and growth in hatchery and stream environments, and in maturation of residuals in a stream, between progeny of hatchery and wild steelhead. Chapter 1 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead

and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, and K. D. Stenberg. 2012e. Effect of developmental stage of unfed fry on survival and growth of steelhead released in a stream and in hatchery ponds. Chapter 6 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, and K. D. Stenberg. 2012f. Effect of size of unfed fry at release on survival and growth of juvenile steelhead in streams and a hatchery. Chapter 7 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, K. D. Stenberg, and B. M. Baker. 2012g. Effect of incubation temperature on post-embryonic survival and growth of steelhead in a natural stream and a hatchery. Chapter 5 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Tables

Table 1. Estimated regression coefficients, and other statistics associated with quadratic regressions of alevin wet weight on days post-fertilization. Adj = adjusted; Root MSE = (mean square error)$^{0.5}$. *P*-value of coefficient estimates: * <0.05; ** <0.01; *** <0.001; ns >0.05. Overall model fit was highly significant for both regressions (*P*<0.0001). Regressions were fit to data from individual alevins: *N* = 1433 and 1018 for warm and cold, respectively. Regression lines are shown in Figure 2.

Temper-ature	Coefficient estimate (SE)				Adj	Root
	Intecept	Linear	Quadratic	R^2	R^2	MSE
Warm	-0.37360 (0.21106) ns	0.02474 (0.00950) **	-0.0002666 (0.0001068) *	0.020	0.018	0.0185
Cold	-3.01283 (0.33459) ***	0.08115 (0.00849) ***	-0.0005119 (0.0000538) ***	0.086	0.084	0.0169

Table 2. Experimental parameters, estimates, and predictions. Time to maximum alevin wet weight (MAWW) was estimated from samples of alevins and predicted from incubation temperatures. MAWW and days to MAWW were estimated by fitting quadratic regressions to weight versus days post-fertilization. Proportion yolk at MAWW was estimated by linear interpolation from mean proportion yolk on preceding and succeeding sampling dates. See text for a description of temperature model.

Variable	Temperature	
	Warm	Cold
Mean temperature (^{o}C) between fertilization and MAWW	11.4	7.2
Days between fertilization and MAWW estimated from alevin samples	46.4	79.3
Accumulated temperature units (^{o}C) on MAWW date	526	571
MAWW (g)	0.200	0.203
Proportion yolk at MAWW	0.034	0.049
Days between fertilization and MAWW predicted by the temperature model	46.0	79.6
Ratio of predicted to estimated days to MAWW	0.992	1.005

Figures

Figure legends

Figure 1. Mean (±SD) alevin weight, length, and proportion yolk versus sampling date. Sample size ranged from 145-180 per sampling date.

Figure 2. Quadratic regressions of weight against days post-fertilization for alevins incubated at warm (left) and cold (right) temperatures. Regression coefficients are given in Table 1. Horizontal dotted lines indicate maximum alevin wet weight (MAWW) and vertical dotted lines indicate days post-fertilization at MAWW.

Figure 3. Days to MAWW versus egg weight for alevins incubated at cold (top) and warm (bottom) temperatures. Filled circles represent 3-female moving averages. The farthest data point to the left in each panel was derived from females with the first, second, and third smallest eggs; the point immediately to the right of that from females with the second, third, and fourth smallest eggs; etc. Regression lines were fit to the filled circles; regression equations are shown. The open square in each panel represents pooled data for all females; time to MAWW is the same as in Table 2 and Figure 2. The bottom panel contains two less filled circles than the top panel because warm temperature quadratic regressions could not resolve time of MAWW for two of the ten 3-female groupings.

Figure 4. Standardized time to MAWW versus egg weight. On the y-axis is the ratio between temperature model-predicted time to MAWW, and time to MAWW for a particular egg size estimated from the regressions in Figure 3. Alevins from small eggs reach MAWW early and are therefore past MAWW on the model-predicted MAWW date (overdeveloped; $y>1.0$), whereas alevins from large eggs reach MAWW late and fail to reach MAWW by the model-predicted MAWW date (underdeveloped; $y<1.0$).

Figure 5. Proportion yolk versus percent of temperature model-predicted time to MAWW. The two data points for this study are from Table 2. Other data points are from studies employing variable incubation temperatures, typically switching between cold (about 7°C) and warm (about 12°C) water. Also, the other data points originated from a range of egg sizes (0.089-0.124 g). Correlation was tested using all data points (from this and other studies).

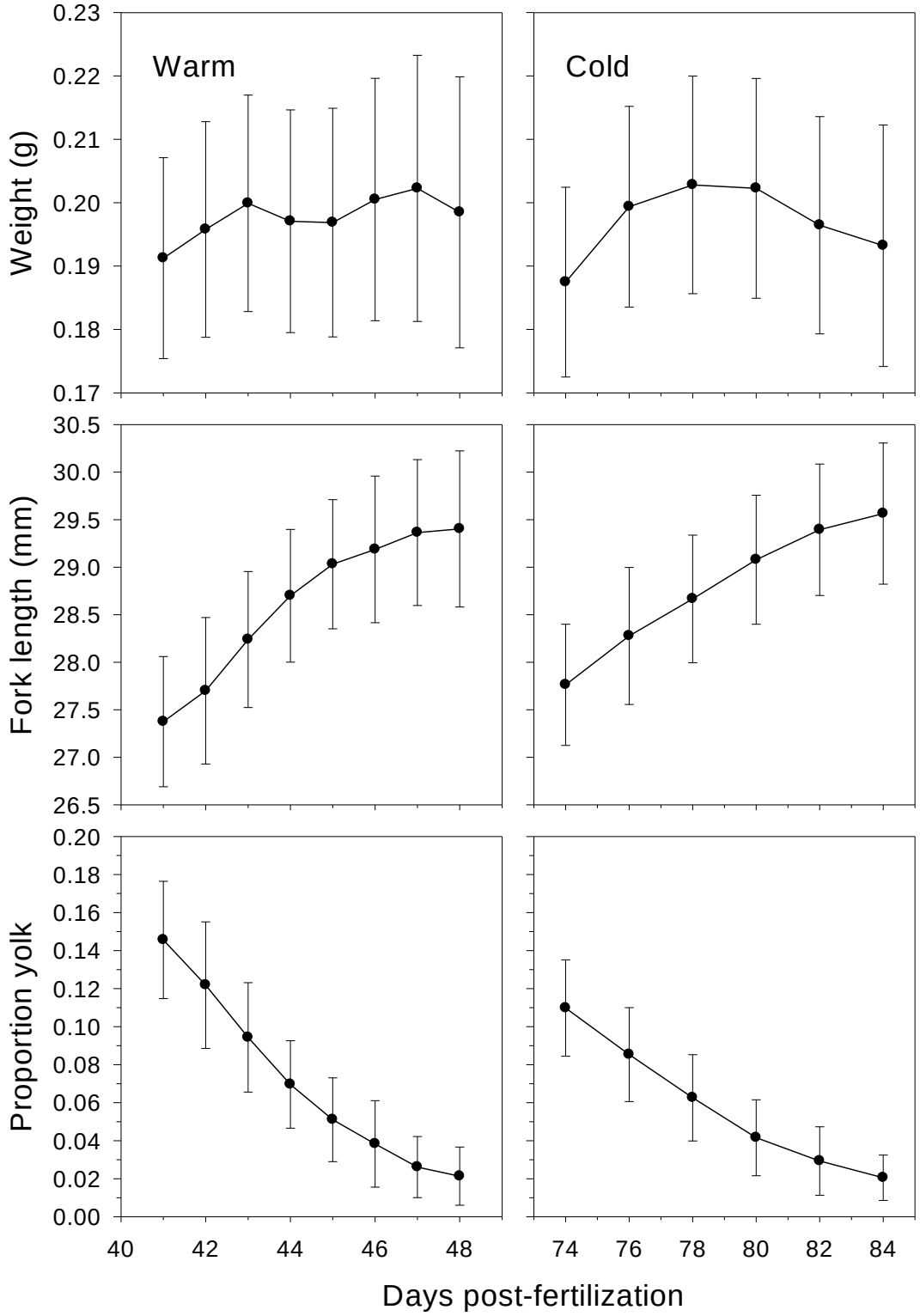
Warm
Cold
Weight (g)
0.23
0.22
0.21
0.20
0.19
0.18
0.17
Fork length (mm)
30.5
30.0
29.5
29.0
28.5
28.0
27.5
27.0
26.5
Proportion yolk
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
40
42
44
46
48
74
76
78
80
82
84
Days post-fertilization

Figure 1.

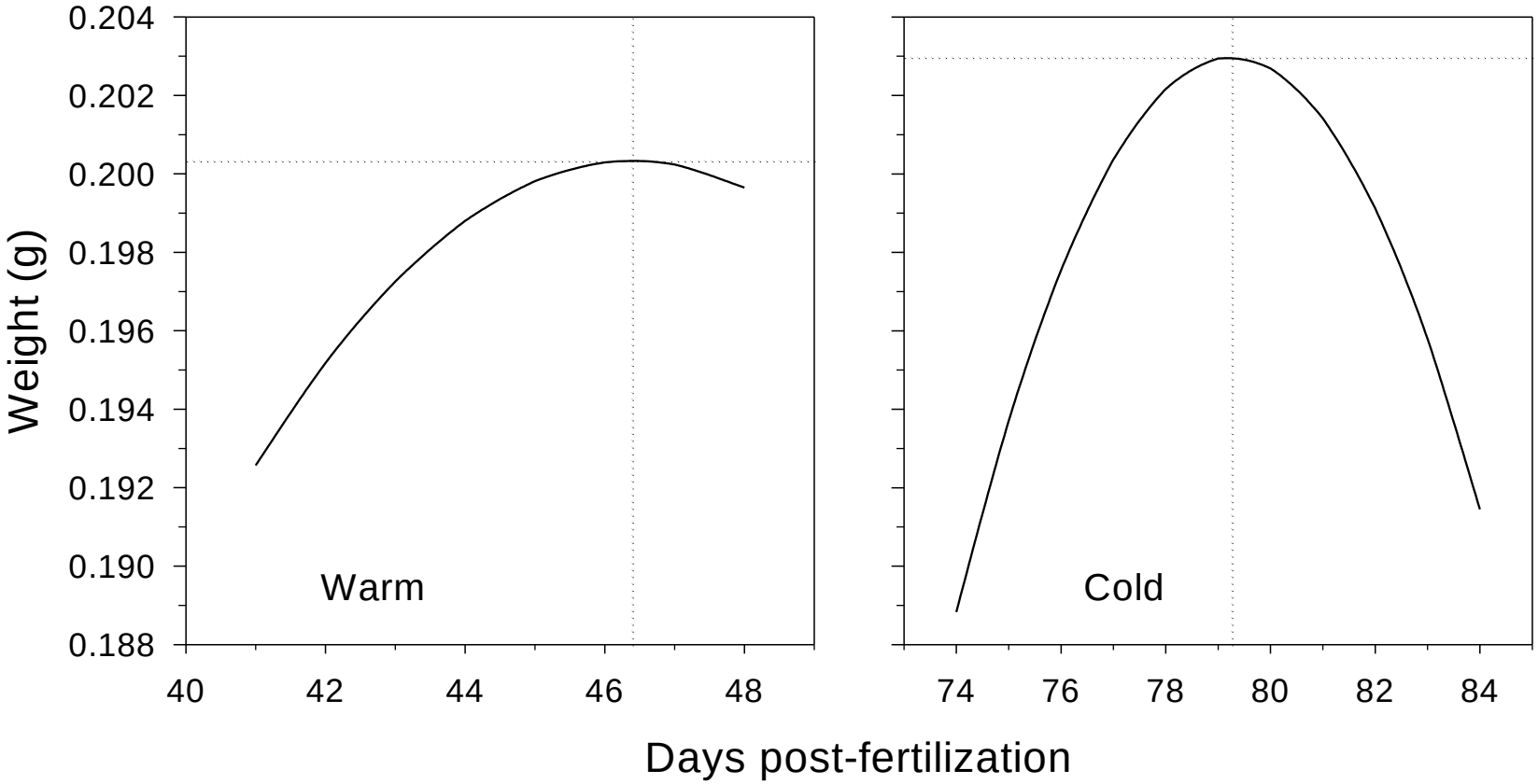
0.204
0.202
0.200
0.198
0.196
0.194
0.192
0.190
0.188
Weight (g)
Warm
Cold
40
42
44
46
48
74
76
78
80
82
84
Days post-fertilization

Figure 2.

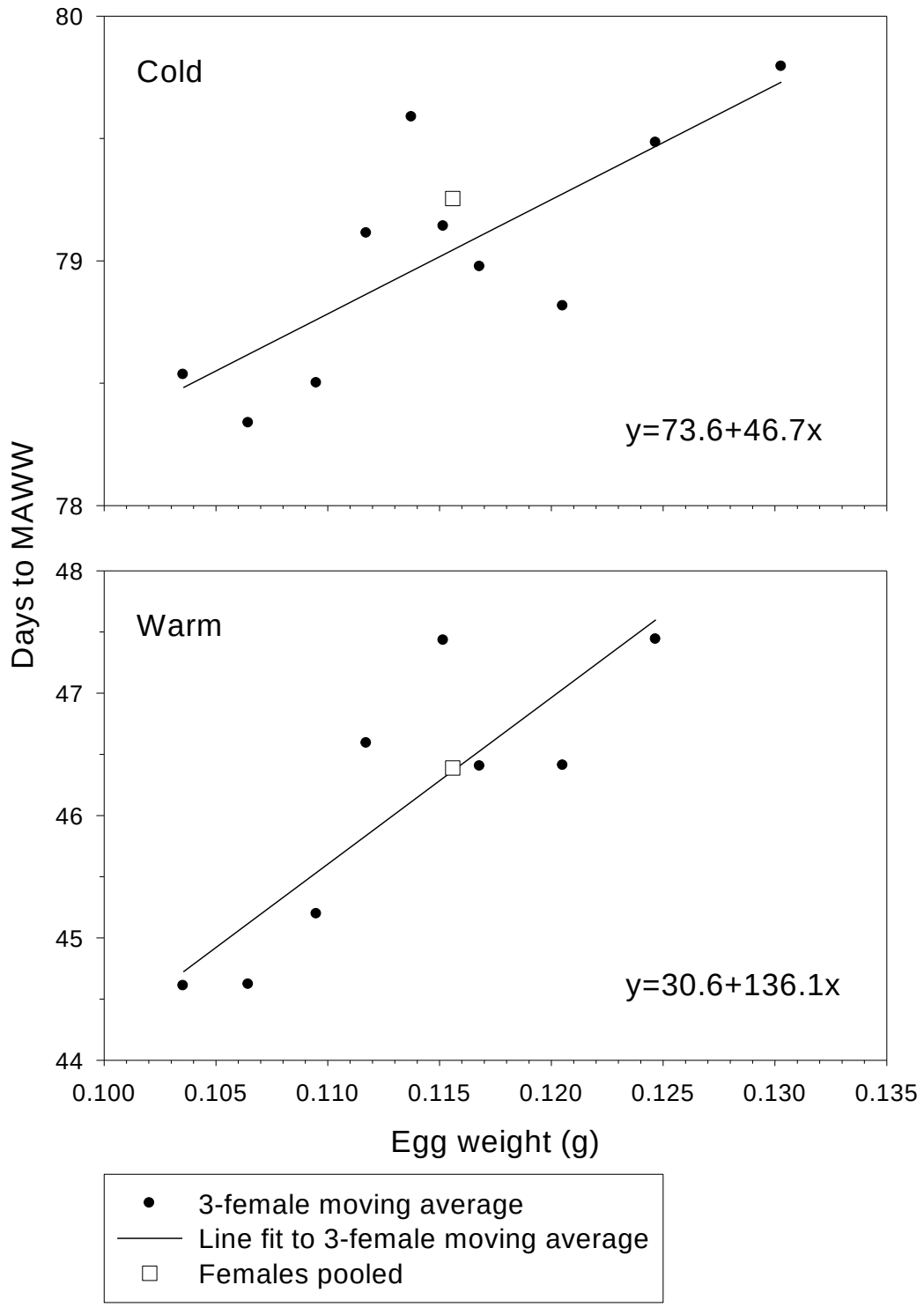
Cold
y=73.6+46.7x
Warm
y=30.6+136.1x
Days to MAWW
Egg weight (g)
80
79
78
48
47
46
45
44
0.100
0.105
0.110
0.115
0.120
0.125
0.130
0.135
3-female moving average
Line fit to 3-female moving average
Females pooled

Figure 3.

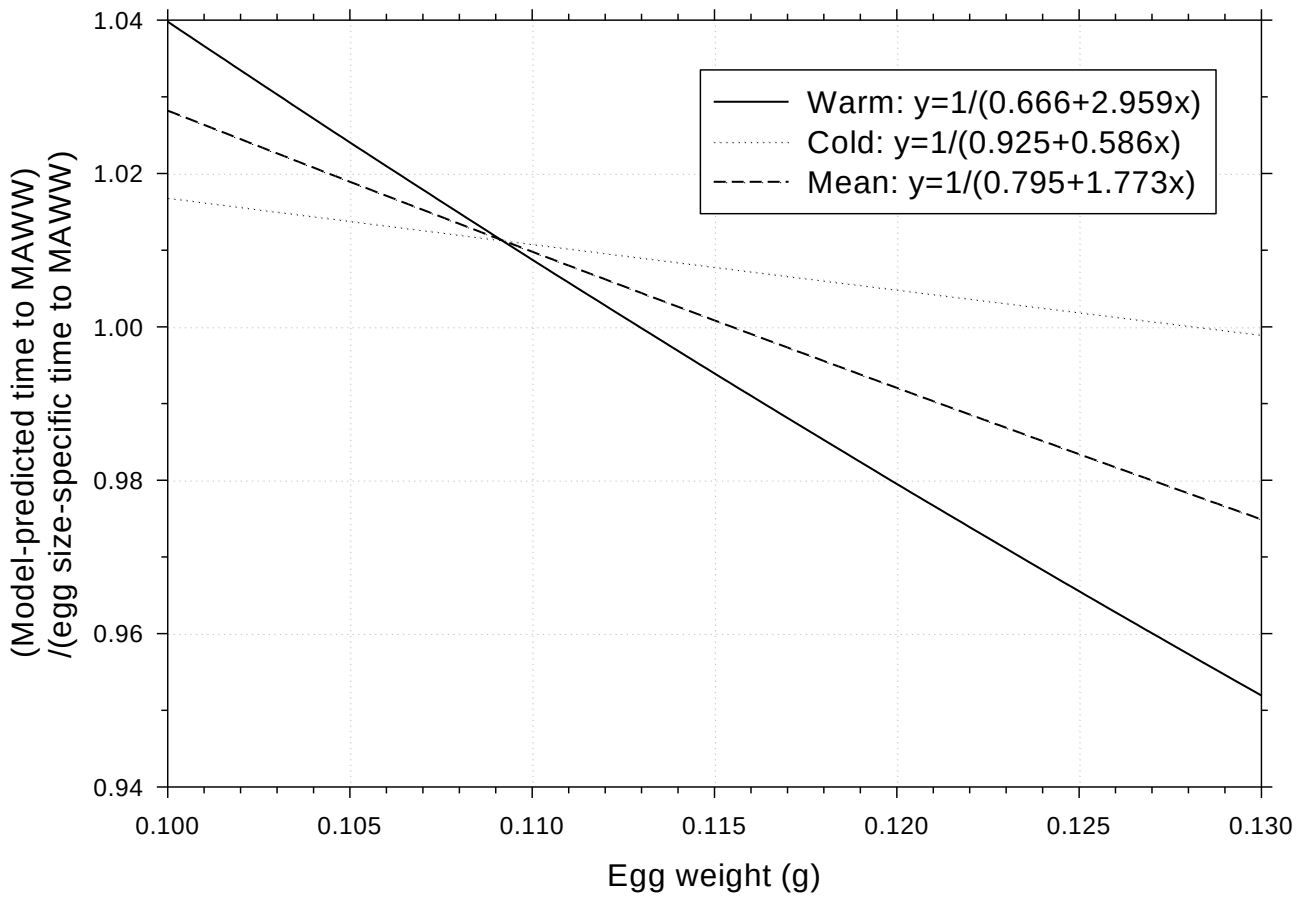
1.04
1.02
1.00
0.98
0.96
0.94
0.100
0.105
0.110
0.115
0.120
0.125
0.130
(Model-predicted time to MAWW)
/(egg size-specific time to MAWW)
Egg weight (g)
Warm: y=1/(0.666+2.959x)
Cold: y=1/(0.925+0.586x)
Mean: y=1/(0.795+1.773x)

Figure 4.

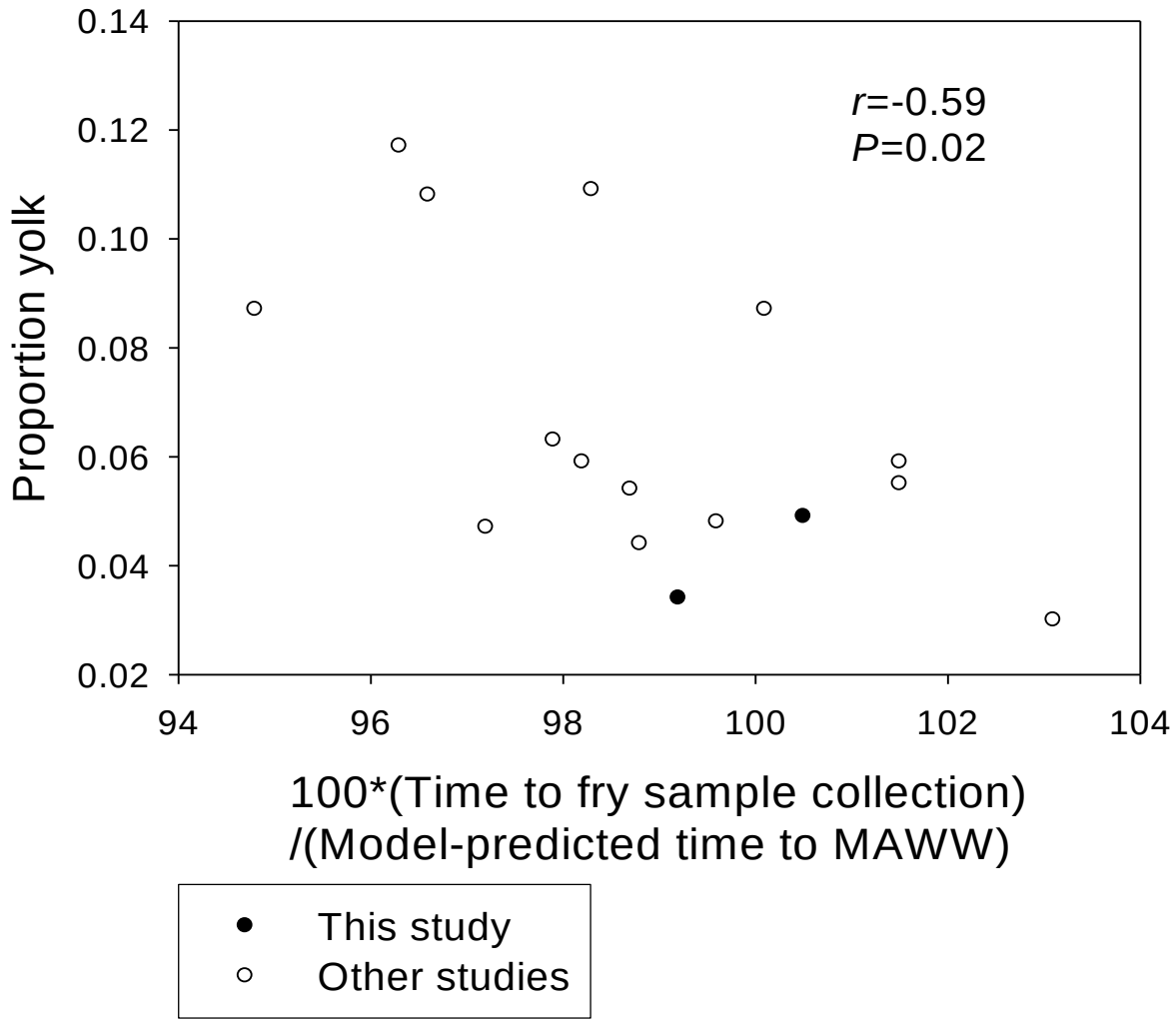
0.14
0.12
0.10
0.08
0.06
0.04
0.02
Proportion yolk
r=-0.59
P=0.02
94
96
98
100
102
104
100*(Time to fry sample collection)
/(Model-predicted time to MAWW)
This study
Other studies

Figure 5.

Chapter 5: Effect of incubation temperature on post-embryonic survival and growth of steelhead in a natural stream and a hatchery *(Study sites: Dworshak Hatchery and North Fork Palouse River; Stocks: Dworshak hatchery; Year classes: 1994 and 1995)*

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Abstract

We tested whether varying incubation temperatures to match development between embryos from different spawning dates affected survival and growth of unfed steelhead *Oncorhynchus mykiss* fry released in a stream and in hatchery ponds. Hatchery steelhead returning to the Clearwater River, Idaho were artificially spawned on two dates separated by a four week interval. Progeny from the early date (ExE, from early males and early females) were incubated in chilled (7°C) water and those from the late date (LxL) in ambient (12°C) water until developmental stage matched. A third group, created by fertilizing eggs from late females with cryopreserved milt from early males (ExL), was included to control for any genetic differences between early and late returning adults. Survival in the stream to 3 and 15 months after release was similar among crosses. Survival in the hatchery to near the end of the standard one year rearing period was similar among crosses for one of two year-classes but different for the other; however, it was difficult to ascribe the differences (ExL>ExE; LxL intermediate but closer to ExE) to incubation temperature differences. We conclude that there was little if any effect of incubation temperature on survival. Length of juveniles of one year-class differed among crosses in the stream and in the hatchery. Length of the other year-class differed among crosses in one pond at the hatchery but not in the other pond or in the stream. When length differed the pattern was always the same: ExE>LxL; ExL intermediate but closer to LxL. We speculate that incubation temperature may have affected growth of juveniles, and in particular that a longer period of incubation in chilled water may have caused fast juvenile growth relative to a shorter incubation period in ambient water.

Introduction

This study was undertaken because incubation temperature differed between progeny of hatchery and wild steelhead *Oncorhynchus mykiss* in experiments comparing performance between these two groups under natural and hatchery rearing (Rubin et al. 2012d). Hatchery females became ripe and were spawned about two weeks before wild females; therefore, we incubated hatchery progeny at lower temperatures than wild progeny so that fry from both groups reached the button-up stage of development (yolk sac absorption nearly complete; fry ready to initiate feeding) simultaneously. Our goal for the current study was to determine to what extent survival and growth of juveniles could be affected by differences in embryo incubation temperature independently of any genetic differences between hatchery and wild stocks.

We produced groups of progeny that differed in the temperature at which they were incubated but were similar in other respects, and then compared survival and growth of juveniles among progeny groups under rearing conditions similar to those experienced by the hatchery and wild progeny. Differences in spawning time were created by spawning females that ripened early and late in the season. Paternal differences between early and late season progeny were minimized by cryopreserving milt from males used in early season crosses (ExE, from early males and early females) and then thawing it to fertilize late season eggs (ExL). A third progeny group—from late season females crossed with late season males (LxL)—was included to control for genetic differences between adults returning to the hatchery and ripening early compared to adults from the later part of the run. A difference between ExL and LxL

progeny would indicate a genetic difference between early and late males. Such a difference would in turn suggest that any differences between ExE and ExL progeny could be due to genetic differences between early and late females rather than to incubation temperature effects.

Study area

Gametes were obtained from adults returning to Dworshak National Fish Hatchery, located in Ahsahka, Idaho at the confluence of the North Fork and mainstem Clearwater rivers. Embryo incubation and juvenile rearing also occurred at the hatchery.

Natural rearing occurred in the North Fork Palouse River (NFPR), Idaho. The NFPR is located above Palouse Falls, an impassible barrier to anadromous fish. The study reach comprised the lower 7 km of stream (midpoint: UTM zone 11, 52-04-100N, 5-34-100E). Brook trout *Salvelinus fontinalis* and rainbow trout *O. mykiss* were the only resident salmonids we found in the study reach. NFPR is described in more detail by Reisenbichler et al. (2006).

Methods

Experimental crosses

1994. — Progeny groups were marked with genotypes at the cytosol non-specific dipeptidase locus (*PEPA**; Shaklee et al. 1990) so that the groups could be differentiated from each other after being mixed as button-up fry. Adults were screened for *PEPA** genotype prior to spawning by excising a small piece of anal fin tissue and analyzing it electrophoretically (Aebersold et al. 1987). Only early adults homozygous for the alternate (**111*) allele and late adults homozygous for the common (**100*) allele were spawned, thus creating distinct genotypes for each cross (ExE = **111*/**111*, ExL = **100*/**111*, and LxL = **100*/**100*).

ExE crosses were made on 21 March (Table 1) with fish returning to the hatchery less than two weeks prior. The eggs from each female were divided in half, and each half was fertilized with milt from a different male. Additional milt from each male was mixed with extender at a 3:1 ratio and cryopreserved in 4 ml plastic straws (Wheeler and Thorgaard 1991). ExL and LxL crosses were made on 18 April (Table 1) with returns from within two weeks of that date. Half of the eggs from each female were fertilized with thawed milt from an early male and the other half with fresh milt from a late male. Thus maternal contribution was nearly identical between ExL and LxL progeny (Table 1), and paternal contribution was similar between ExE and ExL progeny although males varied in their relative contribution to the two groups (Figure 1). When a group of eggs was fertilized with thawed milt, batches of about 600 eggs were combined with single straws of milt until all the eggs were fertilized. All eggs from each female were weighed collectively before fertilization, and $\geq$30 unfertilized eggs from each female were preserved in 10% buffered formalin and >90 days later counted and weighed collectively to provide estimates of fecundity and egg size (see below).

Fertilized eggs were placed in trays in vertical incubators. Each tray contained two full-sib families separated by a divider (i.e., half of a female's eggs on one side, and half of the same or a different female's eggs on the other). Embryos were incubated in three stacks, each containing one of the three crosses and plumbed to receive ambient (~12°C) or chilled (~7°C) water. ExE embryos were incubated on chilled water and ExL and LxL on ambient water until development was matched among groups (see below). A model that predicts developmental stage from incubation temperature (Rubin et al. 2012c) was used to synchronize development.

When embryos reached the eyed stage, dead embryos were removed (all families) and hand counted (subset of families), and the live embryos were counted electronically (all families; counter

manufactured by Jensorter) and returned to the same tray and stack position. For families where dead embryos were not counted, the total number of embryos was estimated from total weight of unfertilized eggs, mean weight per egg from preserved eggs, and a correction factor based on measurements from families for which dead embryos were counted. Percent eye-up and therefore number of embryos per half-tray after eye-up was about half as great for ExL embryos as for the other two crosses (Table 2).

When embryo development became synchronized among groups after hatch, incubation temperature was equalized and all embryos were subjected to a sequence of alternating ambient and chilled water (Figure 2) to induce a banding pattern on their otoliths (Volk et al. 1990) as a secondary mark to differentiate experimental steelhead from resident rainbow trout in NFPR. Mean temperature between fertilization and button-up was 4.0 ^{o}C less for ExE embryos than for those from the other two groups (Table 2).

Fry reached the button-up stage in early June. Samples of fry were collected from each family $\leq$3 days before release in hatchery ponds or NFPR. Samples were preserved in 10% buffered formalin, and >90 days later individuals were measured for length, whole body weight, and after dissection, yolk weight. In addition to proportion yolk, temperature model predictions provided a second measure of how well development was matched among groups at release (Rubin et al. 2012c). Model predictions were in units of percentage of total time to maximum alevin wet weight (MAWW); for example, 90% of predicted time to MAWW indicates underdevelopment and 110% indicates overdevelopment. MAWW approximates the button-up fry stage of development (Jensen 1988).

Fry were counted electronically (Applebee and Tipping 1991) on the day of release into one nursery tank at the hatchery and 1-2 days before release in NFPR. Each family was sampled proportionately to equalize cross and family composition between the two rearing environments. Numbers of fry released are shown in Tables 3 and 4. We had to release more ExE and LxL than ExL fry to attain target densities. Nearly equal numbers of fry were released in NFPR at each of eight sites approximately equally spaced over the study reach. Cross and family composition was similar among release sites.

1995. — Each female's eggs were allocated to crosses in a way that compensated for the expected lower percent eye-up of ExL embryos (i.e., that resulted in a similar number of eyed embryos from all three crosses). Also, otolith marks rather than *PEPA** genotypes were used to differentiate the crosses, and fry were counted into release groups for two rather than one nursery tank at the hatchery. Otherwise procedures were similar to 1994.

The number of eggs from each early female was divided into thirds, one third was discarded, and the other two thirds were each fertilized with milt from a different early male. The number of eggs from each late female was divided into thirds, one third was fertilized with milt from a late male, and the other two thirds were fertilized with thawed milt from one or more (up to six) early males. Thus twice as many eggs were allocated to the ExL as to the LxL group. Density during incubation was kept similar among crosses by holding each third of a late female's eggs in a separate tray compartment between fertilization and eye-up, and then consolidating the two compartments with ExL embryos at eye-up. ExL compartments sometimes contained embryos from more than one sire but were treated similarly to full-sib families for purposes of sampling fry for length and weight measurements and counting fry into release groups.

After development was matched among the three crosses and the embryos had hatched, their otoliths were marked to differentiate the crosses. Three unique banding patterns were created by alternating between ambient and chilled water. Dark bands were formed by 8 h exposure to chilled water, and light areas of varying width were formed between the bands by exposure to ambient water for 1-4 d.

Resulting patterns, expressed as days of exposure to ambient water separated by dashes that represent exposure to chilled water, were 2-2-3 (ExE), 4-2-1 (ExL), and 2-4-1 (LxL). Marking started and ended on the same day for all crosses.

Each family was sampled proportionately to provide fry from each cross for each of three release groups: one for NFPR and two for a nursery tanks at the hatchery (Tables 3 and 4).

Hatchery rearing

Juveniles at the hatchery were reared using standard production methods. They were held indoors in rectangular tanks and fed by hand until late August, then moved outside into Burrows ponds fitted with automatic demand feeders and held there until release the following spring. As fish grew some of them were moved into additional inside tanks, but when moved outside fish initially ponded in the same nursery tank were recombined into a single pond for the duration of rearing. During the fall each fish was marked by clipping the adipose fin and the right ventral fin and inserting a coded wire tag. Survival between when fry were put in nursery tanks and smolt release was 62% for the 1994 year-class, 77% for one of the 1995 year-class ponds, and 80% for the other. Release dates were 27 April 1995 and 3 May 1996.

Juvenile sampling

Juveniles were sampled at the hatchery on 26 April 1995 and 2-3 April 1996. Samples were dipnetted from each pond after crowding fish to one end. Each fish in the samples was measured, weighed, and sampled for fin tissue that was frozen for later analysis of *PEPA** genotype (1995) or euthanized and their heads preserved in 95% ethanol for subsequent otolith extraction and thermal mark determination (1996).

Juveniles were captured from NFPR by backpack electrofishing during 28 August-8 September 1994, 7-10 September 1995, and 4-6 September 1996. For each year-class, about 40 each of age-0 and age-1 juveniles were collected near each of the eight release sites. Liberal size limits were used to ensure that the smallest and largest fish of our target age classes were sampled. Individuals were measured, weighed, euthanized, and their bodies frozen and heads preserved in ethanol. Older steelhead from previous studies (Reisenbichler et al. 2006) and resident rainbow trout were excluded based on later otolith analysis.

Otoliths were processed following Volk et al. (1990). Measurements of reference specimens collected before fry release, including unmarked controls, indicated that marked otoliths from experimental steelhead could be discriminated from unmarked otoliths of resident trout, and that the three marks on otoliths of 1995 year-class steelhead could be differentiated. For 1994 year-class specimens, variation in distance from the core to the inner edge of the mark and in the width of the mark was low (CV=1%; n=30) indicating that the mark was consistently visible on the same part of the otolith. For 1995 year-class specimens, distributions of distances between dark bands within the mark were non-overlapping among the three progeny groups (i.e., the interval mean$\pm$2*SD for one group did not overlap with that interval for another; n=49).

Analyses

The year-classes were analyzed separately. Survival of ExL relative to ExE fish ($RS_{EL/EE}$) was computed as:

$(P_{EL2}*P_{EE1})/(P_{EL1}*P_{EE2})$;

P_{EL1} = proportion of ExL fish at release, P_{EL2} = proportion of ExL fish at time 2 (i.e., juvenile sampling), P_{EE1} = proportion of ExE fish at release, and P_{EE2} = proportion of ExE fish at time 2. Survival of LxL relative to ExE fish was computed similarly. Relative survival was termed "apparent" for naturally reared juveniles because estimates reflected emigration as well as mortality. Relative survival was tested with *G*-tests for goodness of fit (Sokal and Rohlf 1995); expected frequencies were the number of fish sampled times the proportion of each cross from the original number of fry released. A replicated *G*-test for goodness of fit (Sokal and Rohlf 1995) was used for the 1995 year-class at the hatchery; expected frequencies for the pooled sample were the sum of the expected frequencies in the replicate ponds. Exact *P*-values of goodness of fit tests were obtained from StatXact-3 (Cytel Software Corporation 1997); sequential Bonferroni adjustments were made to account for multiple comparisons (Sokal and Rohlf 1995).

Length was tested with one-way analysis of variance (AOV) except for the 1995 year-class at the hatchery where pond was included as a second factor; paired comparisons were made with Tukey's studentized range test. Condition (weight at length) was tested with analysis of covariance (AOCV) on $\log_{10}$ transformed weight and length. If slopes of the weight-length relation were similar among crosses ($P>0.05$), intercepts were compared among crosses using the "common slope" model (i.e., with the cross*length interaction term removed). Table entries were obtained by evaluating weight at a common length (the geometric mean of lengths pooled over crosses). Relations from the common slope AOCV were used if slopes were similar, or from the model including the cross*length interaction term if not. Condition factor (*k*) was computed from weight at common length and common length. This method avoids the problem of correlations between *k* and length that occur when the exponent of the weight-length relation differs from 3 (Cone 1989).

Results

Development and size of fry at release

In 1994, proportion yolk was greater for ExE than for ExL or LxL fry, and percent of time between fertilization and MAWW predicted by the temperature model was less (Table 2), indicating that ExE fry were less developed than ExL or LxL fry. Apparently, we waited slightly too long to switch ExE embryos from chilled to ambient water (Figure 2). In 1995, development was more closely matched among crosses. Temperature model predictions were similar among crosses, and although proportion yolk was significantly greater for ExL than for ExE or LxL fry, the difference in mean proportion yolk between ExL and the other two crosses was only a third as large as the difference between ExE and the late female crosses in 1994 (Table 2). In both years, eggs from early females were larger than eggs from late females, and ExE fry were longer and heavier than ExL or LxL fry (Table 2).

Juvenile survival and growth

There was no difference among crosses in apparent survival to age-0 or to age-1 in NFPR for either year-class (Table 3), or in survival at the hatchery for the 1995 year-class; however, survival at the hatchery was higher for ExL than for ExE fish of the 1994 year-class (Table 4). Survival at the hatchery for LxL fish of the 1994 year-class was intermediate and not significantly different from either of the other groups. In NFPR, length was greater for ExE than for LxL fish for both ages of the 1994 year-class but was similar among crosses for both ages of the 1995 year-class (Table 3). Length of 1994 year-class ExL fish in NFPR was closer to LxL than to ExE length and was significantly less than ExE length ($P<0.05$) when both ages were included in a two-way AOV. At the hatchery, length was greater for ExE than for LxL fish in the only 1994 year-class pond and in one but not the other 1995 year-class pond (Table 4). Condition was similar among crosses except in one of the two 1995 year-class ponds at the hatchery where condition was lower for ExE fish than for fish from either of the other groups (Tables 3 and 4).

Discussion

Development and size of fry at release

The general similarity in development and size between ExL and LxL fry (no significant difference between the two on any measurement except proportion yolk for the 1995 year-class [ExL>LxL; Table 2]), suggests genetic similarity between early and late males with respect to embryo development rate and yolk conversion efficiency. The greater size of ExE fry compared to ExL or LxL fry probably resulted from greater egg size for early than for late females. Size of fry at the button-up stage of development is positively correlated with egg size (Rubin et al. 2012f). Further, yolk conversion efficiency is inversely related to incubation temperature (Rubin et al. 2012c) which may have increased size differences between fry from early and late females since incubation temperature was lower for ExE than for ExL or LxL embryos. The consistent difference in size between eggs from early and late females was unexpected and its cause is unknown to us.

Survival

The similarity in apparent survival among crosses for both year-classes in NFPR suggests that both survival and emigration were similar among crosses. At the hatchery, survival was similar among crosses for the 1995 year-class but differed for the 1994 year-class (Table 4). The difference for the 1994 year-class is difficult to interpret. Survival was higher for ExL than for ExE and trending higher for ExL than for LxL. The latter difference was not significant (P=0.11 after adjusting for multiple comparisons). Survival was similar between LxL and ExE. We offer three possible explanations for the survival ranking described above (ExL>LxL=ExE):

1. ExL and LxL differed only in paternity (temperature and maternity identical), so higher survival of ExL implies an advantage of early paternity (i.e., early genes). Higher survival of ExL than ExE implies an advantage of late (warm) temperature that more than counteracted the disadvantage of late genes. Similar survival of LxL and ExE implies that the disadvantage of a double dose of late genes counteracted the advantage of late temperature.
2. For unknown reasons, progeny of late females were favored whereas progeny of late males were disfavored; progeny of early parents of either sex were neutral. Then ExL (neutral + favored) would have an advantage over LxL (disfavored + favored) and ExE (neutral + neutral).
3. Genetic interactions between early males and late females were synergistic rather than additive such that ExL progeny were genetically superior to both LxL and ExE progeny.

The key point is that we find it difficult to ascribe the survival differences to an effect of incubation temperature. Temperature figures only in the first explanation, and that explanation seems no more plausible to us than the others.

Importantly, the experiment was better executed in 1995, when no survival differences among crosses were found, than in 1994. Development was well matched between progeny from early and late females in 1995 but less so in 1994, and three times more male parents were used in 1995 than in 1994, thus lessening the chance of small population effects. Further, density of eyed embryos in incubation trays and number of fry released were more similar among crosses in 1995 than in 1994. We conclude that the incubation temperature treatments had little if any effect on survival.

Growth

Length of 1994 year-class juveniles differed among crosses in NFPR and at the hatchery, and length of 1995 year-class juveniles differed among crosses in one pond at the hatchery but not in the second pond or in NFPR. Where length differed significantly among crosses the pattern was always the same: ExE>LxL; ExL intermediate but closer to LxL (Tables 3 and 4). Thus we found more evidence

for an effect of incubation temperature on growth than on survival, but growth comparisons among crosses were inconsistent between year-classes and within the 1995 year-class.

Factors that could account for the inconsistency between year-classes include fry size and development at release, small population effects (fewer parents in 1994 than in 1995), and the genetic marks (used in 1994 but not in 1995). In 1994, eggs and fry from late females were smaller than those from early females, and fry from late females were overdeveloped compared to those from early females which were closer to optimally developed (Table 2). These differences were of sufficient magnitude to affect growth in streams (Rubin et al. 2012e; 2012f) and could have accounted for the growth differences among 1994 year-class crosses in NFPR. In 1995, fry from late females were again smaller than those from early females, but development was similar and near optimal for fry from both groups of females. Perhaps growth in NFPR differed among 1994 but not 1995 year-class crosses because both fry size and development differed in 1994 whereas only size differed in 1995. Neither size nor development of fry at release were found to affect growth in the hatchery in companion studies (Rubin et al. 2012e; 2012f); but size was tested separately from development. Perhaps the growth differences among 1994 year-class crosses at the hatchery were due to the combined effects of differences in fry size and development.

Growth of 1994 year-class ExL fish was always intermediate but closer to growth of LxL than to growth of ExE fish. This pattern suggests a combination of incubation temperature (or maternal) and genetic effects. In 1994, ExE progeny originated from only 7 males and 9 females, and ExL progeny originated from the same 7 males. If by chance a few of the early males and females happened to carry genes for fast growth, then growth differences between ExE and ExL fish would be greater than from incubation temperature or maternal differences alone, and growth differences between ExE and LxL fish would be greater still. Such a chance event was less likely in 1995 due to the larger number of early parents, particularly males. When growth differences occurred among 1995 year-class crosses, the pattern for ExL fish was the same as for the 1994 year-class (ExL intermediate but closer to LxL), raising the possibility that consistent differences in genetic growth rate between early and late returning adults rather than chance events may have contributed to growth differences among crosses.

The *PEPA** genotypes used to mark the crosses in 1994 were found not to be selectively neutral in a companion study (Reisenbichler et al. 2006). The genotypes didn't affect survival or growth of juveniles in NFPR or at the hatchery, but survival to adult of smolts released from the hatchery was highest for fish carrying the ExE mark, intermediate for fish with the ExL mark, and lowest for fish with the LxL mark. Juvenile rearing conditions in the current study were nearly identical to those in the companion study, but we cannot exclude the possibility that the current study somehow elicited non-neutrality of the genetic marks with respect to juvenile growth. If so, the effect would have been indistinguishable from that of genetic differences in growth between early and late parents (i.e., growth would be fastest for ExE progeny and slowest for LxL progeny regardless of whether from non-neutrality of the genetic marks or from genetic differences between early and late parents).

The inconsistency of growth results from the two 1995 year-class hatchery ponds is perplexing. In the pond where length was greater for ExE than for LxL fish, condition (weight at common length) was nearly identical between ExE and LxL fish (Table 4). The difference in length coupled with no difference in condition indicates faster growth for ExE fish even though weight didn't differ statistically between ExE and LxL fish (data not shown). In the other pond there was some evidence for the opposite effect, that growth may have been slower for ExE than for LxL fish. Mean length was less for ExE than for LxL fish although not significantly less, and condition was significantly lower for ExE than for LxL fish; thus the difference in weight between ExE and LxL fish was more pronounced than for length although still not significant. As a comparison of relative growth between ponds, mean weight was 16.5% greater for ExE than for LxL fish in one pond whereas it was 11.5% greater for LxL than for ExE fish in the other. The ponds were true replicates in that each originated with nearly identical numbers of fry from each

cross and full-sib family. Rearing conditions were meant to be similar between ponds; however, overall growth was considerably faster in one pond than in the other (mean length 5% greater in one pond than in the other; $P<0.05$) suggesting differences in ration level that may have contributed to the discrepancy between ponds in growth of ExE relative to LxL fish.

The fact that 1994 year-class growth differences may have been from factors other than our intended incubation temperature treatments, and the inconsistency of 1995 year-class growth differences between hatchery ponds make it difficult to draw conclusions about the effect of incubation temperature on juvenile growth. The growth difference among 1995 year-class crosses in one of two ponds at the hatchery seems to us a key result. Although this was the only significant difference in growth for the 1995 year-class, the pattern of growth differences among crosses was the same as for all 1994 year-class comparisons. We therefore speculate that incubation temperature may have affected growth of juveniles, and in particular that a long period of incubation in chilled water may have caused fast growth relative to a shorter incubation period in ambient water. We know of no other studies where experimenters attempted to isolate the effect of incubation temperature on juvenile growth, but several studies have shown that depressing growth of juveniles by restricting food or reducing temperature, and then restoring initial conditions (rations or temperature) triggers compensatory, faster growth, relative to controls held at initial conditions throughout (e.g., Nicieza and Metcalfe 1997). Incubation in chilled water slowed embryonic development and may have elicited a compensatory response (fast growth) in juveniles.

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References

Aebersold, P. B., G. A. Winans, D. J. Teel, G. B. Milner, and F. M. Utter. 1987. Manual for starch gel electrophoresis – a method for the detection of genetic variation. NOAA Technical Report NMFS 61.

Applebee, A. E., and J. M. Tipping. 1991. Use of electronic counters for coho and Chinook salmon, steelhead, and cutthroat trout smolts. The Progressive Fish Culturist 53:195-198.

Cone, R. S. 1989. The need to reconsider the use of condition indices in fisheries science. Transactions of the American Fisheries Society 118:510-514.

Cytel Software Corporation. 1997. StatXact-3, Version 3.1. Copyright 1989-1997.

Jensen, J. O. T. 1988. A microcomputer program for predicting embryonic development in Pacific salmon and steelhead trout. World Aquaculture 19:80-81.

Nicieza, A. G., and N. B. Metcalfe. 1997. Growth compensation in juvenile Atlantic salmon: responses to depressed temperature and food availability. Ecology 78:2385-2400.

Reisenbichler, R. R., M. C. Hayes, S. P. Rubin, L.A. Wetzel, and B. M. Baker. 2006. *PEPA** genotype affects return rate for hatchery steelhead. Transactions of the American Fisheries Society 135:28-39.

Rubin, S. P., R. R. Reisenbichler, and S. L. Slatton. 2012c. Predicted time from fertilization to maximum wet weight for steelhead alevins based on incubation temperature and egg size. Chapter 4 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, J. E. Hensleigh, F. E. Leonetti, B. M. Baker, S. L. Slatton, and K. D. Stenberg. 2012d. Differences in survival and growth in hatchery and stream environments, and in maturation of residuals in a stream, between progeny of hatchery and wild steelhead. Chapter 1 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, and K. D. Stenberg. 2012e. Effect of developmental stage of unfed fry on survival and growth of steelhead released in a stream and in hatchery ponds. Chapter 6 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, and K. D. Stenberg. 2012f. Effect of size of unfed fry at release on survival and growth of juvenile steelhead in streams and a hatchery. Chapter 7 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Shaklee, J. B., F. W. Allendorf, D. C. Morizot, and G. S. Whitt. 1990. Gene nomenclature for protein-coding loci in fish. Transactions of the American Fisheries Society 119:2-15.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry, 3rd edition. W. H. Freeman and Company, New York.

Volk, E. C., S. L. Schroder, and K. L. Fresh. 1990. Inducement of unique banding patterns as a practical means to mass-mark juvenile pacific salmon. American Fisheries Society Symposium 7:203-215.

Wheeler, P. A. and G. H. Thorgaard. 1991. Cryopreservation of rainbow trout semen in large straws. Aquaculture 93:95-100.

Tables

Table 1. Number of parents spawned and full-sib families produced. Milt from the same early males used for ExE crosses was cryopreserved and then thawed to produce ExL crosses. Thus early males appear in the table twice, once in the early spawning date row and again in the late spawning date row. In 1994, each family represents one half of a female's eggs fertilized with milt from one male (the other half of the female's eggs were fertilized with milt from a different male). In 1995, each ExE and LxL family represent one third of a female's eggs fertilized with milt from one male, and each ExL family represents two thirds of a female's eggs fertilized with thawed milt from up to six males.

	Date		Males		Families		
Year	spawned	Females	Early	Late	ExE	ExL	LxL
1994	21 Mar	8.5[a]	7		17		
	18 Apr	16.5	7	9		16[b]	17
1995	21 Mar	11	22		22		
	18 Apr	15	21[c]	15		15	15

[a]Half of one female's eggs were not fertilized.

[b]One family was excluded because it failed our genetic mark confirmation test (of 10 alevins sampled not all had the **100/*111* genotype). The other half of that female parent's eggs, which were fertilized by a late male (LxL group), remained in the experiment.

[c]One early male was used in ExE but not ExL crosses.

Table 2. Mean (SD) egg size; density, survival, and temperature during incubation; and final size, yolk weight as a proportion of whole body weight, and temperature model prediction of development, of fry at release. Fry measurements were taken on 10 (1994) or 20 (1995) fry from each family (1994: *n*=500; 1995: *n*=1040). Egg weight was the same for ExL and LxL crosses because the same females were used in both crosses. Survival from eyed embryos to button-up fry in 1995 was computed from counts of dead embryos removed from each half-tray. These data were not collected in 1994 and thus survival could not be computed. Letters (x, y, z) indicate significance of paired comparisons; means without a letter in common differ ($P<0.05$); na=not applicable (test conducted between early and late females). Tests were conducted only for egg size and for fry length, weight, and proportion yolk.

	1994			1995		
Variable	ExE	ExL	LxL	ExE	ExL	LxL
Egg weight (mg)[a]	121 (10) x	110 (15) na	110 (15) y	111 (10) x	104 (12) na	104 (12) y
Number of fertilized eggs placed in each half-tray[b]	3127 (628)	3617 (858)	3686 (1008)	2033 (404)	2375 (478)	2448 (459)
Percent eye-up[b]	79.8 (11.9)	36.2 (17.9)	74.6 (26.5)	99.5 (2.6)	42.0 (11.0)	93.6 (4.6)
Number of eyed embryos returned to each half-tray after removing dead embryos[b]	2527 (775)	1286 (685)	2653 (1132)	2023 (403)	1999 (674)	2297 (478)
Survival from eyed egg to button-up fry (%)[b]	-	-	-	99.2 (0.6)	97.2 (2.7)	97.2 (4.1)
Incubation temperature between fertilization and button-up	8.0	12.0	12.0	7.8	11.3	11.4
Fry length (mm)[c]	28.9 (1.0) x	28.5 (1.1) y	28.4 (1.1) y	29.0 (0.8) x	28.0 (0.9) y	27.8 (0.9) y
Fry weight (mg)[c]	195 (23) x	185 (28) y	180 (25) y	189 (18) x	170 (21) y	169 (20) y
Proportion yolk[cd]	0.087 (0.055) x	0.055 (0.030) y	0.059 (0.038) y	0.048 (0.018) x	0.059 (0.026) y	0.054 (0.040) x
Temperature model prediction of fry development (percent of time between fertilization and MAWW[e])	104.8	110.6	110.6	102.8	104.5	104.5

[a]Standard deviation among females.
[b]Standard deviation among half-trays (i.e., families).
[c]Standard deviation among individuals.
[d]AOV on arcsine squareroot transformed proportions.
[e]Maximum alevin wet weight.

Table 3. Apparent relative survival, length, and condition of juveniles in NFPR. Juveniles were sampled three months after release and again one year later. Letters (x, y, z) indicate significance of paired comparisons; values without a letter in common differ (P<0.05). N = total number of fry released; n = number of juveniles sampled.

Year-class	Release date	Cross	N or proportion of fry released	n or proportion of juveniles sampled		Apparent relative survival		Mean (SD) fork length (mm)		Weight[a] (k[b]) at common length[c]	
				Age-0	Age-1	Age-0	Age-1	Age-0	Age-1	Age-0	Age-1
1994	3 Jun	ExE	0.423	0.397	0.440	1.00 x	1.00 x	66.7 (7.6) x	120 (12) x	3.04 (1.12) x	17.5 (1.11) x
		ExL	0.214	0.213	0.194	1.06 x	0.87 x	64.6 (7.0) xy	115 (13) xy	3.02 (1.11) x	17.7 (1.13) x
		LxL	0.363	0.389	0.366	1.14 x	0.97 x	63.8 (6.9) y	115 (11) y	3.02 (1.12) x	17.3 (1.10) x
		Total	42,048	239	191						
1995	4 Jun	ExE	0.339	0.324	0.358	1.00 x	1.00 x	70.7 (6.8) x	113 (11) x	3.90 (1.15) x	15.5 (1.10) [d]
		ExL	0.311	0.346	0.297	1.17 x	0.90 x	70.1 (7.3) x	112 (11) x	3.95 (1.16) x	15.7 (1.12) [d]
		LxL	0.350	0.330	0.346	0.99 x	0.94 x	69.7 (7.7) x	113 (9) x	3.85 (1.13) x	15.2 (1.09) [d]
		Total	36,380	309	246						

[a]Weight (in grams) evaluated at common length with weight-length relations from AOCV; slopes similar among crosses (P>0.05) unless otherwise indicated.
[b]k=100,000*W/L^3; W=weight at common length; L=common length.
[c]Geometric mean of lengths pooled over crosses.
[d]Slope of weight-length relation differed among crosses (P<0.05); paired comparisons not made.

Table 4. Relative survival, length, and condition of juveniles at DNFH. Juveniles were sampled in April, 10 months after placement in nursery tanks at the hatchery and 1-4 weeks before release from the hatchery. Letters (x, y, z) indicate significance of paired comparisons; values without a letter in common differ (P<0.05). Relative survival was similar between 1995 year-class ponds, and thus frequency data were pooled before testing for differences among crosses. For both length and condition there was a significant interaction between cross and pond (P<0.05), so paired comparisons were conducted separately for each pond. N = total number of fry released; n = number of juveniles sampled.

Year-class	Ponding dates	Pond	Cross	*N* or proportion of fry ponded	*n* or proportion of juveniles sampled	Relative survival	Mean (SD) fork length (mm)	Weight[a] (k[b]) at common length[c]
1994	1-2 Jun	1	ExE	0.431	0.397	1.00 x	196 (31) x	66.4 (1.01) x
			ExL	0.224	0.271	1.31 y	190 (31) xy	66.4 (1.01) x
			LxL	0.344	0.332	1.05 xy	185 (36) y	67.1 (1.02) x
			Total	23,704	491			
1995	3-4 Jun	1	ExE	0.352	0.360	1.00	157 (23) x	41.0 (1.03) x
			ExL	0.305	0.333	1.07	161 (19) x	42.8 (1.07) y
			LxL	0.343	0.307	0.87	162 (20) x	43.1 (1.08) y
			Total	25,144	150			
		2	ExE	0.347	0.411	1.00	173 (20) x	48.2 (1.04) x
			ExL	0.300	0.298	0.84	166 (18) xy	49.1 (1.06) x
			LxL	0.354	0.291	0.70	163 (24) y	48.4 (1.04) x
			Total	25,450	151			
		Pooled	ExE			1.00 x		
			ExL			0.95 x		
			LxL			0.78 x		

[a]Weight (in grams) evaluated at common length with weight-length relations from AOCV; slopes always similar among crosses (P>0.05).
[b]k=100,000*W/L^3; W=weight at common length; L=common length.
[c]Geometric mean of lengths pooled over crosses.

Figures

Figure legends

Figure 1. Proportion of total ExE eyed embryos sired by each early male, and the same for the ExL group. In 1995, separate counts of ExL eyed embryos were made for each female parent, but each female was often crossed with more than one male. In these cases it was assumed that male contribution was proportional to number of straws of milt used.

Figure 2. Daily mean temperature during embryo incubation. Development of crosses from early and late females matched, and the ExE group switched to ambient (12°C) water, on 17 May 1994 and 19 May 1995, after which a schedule of alternating ambient and chilled water was employed for otolith marking.

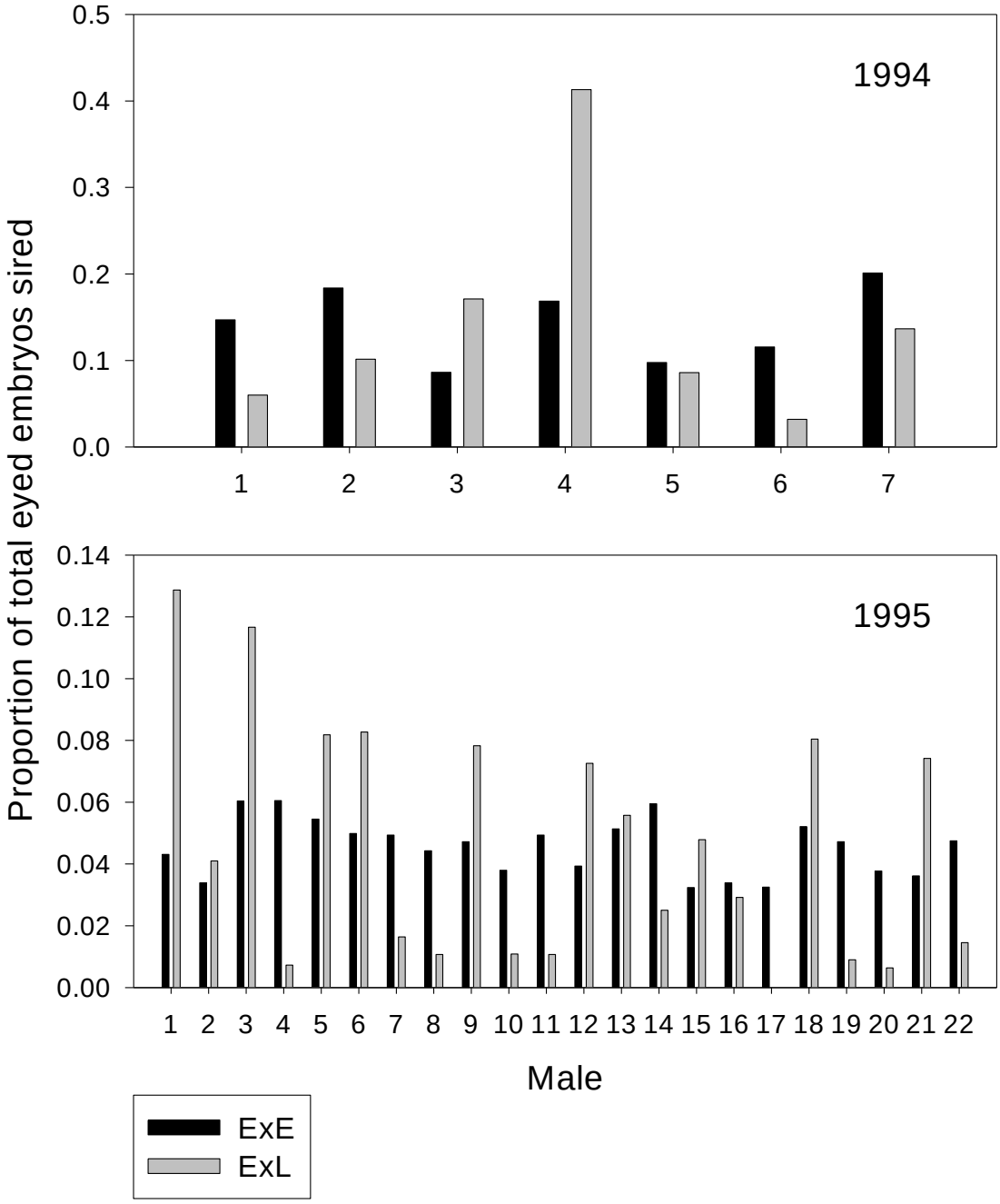
1994
1995
Proportion of total eyed embryos sired
Male
ExE
ExL

Figure 1.

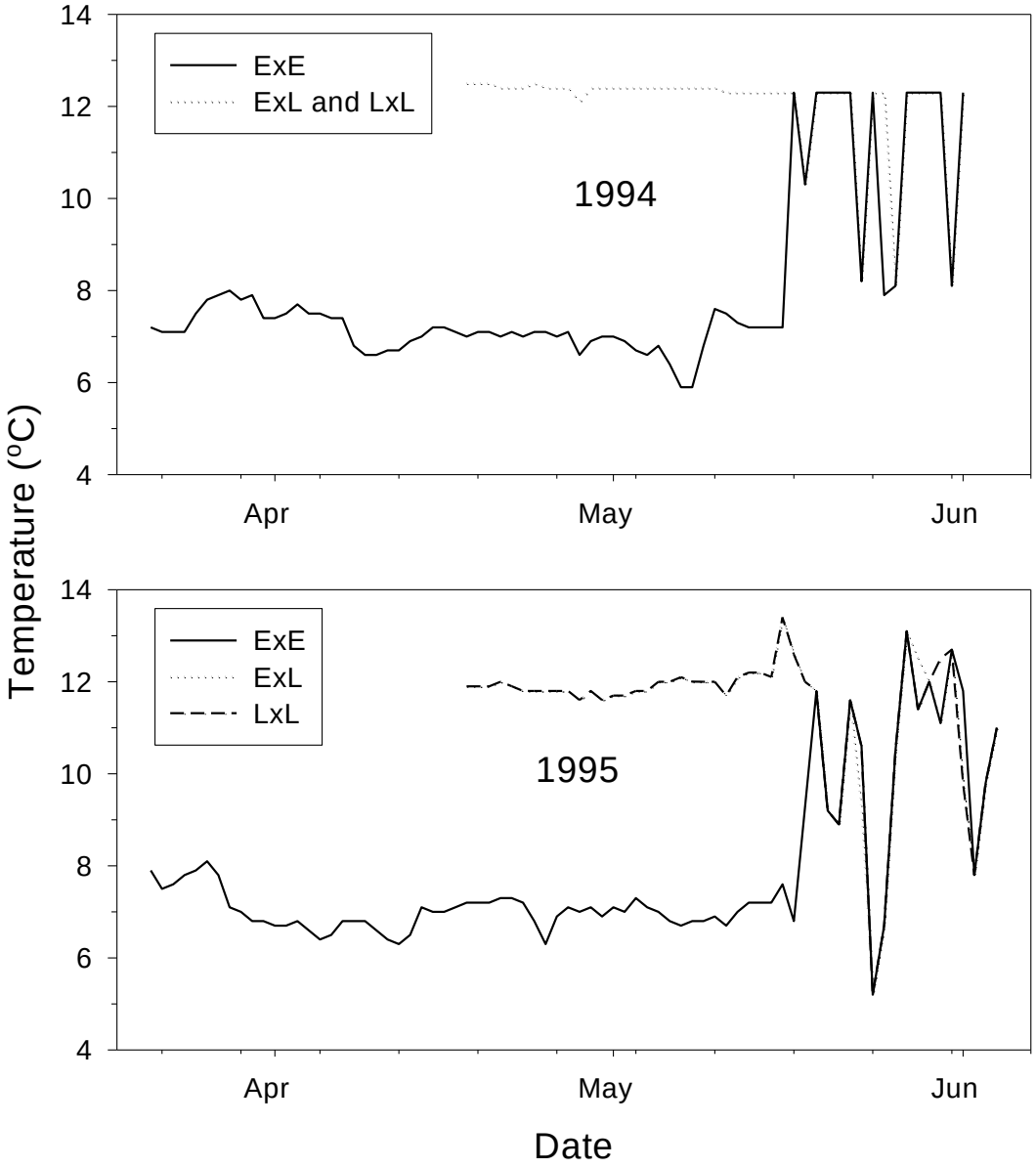
14
12
10
8
6
4
ExE
ExL and LxL
1994
Apr
May
Jun
Temperature (°C)
14
12
10
8
6
4
ExE
ExL
LxL
1995
Apr
May
Jun
Date

Figure 2.

Chapter 6: Effect of developmental stage of unfed fry on survival and growth of steelhead released in a stream and hatchery ponds *(Study sites: Dworshak Hatchery and North Fork Palouse River; Stock: Dworshak hatchery; Year class: 1996)*

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Abstract

We tested whether differences in developmental stage of unfed fry at release affected subsequent survival and growth of steelhead *Oncorhynchus mykiss* in a stream and hatchery ponds. Differences in development were created by artificially spawning hatchery steelhead from the Clearwater River, Idaho, and incubating their progeny at three different temperatures (means=10.9, 11.3, and 11.7°C). Time between fertilization and maximum alevin wet weight (MAWW) was predicted from incubation temperature using a model. MAWW is equivalent to the button-up fry stage of development. Developmental stages at release were "underdeveloped" (97.7% of model-predicted time to MAWW, mean weight=0.177 g, proportion yolk=0.087), "intermediate" (102.5%, 0.179 g, 0.044), and "overdeveloped" (107.9%, 0.156 g, 0.030). Neither survival nor growth in the hatchery to near the end of the standard one year rearing period differed among groups. In the stream, frequency of overdeveloped fish relative to the other two groups decreased from release in May to September, probably indicating lower survival for the overdeveloped fish during that interval since emigration of sub-yearlings is typically negligible. Length in September was less for overdeveloped than for intermediate fish and was in between for underdeveloped fish, suggesting that growth between May and September was less for overdeveloped fish than for intermediate fish. Although changes in relative frequency and size occurred among fry development groups from September to one year later, those changes may have reflected differences in emigration rate during the interval rather than differential survival or growth. Our results show a cost to survival and growth in a stream, but not in a hatchery, from overdevelopment characterized by loss of weight and yolk reserves relative to fry closer to MAWW at release. We didn't find any cost from underdevelopment; however, our underdeveloped fry were closer to MAWW than the overdeveloped fry, and they may have been no farther from MAWW than the intermediate fry based on percentage of model-predicted time to MAWW at release.

Introduction

This study was undertaken because developmental stage of unfed fry at release differed between hatchery and wild progeny of steelhead *Oncorhynchus mykiss* in experiments comparing those groups under natural and hatchery rearing (Rubin et al. 2012a; 2012b). It is important to know how initial differences in development, as opposed to stock differences, affected survival and growth in those studies. Accordingly, we created groups of fry with developmental stages ranging from incomplete yolk absorption (underdeveloped) to loss of weight following complete yolk absorption (overdeveloped) at the time of release, and then compared their survival and growth under rearing conditions similar to those experienced by the hatchery and wild progeny. Differences in development were created by varying temperatures during incubation.

Study area

Gametes were obtained from adults returning to Dworshak National Fish Hatchery, located in Ahsahka, Idaho at the confluence of the North Fork and mainstem Clearwater rivers. Embryo incubation and hatchery rearing of progeny also occurred there.

The natural rearing area was the North Fork Palouse River (NFPR), Idaho, which is upstream from impassible falls and therefore devoid of anadromous fish. Fry were released and juveniles

recaptured between river kilometers (rkm) 0 and 7 (midpoint: UTM zone 11, 52-04-100N, 5-34-100E). Brook trout *Salvelinus fontinalis* and rainbow trout *O. mykiss* were the only resident salmonids present. NFPR is described in more detail by Reisenbichler et al. (2006).

Methods

Experimental crosses, embryo incubation, and fry release

On 2 April 1996, eggs were stripped from 48 mature females, the total weight of 100 eggs was determined for each female, and the females were ranked by egg weight. The 12 females with the lightest and the 12 with the heaviest eggs were allocated to a different study (Rubin et al. 2012f), and the 24 females with eggs of intermediate weight were used in the current study. We used milt from 24 males to fertilize these intermediate sized eggs in one-to-one matings. A sample of ovarian fluid was collected from each female at spawning and tested for the presence of infectious hematopoietic necrosis virus (IHNV). About 60 unfertilized eggs from each female were preserved in 10% buffered formalin and >90 days later were counted and weighed together to more carefully determine mean egg weight per female.

Fertilized eggs were divided into three groups, and each was incubated at a different temperature so that the groups achieved different stages of development by the release date. Target stages were underdeveloped (yolk not completely absorbed), optimum (yolk absorption nearly complete; equivalent to maximum alevin wet weight or MAWW), and overdeveloped (yolk completely absorbed and loss of weight compared to optimum stage). These stages will henceforth be referred to as underdeveloped, intermediate, and overdeveloped, respectively. A model that predicts developmental stage from incubation duration and temperature (Rubin et al. 2012c) was used to achieve the target stages. One third of the eggs from a female were placed in each of three trays. The trays contained center dividers, and one third of the eggs from a second female were placed on the other side of the divider in each tray. The trays were placed in three separate stacks of vertical incubators, each corresponding to a development group, and the process was repeated for the rest of the females. One female tested positive for IHNV and her progeny were excluded from the experiment. To achieve the targeted differences in development among groups, a temperature difference of about 1°C was maintained between the "underdeveloped" and "intermediate" stacks, and between the "intermediate" and "overdeveloped" stacks, for about three weeks (until temperatures were equalized prior to otolith marking, see below).

When embryos reached the eyed stage, dead embryos were counted and removed and live embryos counted. Each half-tray was sampled proportionately to provide an equal number of embryos from each development group for a given rearing environment and to ensure that family composition was equivalent between rearing environments. Embryos were then returned to undivided trays with families mixed but groups still held in separate stacks at the different temperatures. After all embryos were hatched, temperatures was equalized among stacks (i.e., among groups), and a week later we commenced thermal inducement of otolith marks (Volk et al. 1990) to differentiate the development groups. Three unique banding patterns were created by alternating between warm (12°C) and cold (7°C) temperatures: wide-narrow-narrow (underdeveloped), narrow-wide-narrow (intermediate), and narrow-narrow-wide (overdeveloped). Bands were formed by 1 d exposure to the cold temperature, followed by 2 d warm for narrow intervals or 4 d warm for wide intervals and 1 d cold again. All groups were marked during the same time period (i.e., cold days at the beginning and end of the marks were the same for all groups).

Samples of fry were collected from each group 0-1 day before release. Some of the fry from each sample were preserved in 10% buffered formalin, and >90 days later measured for length, whole body weight, and after dissection, yolk weight. Otoliths were extracted from the rest to provide reference specimens for testing accuracy of mark differentiation (see below). Known and nearly equal numbers of fry from each group were released on 18 May in two tanks at the hatchery and on 19 May at eight sites approximately equally spaced over the NFPR study reach (numbers of fry released are shown in Tables 2

and 3). Mean temperature from fertilization to release ranged from 10.9-11.7^{o}C among groups (Table 1). Survival from fertilization to release ranged from 94-96% among groups.

Hatchery rearing

Juveniles at the hatchery were reared using standard production methods. They were kept indoors in rectangular tanks and fed by hand until early September, then moved outside into Burrows ponds fitted with demand feeders and held there until release the following April. Although some fish were moved into additional inside tanks as they grew, all fish were recombined into a single pond when moved outside. In October, 81% of the fish were marked with adipose and right ventral fin clips and coded wire tags, and about half of the marked fish were moved to a second pond. The remaining 19% were given adipose fin clips and transferred to production lots at the hatchery. Based on counts of fish marked and subsequent mortalities, survival was 92% from ponding to marking and 97% from marking to release, or 89% from ponding to release.

We sampled juveniles at the hatchery on 15-16 April 1997, two weeks before they were released to migrate seaward. Samples were randomly dipnetted from each pond after crowding fish to one end. Individuals were measured, weighed, euthanized, and their heads preserved in 95% ethanol for subsequent otolith extraction.

Natural rearing

Juveniles were captured by backpack electrofishing in summer four months after release and again one year later (dates of electrofishing are shown in Table 4). About 40 age-0 (in 1996) or age-1 (in 1997) steelhead were collected near each release site. Resident rainbow trout, and older steelhead from a previous study (Rubin et al. 2012g), were identified later by virtue of their unmarked or differently marked otoliths and excluded from our analyses. Sampling procedures for individuals captured in NFPR were the same as for fish at the hatchery.

Otoliths

Otoliths were sectioned and ground to permit viewing of the marks (Volk et al. 1990). Blind tests with 40 reference specimens collected before fry release (10 from each treatment group and 10 unmarked controls) indicated 95% scoring accuracy. For all collections combined, 3.4% of the samples couldn't be scored due to damaged or lost otoliths, clear otoliths (composed of vaterite rather than aragonite) that did not show the marks, or over-processing.

Analyses

Survival of overdeveloped relative to underdeveloped fish ($RS_{O/U}$) was computed as:

$(P_{O2}*P_{U1})/(P_{O1}*P_{U2})$;

P_{O1} = proportion of overdeveloped fish at release, P_{O2} = proportion of overdeveloped fish at time 2 (i.e., juvenile sampling), P_{U1} = proportion of underdeveloped fish at release, and P_{U2} = proportion of underdeveloped fish at time 2. Survival of intermediate fish relative to underdeveloped fish was computed similarly. Relative survival was termed "apparent" for naturally reared juveniles because estimates reflected emigration as well as mortality. Relative survival was tested with *G*-tests for goodness of fit (Sokal and Rohlf 1995); expected frequencies were the original number of fry released from each group. Differences in relative frequency of the groups between samples of juveniles collected at different times (e.g., age-0 and age-1 parr) were tested with contingency table *G*-tests of independence (Sokal and Rohlf 1995). Exact *P*-values for goodness of fit and independence tests were obtained from StatXact-3 (Cytel Software Corporation 1997); sequential Bonferroni adjustments were made to account for multiple comparisons (Sokal and Rohlf 1995).

Differences in length were tested with analysis of variance (AOV); sequential Bonferroni adjustments again accounted for multiple comparisons. Condition (weight at common length) was tested with analysis of covariance (AOCV) on $\log_{10}$ transformed weight and length. If slopes of the weight-length relation were similar among groups (P>0.05), intercepts were compared among groups using the "common slope" model (i.e., with the cross*length interaction term removed). Table entries of condition were obtained by calculating weight at common length (the geometric mean of lengths pooled over groups) using relations from the AOCVs. Relations from the common slope AOCV were used if slopes were similar, or from the model including the cross*length interaction term if not. Condition factor (k = 100,000*W/L^3; W = weight [g]; L = fork length [mm]) was computed from weight at common length and common length. This method avoids the problem of correlations between k and length that occur when the exponent of the weight-length relation differs from 3 (Cone 1989).

Results

Egg and fry characteristics

Mean weight of preserved eggs ranged from 0.098-0.121 g among the 23 females used in the study (mean = 0.108; SD = 0.007). Development of fry on the release dates differed among groups (Table 1). Weight was less for the overdeveloped than for the intermediate group indicating weight loss following MAWW for the former. Proportion yolk was greatest for the underdeveloped and least for the overdeveloped group. According to temperature model-predicted time to MAWW, underdeveloped alevins were less developed than MAWW (97.7% of total time to MAWW), intermediate alevins were more developed than MAWW by about the same amount (102.5%), and overdeveloped alevins were even farther past MAWW at release (107.9%).

Hatchery rearing

Survival, length, and condition of juveniles reared at the hatchery were similar among groups (Table 2). Relative frequency of the groups was independent of pond (G-test of independence), and there was no interactive affect between group and pond on length or condition (2-way AOVs; P>0.1), indicating that the similarity among groups was consistent between rearing ponds.

Natural rearing

Apparent survival from release in May to age-0 sampling in September was lower for the overdeveloped group than for either of the other two groups which were similar to each other (Table 3). The frequency of overdeveloped fish relative to intermediate fish increased from age-0 sampling in September to age-1 sampling a year later (P=0.02; G-test of independence), reversing the disadvantage of overdeveloped fish compared to intermediate fish and accounting for similarity in apparent survival among groups from release to age-1 (Table 3).

Length at age-0 was less for overdeveloped than for intermediate fish, and was in between for underdeveloped fish but not significantly different from either of the other groups (Table 3). Length at age-1 was similar among groups, as was condition at both ages (Table 3).

Discussion

Apparent survival from release in NFPR in May to age-0 sampling in September was lower for overdeveloped fry than for intermediate or underdeveloped fry. This difference in apparent survival likely reflected a true survival difference rather than differential emigration because results from other studies showed emigration of age-0 steelhead between May and September to be negligible. For these other studies, we released four different year-classes of unfed fry in comparably sized Idaho streams,

trapped emigrants, and found that emigration rate between release and late summer (percent of released fry that emigrated) was <1% in each case (Rubin et al. 2012b; 2012d; 2012f).

Poor survival of the overdeveloped group probably resulted from weight loss and lack of yolk reserves at release relative to the other groups (Table 1). It is interesting, though, that survival in the hatchery was not affected. We speculate that the absence of predators in the hatchery accounted for the difference in results between environments. Energy depletion may have left overdeveloped fry more vulnerable to predation in NFPR than fry from the other two groups. Alternatively, perhaps limited food, competition, or the demands of obtaining live food and dealing with current in NFPR prevented overdeveloped fish from eating enough to overcome their initial disadvantage. By comparison, easy access to unlimited food in the hatchery may have allowed the overdeveloped fish to recover. Length at age-0 in NFPR was less for overdeveloped than for intermediate fish, suggesting that growth as well as survival was poorer for overdeveloped than for intermediate fish in the stream environment. As with survival, growth was similar among groups in the hatchery environment.

The decrease in the frequency of intermediate fish relative to overdeveloped fish between age-0 sampling in September and age-1 sampling a year later may have been due to differential emigration consistent with that seen in Twentymile Creek, a comparably sized tributary of the South Fork Clearwater River in Idaho (Rubin et al. 2012b; 2012f). Emigration of yearlings from Twentymile Creek during May-July was considerable (roughly half of the yearlings emigrated) and size dependent, with large fish emigrating and smaller fish remaining in the study reach. In NFPR, intermediate fish were larger than fish from the overdeveloped group at age-0 and may also have been at the onset of yearling emigration. Size dependent emigration similar to that seen in Twentymile Creek could explain the decrease in frequency of intermediate fish relative to overdeveloped fish between age-0 and age-1, as well as the decrease in the difference in length between those groups (length greater for intermediate fish than for overdeveloped fish at age-0 but not at age-1 because large fish from the intermediate group emigrated in between). Note that even the largest yearling emigrants from Twentymile Creek were too small to be smolts migrating to the ocean (Rubin et al. 2012b; 2012f) and therefore were simply dispersing downstream. The same was likely true of yearling emigrants from NFPR based on the size of yearlings sampled in NFPR in September.

Survival was similar between underdeveloped and intermediate fish in NFPR. This isn't surprising from the standpoint of temperature model predictions showing underdeveloped fish to be at 97.7% of total time to MAWW at release and intermediate fish at 102.5% (Table 1), or in other words, equally distant from MAWW. The accuracy of model predictions of time to MAWW was better than $\pm$1% in a companion study (Rubin et al. 2012c). However, measurements of proportion yolk give a somewhat different picture of developmental stage at release. Proportion yolk at MAWW was $\leq$0.049 in the companion study. By comparison, proportion yolk in the current study was 0.087 for underdeveloped fish at release and 0.044 for intermediate fish (Table 1), suggesting that intermediate fish were closer to MAWW than underdeveloped fish and that underdeveloped fish were less developed than indicated by temperature model predictions. Regardless, our results clearly show a cost to survival and growth in streams from more extreme overdevelopment at release (108% of time to MAWW; 13% weight loss compared to the intermediate group; Table 1).

There probably are also costs associated with more extreme underdevelopment than that of our underdeveloped group; however, penalties may be less for underdevelopment than for equivalent overdevelopment (i.e., equal distance from MAWW). Letcher and Terrick (2001) conducted an experiment similar to ours with Atlantic salmon fry and found that survival from spring release to fall sampling in streams was lower for their accelerated (overdeveloped) group than for their delayed (underdeveloped) or nominal (optimal) groups which survived similarly to each other.

Because we varied incubation temperature to produce differences in development, development effects were potentially confounded with temperature effects. However, temperature differences were small, and temperatures were always within the preferred range for steelhead embryos (Kwain 1975). Further, we found no effect of more extreme incubation temperature differences (5°C) on survival of groups of fry released at the same developmental stage (MAWW) in NFPR (Rubin et al. 2012g). It seems unlikely that incubation temperature differences influenced survival or growth in the current study beyond our intended effect of creating differences in developmental stage at release.

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References

Cone, R. S. 1989. The need to reconsider the use of condition indices in fisheries science. Transactions of the American Fisheries Society 118:510-514.

Cytel Software Corporation. 1997. StatXact-3, Version 3.1. Copyright 1989-1997.

Kwain, W. 1975. Embryonic development, early growth, and meristic variation in rainbow trout (Salmo gairdneri) exposed to combinations of light intensity and temperature. Journal of the Fisheries Research Board of Canada 32:397-402.

Letcher, B. H., and T. D. Terrick. 2001. Effects of Developmental Stage at Stocking on Growth and Survival of Atlantic Salmon Fry. North American Journal of Fisheries Management 21:102-110.

Reisenbichler, R. R., M. C. Hayes, S. P. Rubin, L.A. Wetzel, and B. M. Baker. 2006. *PEPA** genotype affects return rate for hatchery steelhead. Transactions of the American Fisheries Society 135:28-39.

Rubin, S. P., R. R. Reisenbichler, J. E. Hensleigh, L. A. Wetzel, and B. M. Baker. 2012a. Genetic differences between hatchery and wild steelhead for growth and survival in the hatchery and seaward migration after release. Chapter 2 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, J. E. Hensleigh, L. A. Wetzel, B. M. Baker, F. E. Leonetti, K. D. Stenberg, and S. L. Slatton. 2012b. Genetic differences between hatchery and wild steelhead for survival, growth, dispersal, and male maturation in a natural stream. Chapter 3 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, and S. L. Slatton. 2012c. Predicted time from fertilization to maximum wet weight for steelhead alevins based on incubation temperature and egg size. Chapter 4 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, J. E. Hensleigh, F. E. Leonetti, B. M. Baker, S. L. Slatton, and K. D. Stenberg. 2012d. Differences in survival and growth in hatchery and stream environments, and in maturation of residuals in a stream, between progeny of hatchery and wild

steelhead. Chapter 1 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, and K. D. Stenberg. 2012f. Effect of size of unfed fry at release on survival and growth of juvenile steelhead in streams and a hatchery. Chapter 7 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, K. D. Stenberg, and B. M. Baker. 2012g. Effect of incubation temperature on post-embryonic survival and growth of steelhead in a natural stream and a hatchery. Chapter 5 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry, 3rd edition. W. H. Freeman and Company, New York.

Volk, E. C., S. L. Schroder, and K. L. Fresh. 1990. Inducement of unique banding patterns as a practical means to mass-mark juvenile pacific salmon. American Fisheries Society Symposium 7:203-215.

Tables

Table 1. Temperature and duration of incubation, temperature-model-predicted time to maximum alevin wet weight (MAWW), and mean (SD) of fry characteristics 0-1 day before release.

		Days post-fertilization			Fry characteristics			
Group	Mean incubation temperature (°C)	To release	To MAWW[a]	Release/ MAWW	*n*	Fork length (mm)	Weight (g)	Proportion yolk
Underdevel	10.9	47.5	48.6	0.977	60	27.8 (0.8)	0.177 (0.014)	0.087 (0.025)
Intermediate	11.3	47.5	46.3	1.025	60	28.5 (0.7)	0.179 (0.014)	0.044 (0.022)
Overdevel	11.7	47.5	44.0	1.079	60	28.3 (0.7)	0.156 (0.016)	0.030 (0.019)

[a]Predicted by temperature model (Rubin et al. 2012c).

Table 2. Relative survival, length, and condition of juveniles reared at the hatchery. Juveniles were sampled in April, 11 months after ponding and two weeks before release. Letters (x, y, z) indicate significance of paired comparisons: values without a letter in common are different (P<0.05).

Group	*N* or proportion fry ponded	*n* or proportion yearlings sampled	Relative survival	Mean (SD) fork length (mm)	Weight[a] (k[b]) at common length[c]
Underdevel	0.333	0.343	1.00 x	205 (16) x	85.1 (0.98) x
Intermediate	0.330	0.347	1.02 x	206 (15) x	84.9 (0.98) x
Overdevel	0.336	0.310	0.90 x	207 (14) x	84.7 (0.98) x
Total	57830	429			

[a]Weight (in grams) evaluated at common length with weight-length relations from AOCV; slopes always similar among groups (P>0.05).
[b]k=100,000*W/L^3; W=weight at common length; L=common length.
[c]Geometric mean of lengths pooled across groups.

Table 3. Apparent relative survival, length, and condition of juveniles reared in NFPR. Juveniles were sampled four months after release and again one year later (Table 4). Letters (x, y, z) indicate significance of paired comparisons; values without a letter in common are different (P<0.05).

Group	*N* or proportion fry released	*n* or proportion juveniles sampled		Apparent relative survival		Mean (SD) fork length (mm)		Weight[a] (*k*[b]) at common length[c]	
		Age-0	Age-1	Age-0	Age-1	Age-0	Age-1	Age-0	Age-1
Underdevel	0.334	0.374	0.358	1.00 x	1.00 x	70.3 (8.6) xy	121 (13) x	4.00 (1.14) x	21.2 (1.11) x
Intermediate	0.332	0.401	0.312	1.08 x	0.88 x	72.5 (7.6) x	124 (15) x	4.01 (1.14) x	21.2 (1.11) x
Overdevel	0.334	0.226	0.330	0.60 y	0.92 x	69.3 (8.0) y	123 (14) x	4.00 (1.14) x	21.1 (1.11) x
Total	74,478	297	218						

[a]Weight (in grams) evaluated at common length with weight-length relations from AOCV; slopes always similar among groups (P>0.05).
[b]k=100,000*W/L^3; W=weight at common length; L=common length.
[c]Geometric mean of lengths pooled across groups.

Table 4. Dates of electrofishing at sites in NFPR.

Year	Dates	Sites
1996	4-6 Sep	All
1997	12 Aug	One of eight
	17-18 Sep	Seven of eight

Chapter 7: Effect of size of unfed fry at release on survival and growth of juvenile steelhead in streams and a hatchery *(Study sites: Dworshak Hatchery, Silver Creek, and Twenty-Mile Creek; Stock: Dworshak hatchery; Year classes: 1996 and 1999)*

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Abstract

We tested whether differences in size of unfed fry at release affected survival and growth of juvenile steelhead *Oncorhynchus mykiss* in hatchery ponds and streams. Differences in fry size were produced by selecting and spawning females that differed in the mean size of their eggs. Experiments were initiated in 1996 and 1999 with hatchery steelhead returning to the Clearwater River, Idaho. Fry size groups were small (mean fork length=26.7 mm, mean weight=0.149 g) and large (28.1 mm, 0.197 g) in 1996 and small (27.5 mm, 0.159 g), medium (28.2 mm, 0.190 g), and large (28.9 mm, 0.201 g) in 1999. Survival in the hatchery to near the end of the standard one year rearing period and in streams to late summer, three months after release, was higher for the large than for the small group in 1996 but was similar among groups in 1999. Survival in streams to age-1 appeared to show the same pattern (large>small in 1996; no difference in 1999), but differences among fry size groups in emigration as well as mortality may have been involved. The inconsistency between years may have resulted because some 1996 female parents of the small group had exceptionally small eggs and were a year younger than the other 1996 females and all 1999 females. Growth in the hatchery was similar among groups in both years whereas growth in streams was faster for the large than for the small group in both years and intermediate for the medium group in 1999. Growth in streams appeared to be limited by food availability. Initially large fry probably out-competed smaller fry for limited food; however, we found no evidence that dispersal from release sites or emigration from streams was caused by competitive displacement of small by larger fish.

Introduction

This study was undertaken because size of unfed fry at release differed between progeny of hatchery and wild steelhead *Oncorhynchus mykiss* in experiments comparing those groups under natural and hatchery rearing (Rubin et al. 2012a; 2012b). Our goal for the current study was to determine whether initial differences in size affected survival and growth of juveniles independently of any genetic differences between hatchery and wild populations. Accordingly, we manufactured groups of fry that differed in size but were as similar as possible in other respects, and then compared their performance under rearing conditions similar to those of the hatchery-wild study. Fry size differences were created by selecting and spawning females that differed in the mean size of their eggs. Ideally we would have selected different sized eggs from a female and then repeated the process with other females, thus eliminating maternal differences among egg size groups (e.g., Einum and Fleming 1999), but this wasn't feasible due to the large number of eggs needed for our experiments.

Natural rearing comparisons were made in two different study streams. Juveniles in one stream were sampled during summer, two months after release and again one year later. Because outmigrants were not sampled, changes in relative abundance between release and juvenile sampling were the product of survival and emigration differences, which could not be separated. The second stream was more intensively monitored to mimic our earlier comparison of hatchery and wild progeny in that stream (Rubin et al, 2010b). Outmigrants as well as fish residing in the stream were sampled, and population estimates were made for each to differentiate survival from emigration.

We also addressed the following questions, raised by the hatchery-wild comparison study, in the intensively monitored stream:

1. Does initial size of fry affect dispersal from the release site? We hypothesized that if large body size imparts a competitive advantage and competition occurs at release sites due to high densities, then smaller individuals might be forced to disperse.
2. Does size of yearlings affect emigration from the study stream? We hypothesized that competition might cause smaller individuals to emigrate.

Our method of producing differently sized fry (starting with differently sized eggs and then incubating them at a common temperature) resulted in fry that differed in development as well as size because development rate is inversely related to egg size (Rubin et al. 2012c). The effect of differences in developmental stage of fry at release was evaluated in a separate study (Rubin et al. 2012e). Findings from that study were used to assess what portion of the current study's results may have been due to development rather than to size.

Study area

Gametes were obtained from adult steelhead returning to Dworshak National Fish Hatchery, located in Ahsahka, Idaho at the confluence of the North Fork and mainstem Clearwater rivers. Embryo incubation and rearing of progeny allocated to the hatchery environment also occurred there.

Natural rearing occurred in two tributaries of the South Fork Clearwater River, Idaho: Silver and Twentymile creeks. Both streams were devoid of anadromous fish due to impassible falls near their mouths. Fry were released and juveniles recaptured between river kilometers (rkm) 3 and 6 in Silver Creek (midpoint: UTM zone 11, 50-76-400N, 5-95-400E), and 3 and 8.5 in Twentymile Creek (50-68-200N, 5-97-200E). Elevation in the Silver Creek study reach ranged from 1146 m to 1244 m (mean gradient=3.0%). Mean wetted width was 8.3 m in July 2000, and mean of water temperature logged every 4.8 hr from 1 July 1999 to 30 June 2000 was 4.8 ^{o}C. Brook trout *Salvelinus fontinalis* were the only fish present in Silver Creek prior to releasing our study fish. Elevation in the Twentymile Creek study reach ranged from 1317 m to 1540 m (mean gradient=4.1%). Mean wetted width in the reach was 7.6 m in July 1998, and mean water temperature from 1 July 1995 to 30 June 1996 was 3.8 ^{o}C (temperature logging interval ≤4.8 hr). The lower half of the reach meandered through a meadow whereas the upper half consisted primarily of cascades and plunge pools and has a steep gradient (Figure 1). Hybrids of westslope cutthroat *Oncorhynchus clarki lewisi* and rainbow trout *O. mykiss* were the only non-study fish encountered in Twentymile Creek.

Methods

Experimental crosses, embryo incubation, and fry release

On 2 April 1996, eggs were stripped from 48 females, the total weight of 100 eggs was measured for each female, and the females were ranked by egg weight. The 12 females with the lightest eggs and the 12 with the heaviest eggs were used to form small and large fry size groups, respectively (Figure 2; Table 1). Paternal differences between size groups were minimized by crossing each of 12 males with one female from the small group and one female from the large group. The contribution of a male within a group (the proportion of total eggs for that group fertilized by that male) was not equal between groups but rather depended on the fecundity of his mate relative to the fecundity of the other females in the group. About 60 unfertilized eggs from each female were preserved in 10% buffered formalin and >90 days later counted and weighed together to more carefully determine mean egg weight per female. We also measured the fork length of each female.

Embryos from each female were incubated in separate trays in vertical incubators. When embryos reached the eyed stage, live and dead embryos were counted and dead embryos were removed. Each family (i.e., tray) was sampled proportionately to provide an equal number of embryos from each size group for a given rearing environment (family composition equivalent between rearing environments). Embryos were then returned to trays for further incubation. Size groups were placed in separate trays and families were mixed within size groups. Trays with embryos from the large size group were held in separate vertical stacks from trays with the small group to facilitate otolith marking. After all embryos had hatched, otolith marks were thermally induced (Volk et al. 1990) to differentiate the large and small size groups. Two unique banding patterns were created by alternating between ambient (12°C) and cold (7°C) temperatures: narrow-narrow-wide (large group) and wide-narrow-narrow (sma1l group). Dark bands were formed by 1 d exposure to the cold temperature, and lighter intervals between the bands were formed by exposure to either 2 d ambient water (narrow interval) or 4d ambient water (wide interval). Both size groups were marked during the same time period (i.e., cold days at the beginning and end of the marks were the same for both groups). Mean temperature from fertilization to release was 8.6^{o}C for both groups.

The experiment was repeated in 1999 but with a third, medium sized group included. On 6 April, eggs were stripped from 75 females, mean egg weight was determined for each, and nine females with small, nine with average sized, and nine with large eggs were selected (Figure 2; Table 1). Nine males were each crossed with one female from each size group. Eyed embryos to include in the study were selected from each family such that male contribution was identical among groups (i.e., if a male fertilized 10% of the eggs in the small size group he also fertilized 10% in the medium and large groups). Otolith marks used in 1999 were narrow-wide-narrow, wide-narrow-narrow, and narrow-narrow-wide for the small, medium, and large groups, respectively. Mean temperature from fertilization to release was 8.6^{o}C for all groups.

Survival from fertilization to release in streams or hatchery ponds was $\geq$92% for all size groups in both years. Samples of fry were collected from each group for length and weight measurements $\leq$3 days before release. Samples were preserved in 10% buffered formalin, and >90 days later individuals were measured for length, weight, and yolk weight. Proportion yolk was computed as yolk weight divided by whole body weight. Release dates were selected using a temperature model that predicts date of maximum alevin wet weight (MAWW) from incubation temperature (Rubin et al. 2012c). MAWW occurs when yolk absorption is nearly complete and approximately corresponds to when fry would emerge from redds (Jensen 1988). Fry were released within two days of the predicted date of MAWW.

Proportion yolk provided an index of developmental stage of fry at release. We adjusted temperature model-predicted time to MAWW for egg size effects (Rubin et al. 2012c) to provide a second index of developmental stage at release. The units of the index are proportion of total time to MAWW (e.g., 0.9 means 90% of estimated time to MAWW [underdeveloped], 1.1 means 110% of estimated time to MAWW [overdeveloped]). The index was computed by entering mean egg size (from Table 1) into the intermediate equation from Figure 4 of Rubin et al. (2012c). This was an appropriate calculation since mean incubation temperature in the current study (8.6 ^{o}C) was similar to that used to derive the intermediate equation (9.3 ^{o}C; Rubin et al. 2012c).

Dates and numbers of fry released in hatchery rearing vessels and in the study streams are given in Tables 2-4. Fry were placed in each of two tanks at the hatchery in 1996 and in one tank in 1999. Fry were released at two points (rkm 4 and 5) in Silver Creek in both 1996 and 1999. Fry were released in Twentymile Creek in 1999 only. The study reach in Twentymile Creek was divided into 54 contiguous 100 m-long sites. Starting at the upstream end of the reach, fry were released throughout the first two sites, the next two were skipped, the next two were stocked, and the next three were skipped. This pattern

was repeated to the downstream end of the reach. In both Silver and Twentymile creeks, approximately equal numbers of fry from each size group were released at each site.

Hatchery rearing

Juveniles at the hatchery were reared using standard production methods. Fish were initially placed in rectangular tanks indoors and fed by hand until late August-early September, then moved outside into Burrows ponds fitted with demand feeders and held there until release the following April. Each year-class was initially placed in a single outside pond. In October 1996, 80% of the fish of that year-class were marked with fin clips and coded wire tags, and transferred into two ponds with approximately equal numbers of fish in each. The remaining 20% were removed from our experiment and added to production lots at the hatchery. All experimental juveniles of the 1999 year-class were marked with fin clips when moved outside and held in one pond until release from the hatchery. Based on counts of fish marked and subsequent mortalities, survival from placement of fry in tanks to release of juveniles from the hatchery was 90% for the 1996 year-class (94% to marking, 96% from marking to release) and 89% for the 1999 year-class (96%, 93%).

Juveniles were sampled at the hatchery on 16-17 April 1997 and 5 April 2000. Samples were collected from each pond by dipnet after crowding fish to one end. Individuals were euthanized, measured, weighed, and their heads preserved in 95% ethanol for subsequent otolith extraction. Otoliths were processed following Volk et al. (1990). Blind tests with reference specimens collected before fry release, including unmarked controls, indicated 100% accuracy of scoring (n=30 in 1996; n=40 in 1999). Over all collections, including those made in streams (see below), 1.3% of the samples could not be scored because the otoliths were damaged, lost, or unreadable.

Natural rearing

Juveniles in streams were captured by backpack electrofishing during summer two months after release and again one year later (Table 5). Collection methods in Silver Creek differed among years. In 1996, the stream was divided into 43 contiguous 50 m-long sites between rkm 3.4 and 5.5. All odd numbered sites were electrofished, and about 18 of the sub-yearlings captured from each were sampled similarly to juveniles at the hatchery. In 1997, all yearlings captured from the even numbered sites were sampled. In 1999 and 2000 the entire stream between rkm 2.7 and 6 was electrofished. One of every four sub-yearlings captured in 1999 and all yearlings captured in 2000 were sampled.

Electrofishing in Twentymile Creek was oriented around the same 54 sites laid out during fry release (see above). We numbered the sites consecutively starting with the site farthest downstream. In each year, half of the sites were electrofished in sets of two: 1 and 2, 5 and 6, etc., in 1999; 2 and 3, 6 and 7, etc. in 2000. Because gaps of two and three sites were alternated between sites where fry were released but gaps of two sites were maintained between electrofished sites, 50 and 37% of the sites electrofished in 1999 and 2000, respectively, were also fry release sites. This allowed us to test for differences between release and non-release sites. In both 1999 and 2000, at three of every four electrofishing sites a blocknet was placed at the upstream end and one upstream pass was made. At every fourth site blocknets were placed at the upstream and downstream ends and 3-pass removal was conducted. Half of the sub-yearlings captured in 1999 were measured and weighed, and half of those (one quarter of the total number captured) were sampled for otoliths. All of the yearlings captured in 2000 were measured and weighed, and two thirds were sampled for otoliths. In both years, surface area of sites was estimated from site length and the mean width of cross-sectional transects spaced every 25 m.

Removal estimates of population size were made for each 3-pass site with the software Microfish (Van Deventer and Platts 1985). The mean ratio of the removal estimates to the number caught on the first pass (Table 6) was used as an expansion factor to estimate population size in each 1-pass site

(Hankin and Reeves 1988). Estimates in 1- and 3-pass sites were then expanded into an estimate for the entire reach using *N*=54 sites as the expansion factor (Hankin and Reeves 1988).

Downstream migrants in Twentymile Creek were captured with a rotary screw trap (1.5 m diameter cone) (Thedinga et al. 1994) located 100 m upstream from the lower end of the study reach and operated from fry release on 9-10 June until 27 October 1999 and again from 30 March to 17 July 2000. The stream was mostly snow bound from November 1999 through March 2000. Daily mean water temperature was <5 ^{o}C during 28 October-20 November 1999 and <1 ^{o}C during 21 November 1999-29 March 2000. We think it unlikely that fish were actively emigrating under these conditions. The trap was checked a minimum of three and more typically seven days per week. Only 21 fish were captured in 1999, of which 15 were sampled for otoliths. In 2000, one of every four fish captured was sampled for otoliths. The rest of the captured fish were given a caudal fin clip and released 0.4 km above the trap, and recapture rate was used to estimate trap efficiency. Upper and lower caudal clips were alternated daily; 91% of recaptures had clips indicating release an odd number of days earlier. Trap efficiency estimates were temporally stratified with one week as the smallest time increment. When assigning recaptures to week of release, we assumed that odd and even day clips indicated release one and two days earlier, respectively. DARR software (Bjorkstedt 2000) was used to decide when to enlarge a stratum by pooling across two or more consecutive weeks as well as to generate population estimates (total number of outmigrants) for resulting temporal strata.

Less than 1% of the age-0 residents (i.e., fish residing in the study reach upstream from the outmigrant trap) and age-1 migrants had unmarked otoliths (i.e., were not our experimental fish) but 8% of the age-1 residents had unmarked otoliths. Population estimates for age-1 residents, which were based in part on counts of fish captured but not sampled for otoliths, were therefore adjusted to remove the non-experimental fish.

Analyses

Survival of the large relative to the small size group ($RS_{L/S}$) was computed as:

$$(P_{L2}*P_{S1})/(P_{L1}*P_{S2});$$

P_{L1} = proportion of large fish at release, P_{L2} = proportion of large fish at time 2 (i.e., juvenile sampling), P_{S1} = proportion of small fish at release, and P_{S2} = proportion of small fish at time 2. Survival of the medium relative to the small size group was computed similarly. Relative survival was termed "apparent" for naturally reared juveniles because estimates reflected emigration as well as mortality. Relative survival was tested with *G*-tests for goodness of fit (Sokal and Rohlf 1995); expected frequencies were the original number of fry released from each group. Differences in relative frequency between samples of juveniles collected at different times or places (e.g., migrants and residents) were tested with contingency table *G*-tests of independence (Sokal and Rohlf 1995). Exact *P*-values for goodness of fit and independence tests were obtained from StatXact-3 (Cytel Software Corporation 1997). Sequential Bonferroni adjustments were made to account for making three paired comparisons for the 1999 year-class (large versus medium, medium versus small, large versus small; Sokal and Rohlf 1995).

Differences in length were tested with analysis of variance (AOV), again with sequential Bonferroni adjustments to account for multiple comparisons. Condition (weight at length) was tested with analysis of covariance (AOCV) on $\log_{10}$ transformed weight and length. If slopes of the weight-length relation were similar among groups (P>0.05), intercepts were compared among groups using the "common slope" model (i.e., with the cross*length interaction term removed). Table entries were obtained by evaluating weight at a common length (the geometric mean of lengths pooled over groups). Relations from the common slope AOCV were used if slopes were similar, or from the model including the cross*length interaction term if not. Condition factor (k) was computed from weight at common

length and common length. This method avoids the problem of correlations between *k* and length that occur when the exponent of the weight-length relation differs from 3 (Cone 1989).

Size selectivity of capture methods in Twentymile Creek was tested by comparing length between first-time captures released upstream and recaptures (migrant trapping), or between fish caught on pass 1 and those caught on passes 2 and 3 combined (to increase sample size relative to pass 1) at multiple pass sites (resident electrofishing). We note that the test for migrants was less likely to detect size selectivity than the test for residents because recaptured migrants were a subset of first-time captures whereas residents caught on pass 1 were not a subset of residents caught on passes 2 and 3. Capture date was included as a covariate in migrant analyses, and capture site was included as a second factor in resident analyses.

Results

Female length, age, and fecundity

Long-term data on length of known-age returns to Dwoshak Hatchery indicated that most females ≤68 cm and ≥90 cm fork length were three and five years old, respectively (Ralph Roseberg, Idaho Fishery Resource Office, U.S. Fish and Wildlife Service, personal communication). Of the females contributing progeny to our experiments, four were in the age-3 length class and also had small eggs and low fecundity, and two were in the age-5 length class with large eggs and moderate to high fecundity (Figure 3). For the rest of the females (those in the age-4 length class), there were significant correlations between egg weight and female length (r=0.254; P=0.037) and fecundity and length (r=0.503; P<0.001) but not egg weight and fecundity (r=-0.179; P>0.05).

Fry size and developmental stage

Mean length of fry within size groups corresponded to our intentions (small<medium<large; Table 1) but length frequency distributions showed that lengths overlapped considerably among groups (Figure 4). Overlap among groups was less for fry weights than for fry lengths (Figure 4).

Proportion yolk was lower for fry from the small than from the large group (Table 1) indicating a more advanced developmental stage for the former. In 1999, proportion yolk was similar between fry from the medium and large groups indicating similar developmental stages. Percent of time to MAWW indicated that development was more advanced for small-group than for large-group fry and was intermediate for medium fry (Table 1).

Hatchery rearing

Survival was significantly higher for the large than for the small group of the 1996 year-class (Table 2). The relative survival estimate ($RS_{L/S}$=1.21) translated into absolute survivals of 81 and 97% for the small and large groups, respectively, based on 90% absolute survival of the total population (both groups combined) from counts of fish marked and subsequent mortalities. Survival was similar among size groups of the 1999 year-class (Table 2).

Length and condition were similar between size groups of the 1996 year-class, and the same was true among groups for the 1999 year-class (Table 2). There was considerable variation in length of juveniles, but little of it was explained by initial fry size (Figure 5).

Relative survival, size, and condition of the 1996 year-class were consistent between rearing ponds. Relative frequency of the size groups was independent of pond (G-test of independence) and there was no interactive affect of group and pond on fish length or condition (2-way AOVs; P>0.1). The 1999 year-class was reared in a single pond.

Silver Creek

Apparent survival of the 1996 year-class was significantly higher for the large than for the small group at age-0 and at age-1, but apparent survival of the 1999 year-class was similar among size groups at both of those ages (Table 3). Length was always significantly greater for fish from the large than from the small group (true for both ages of both year-classes; Table 3). Length of the medium group was intermediate to that of the other two 1999 year-class groups at both ages (difference significant between medium and large but not between small and medium; Table 3). Condition never differed among size groups (Table 3).

Twentymile Creek

Emigration timing.—Only 21 age-0 migrants were trapped, of which four were from the small group, seven from medium, four from large, and the remainder un-sampled. The low catch indicates negligible emigration of sub-yearlings, consistent with our other studies (Rubin et al. 2012b; 2012d). A much larger number of yearling migrants were trapped, mostly from mid-May through June (Figure 6). Timing of yearling emigration was similar among groups as indicated by similarity of mean capture date (P>0.05; AOV).

Size selectivity of capture methods.—Length of age-1 migrants was similar between first time captures released upstream and recaptures (Table 7), suggesting that the trap was not size selective. At 3-pass electrofishing sites, length of age-0 and of age-1 residents was significantly greater for fish caught on pass 1 than for fish caught on passes 2 and 3 combined (Table 7), indicating that electrofishing selected for large fish.

Population estimate bias.—Migrant and resident population estimates were incompatible in that the estimated number of age-1 migrants was greater than the difference between the estimated numbers of age-0 residents and age-1 residents (Table 4). We suspected that the sharp decline in measured trap efficiency in late June (Figure 6) did not represent a true decrease in efficiency but rather was due to changing behavior of fish released upstream from the trap for efficiency tests. Late in the migration period, some of the fish released upstream may not have resumed their downstream migration. There were no major changes in flow that would cause the trap to operate differently than earlier in the migration (Figure 6). We applied average efficiency before 20 June (52%) to migrants trapped after 20 June to re-estimate total migrants (Table 4); however, there were still too many migrants relative to residents.

Possible explanations for the incompatibility between migrant and resident population estimates were (1) the migrant estimate was positively biased, (2) the age-0 and age-1 resident estimates were negatively biased (increasing each by the same percentage would increase the difference between them), and (3) the age-0 resident estimate was negatively biased relative to the age-1 resident estimate. Results from our previous study in Twentymile Creek (Rubin et al. 2012b) suggested that positive bias of the migrant estimate was moderate, likely no higher than 30% (i.e., true number = estimate/1.3). Bias of resident estimates was likely caused by negative bias of 3-pass removal estimates which recent studies (Peterson et al. 2004; Rosenberger and Dunham 2005) indicate could be considerably greater, perhaps as high as 70% (i.e., true number = estimate/0.3) for yearlings and perhaps even higher for sub-yearlings since negative bias of removal estimates can increase with decreasing fish size (Peterson et al. 2004). Standard statistics generated by the removal estimate software (e.g., probability of capture) were similar between our study (Table 6a) and the two studies just cited, suggesting that 3-pass removal effectiveness was also similar among studies. See Rubin et al. (2012b) for further discussion of population estimate bias.

Size and condition.—Length of age-0 residents differed significantly among all size groups and was greatest for large, intermediate for medium, and least for small-group fish (Table 4). Length of age-1 migrants was similar among size groups, and was positively correlated with capture date ($P<0.001$; slope=0.16 mm/d) but was similar among size groups on any given date ($P=0.27$, AOCV). Note that based on lengths of naturally produced smolts in the Clearwater River drainage (mean fork length during 1993-1999 ranged from 155-189 mm among seven tributaries; Byrne 2001), the age-1 migrants were considerably smaller than is typical for smolts migrating to the ocean. This suggests that the age-1 migrants were simply dispersing downstream. Length of age-1 residents was significantly greater for the large group than for either of the other groups. Condition of age-0 residents was significantly higher for the large group than for either of the other groups (Table 4). Condition was similar among groups for age-1 migrants and for age-1 residents.

It is unlikely that size selectivity of electrofishing exaggerated length differences among fry size groups. A higher proportion of smaller, harder to catch fish were captured at 3-pass sites than at 1-pass sites. When only data from 3-pass sites were used, length differences among groups were similar to those obtained using data from all sites (the same rank order of mean lengths and the same pairs of groups found to differ significantly).

Apparent relative survival.—Apparent survival of age-0 residents was similar among size groups (Table 4). Apparent survival of age-1 migrants differed significantly among all groups and was highest for large, intermediate for medium, and lowest for the small group. Apparent survival of age-1 residents was statistically similar among groups; however, the rank order of apparent relative survival for age-1 residents – large<medium<small – was the inverse of that for migrants, and apparent relative survival differed significantly between age-1 residents and age-1 migrants ($P<0.01$; 2x3 G-test of independence).

Due to size selectivity of electrofishing and persistent size differences among fry size groups, relatively more fish from the large than from the small group were captured on pass 1 than on passes 2 and 3 combined at 3-pass sites (true for sub-yearlings and for yearlings; Table 8). To see how this might have affected apparent relative survival estimates, which were based on data from 1-pass as well as 3-pass sites, we adjusted the proportions of each size group at 1-pass sites according to results from the 3-pass sites. Using sub-yearlings as an example, the proportion of large-group fish caught on all three passes combined was 15% lower (and the proportion of medium- and small-group fish 17% and 7% higher, respectively) than from the first pass alone. We therefore decreased the proportion of large-group fish at 1-pass sites by 15% (and increased the proportion of medium- and small-group fish by 17% and 7%, respectively), and then recombined these adjusted 1-pass site proportions with the original 3-pass site proportions (weighted average; weights were the original number of fish sampled at 1-pass sites [413] and at 3-pass sites [261; Table 9]).

Apparent relative survivals from the adjusted proportions are given in Table 9. Large-group apparent relative survival changed from 1.04 (unadjusted) to 0.92 (adjusted) for sub-yearlings and from 0.85 to 0.77 for yearlings. The latter change accentuated the inverse relation between apparent relative survival for age-1 migrants (large>medium>small) and age-1 residents (large<medium<small).

Relative survival.—Unadjusted apparent survival of age-0 residents was similar among size groups (Table 4), and adjusting for size selectivity of electrofishing didn't appreciably change apparent relative survival (Table 9). Since emigration of sub-yearlings was negligible, apparent relative survival represented true relative survival. We conclude that (1) there was little if any difference among size groups in survival between fry release and age-0 resident sampling, and (2) survival over that interval certainly was not higher for the large than for the small group.

Our ability to estimate relative survival from fry release to age-1 was impaired by population estimate bias of unknown magnitude for age-1 migrants and age-1 residents. The best we could do was to place broad bounds around relative survival. One bound was obtained by assuming no bias of migrant and resident population estimates as well as no mortality for age-1 migrants between emigration (May-July) and age-1 resident sampling (August-September). The total number of age-1 migrants from the small fry size group was computed as the product of the proportion of age-1 migrants from the small group (0.254) and the age-1 migrant population size estimate (6130 which was the more conservative of two estimates; Table 4). The total number of age-1 residents from the small fry size group was computed similarly (proportion from the small fry size group = 0.361; population estimate = 4651; Table 4). The total number of age-1 fish from the small group was computed as the sum small-group age-1 migrants and small-group age-1 residents. The total numbers of age-1 fish from the medium and large fry size groups were computed similarly. Relative survival from fry release to age-1 was then computed using the total number of age-1 fish from each size group. Relative survival computed this way represents a bound because migrant estimates were positively biased, resident estimates negatively biased, and some migrants would have died between emigration and resident sampling; in other words, there could not have been more age-1 migrants and (or) less age-1 residents alive at the end of the interval (age-1 resident sampling in August-September). The other bound was obtained by assuming extreme positive bias of migrant estimates and (or) extreme negative bias of resident estimates. Relative survival to age-1 was computed solely from the total number of age-1 residents from each size group; migrants were excluded. Relative survival computed this way represents a bound because as migrants become less numerous relative to residents (as they would if bias were extreme), the contribution of migrants to the total number of age-1 fish (migrants plus residents) becomes negligible. True relative survival to age-1 fell between these bounds because population estimates were biased but not infinitely so. Note that these bounds on relative survival are not bounds in a statistical sense because they were derived from point estimates of population size for age-1 migrants and age-1 residents and therefore do not account for the variance of the estimates. Even so, the bounds are probably broad enough to include true relative survival given the low variance of the population estimates (CV≤6%; Table 4) and the severe assumptions that the bounds were based on (no estimate bias versus extreme estimate bias).

Relative survival bounds are given in Table 10, including a second set of bounds that was computed using the proportion of age-1 residents from each fry size group after adjustment for size selectivity of electrofishing (Table 9). The upper bounds (those based on migrants and residents combined) showed higher survival to age-1 for large than for small-group fish whereas the lower bounds (those based only on residents) showed the opposite, that survival to age-1 was lower for large than for small-group fish. Thus our results are inconclusive on whether survival to age-1 differed between large and small-group fish. However, the upper bounds indicate that if there was a survival advantage for large over small-group fish to age-1 it was moderate, probably no greater than 20%. Relative survival bounds for medium-group fish were intermediate to those for the other two groups.

Dispersal.—Density of age-0 residents in the lower half of the Twentymile Creek study reach was higher in sites where fry were released than in non-release sites (P=0.01; Figure 7, upper-left panel). Length of age-0 residents in the lower half of the reach was less in release than in non-release sites (Figure 7, middle-left panel; P<0.01), and when the entire study reach was considered, mean length of age-0 residents at a site varied inversely with density (Figure 7, bottom left panel; r=-0.74, P<0.01) suggesting that high density depressed growth. Further, length of age-0 residents in release sites in the lower half of the reach was greatest for large and least for small-group fish (Table 11). Yet despite conditions of high density, slow growth, and greater size for large than for small-group fish in release sites in the lower half of the reach, there was neither a deficit of small-group fish in release sites nor a surfeit of them in non-release sites in the lower half of the reach (Table 11; P=0.7, 2x3 G-test of independence). In other words, there was no evidence that dispersal from release sites was due to competitive displacement of small fish by larger fish. Interestingly, the length differential among groups

(large>medium>small) was present throughout the study reach (Table 11), even in release sites in the upper half of the study reach where densities were lowest and mean lengths greatest (Figure 7). Essentially the same length differential among groups persisted for yearlings throughout the study reach (Table 11).

As noted above, apparent relative survival for age-1 migrants (large>medium>small) was inversely related to apparent relative survival for age-1 residents (large<medium<small; Tables 4 and 9). This difference in apparent relative survival between migrants and residents represented a difference in downstream dispersal among fry size groups. Rate of emigration at age-1 (proportion of total age-1 fish of a given fry size group that emigrated) was highest for large, intermediate for medium, and lowest for small-group fish. The proportion of total age-1 fish of a given fry size group remaining in the study reach upstream from the trap (i.e., the age-1 residents) was therefore lowest for large, intermediate for medium, and highest for small-group fish.

Length of age-0 residents and of age-1 residents was greatest for large, intermediate for medium, and least for small-group fish, and length of age-1 migrants was similar among groups (Table 4); therefore, fish size was greatest for large, intermediate for medium and least for small-group fish at the onset of yearling emigration. This size differential among fry size groups, coupled with the difference in emigration rate among fry size groups, suggests that emigration rate was dependent on fish size. Regardless of fry size group, large individuals emigrated at a higher rate than did small individuals. Emigration rate was higher for the large than for the small fry size group because a higher proportion of large than of small-group fish were large enough to emigrate. Thus our results are inconsistent with competitive displacement of small by larger fish and in fact show the opposite, that large individuals "chose" to disperse downstream to a greater extent than did small individuals.

Discussion

Survival

Survival in the hatchery and apparent survival in Silver Creek were lower for small than for large-group fish of the 1996 year-class (Tables 2 and 3). The difference in apparent survival of the 1996 year-class to age-0 in Silver Creek likely reflected a true survival difference between groups (small<large) rather than differential emigration because emigration of sub-yearlings was found to be negligible for the 1999 year-class in Twentymile Creek and also for three other year-classes of unfed steelhead fry released in comparably sized streams in Idaho for other studies (Rubin et al. 2012b; 2012d). The difference in apparent survival of the 1996 year-class to age-1 in Silver Creek also supported a true survival difference between groups (small<large), although that difference in apparent survival have reflected differential emigration as well as differential mortality.

Survival of the 1999 year-class was similar among size groups in the hatchery and in both study streams. What might have caused the inconsistency between year-classes? One possibility is a difference in egg and resulting fry size between year-classes. Mean egg and fry size for the small group was less in 1996 than in 1999 (Table 1), two of the 1996 female parents had exceptionally small eggs relative to the other 1996 females and all 1999 females (Figures 2 and 3), and the left tail of the fry size distribution for the small group was more pronounced in 1996 than in 1999 (Figure 4). Perhaps the particularly small fry (<0.14 g; Figure 4) from the 1996 year-class experienced poor survival, thereby reducing mean survival for the small group of that year-class.

Alternatively, the inconsistency in relative survival between year-classes may have resulted from maternal differences other than egg size. Four of the 1996 small-group females were short and likely age-3, whereas other 1996 and 1999 females were longer and likely at least age-4 (Figure 3). Perhaps the eggs or fry from the four anomalous 1996 females differed in quality (e.g., chemical composition of yolk,

maternal genetics) from the rest of the 1996 and 1999 eggs and fry. Our results suggest that size of fry had no effect on survival in the hatchery to age-1 or in streams to age-0 (late summer) for the range of fry sizes exhibited by the 1999 year-class; however, fry size and (or) maternal characteristics may have affected survival in those environments to those ages for the broader range of fry sizes and maternal characteristics exhibited by the 1996 year-class (particularly small fry and age-3 female parents in 1996 but not in 1999). Whether fry size affected survival to age-1 in streams is less clear due to uncertainties about the relative contribution of emigration and mortality to apparent relative survival estimates.

Size and growth

The initial size advantage of large over small-group fish was always maintained in streams but it was not maintained in the hatchery. Large-group fish not only maintained their initial size advantage over the small group in streams, but they also grew faster than fish from the small group (Table 12). In both Silver and Twentymile creeks, specific growth rate of the 1999 year-class to age-0 (late summer) was higher for large than for small-group fish. Higher specific growth rate for large than for small-group fish unambiguously indicated faster growth for the former (Elliott 1975). Absolute growth rate of the 1996 year-class to age-0 in Silver Creek was higher for large than for small-group fish but specific growth rate was not (Table 12). In this case, a growth model (e.g., Elliott et al. 1995) is needed to compare growth between fish that differ in initial size; we know of no such model for steelhead. However, specific growth rate in 1996 was only slightly lower for large than for small-group fish, and if mortality that year was higher for particularly small fry than it was for the rest of the small-group fry (see above), then growth rate for small-group fish was overestimated. Growth probably was faster for large than for small-group fish in 1996 as well as in 1999. We did not consider growth rates to age-1 because size of age-1 residents may have been influenced by prior size-dependent emigration.

Growth differences in streams may have resulted from size-related differences in competitive ability. Greater swimming ability due to larger body size may have allowed large-group fish to intercept more food than small-group fish, or social superiority due to larger body size may have allowed large-group fish to occupy better foraging habitat than small-group fish (Fausch 1984; Abbott et al. 1985). The inverse relation between fish size and density in Twentymile Creek (Figure 7) suggests that per capita food availability was limiting growth. Apparently, an initial size advantage conferred greater access to a limited food supply. Food may have been abundant enough in the hatchery to prevent its monopolization by initially large fish. High rations were provided to encourage the fast growth needed to produce smolts in one year.

If food limitation maintains initial size differences, then size differences should decrease with increasing food abundance (Einum and Fleming 1999). In support of this hypothesis, the difference in length between large and small-group sub-yearlings at sites in Silver and Twentymile creeks was negatively correlated with site-specific mean growth rate (Figure 8). Mean growth rate at a site probably reflected per capita food availability at the site. The correlation was not significant in either stream separately but was nearly significant in a combined analysis (r=-0.28; P=0.052; length differences in each stream were standardized [((length difference) - (mean length difference))/(SD length difference)] and then pooled). The correlation couldn't be examined in Silver Creek in 1999 because fish were not captured from discrete sites but rather over the entire length of the study reach (see methods).

Growth was faster in Silver than in Twentymile Creek, and the difference in growth rate among size groups was less in Silver than in Twentymile Creek (Table 12). Part of the reason for faster growth in Silver Creek was higher temperature (mean annual=4.8 ^{o}C in Silver Creek compared to 3.8 ^{o}C in Twentymile Creek); but per capita food availability, either from greater food abundance or lower steelhead density, may also have been higher in Silver Creek, which if true could explain the smaller difference in growth among size groups there compared to Twentymile Creek.

The lack of a difference among size groups in growth at the hatchery indicates that intrinsic growth rate (i.e., growth potential given unlimited food) was similar among groups, which in turn indicates that the females contributing eggs to the different size groups were genetically similar with respect to this trait. Length of age-1 emigrants from Twentymile Creek was similar among groups, suggesting genetic similarity as well. The difference among groups was in the frequency of fish large enough to emigrate, not in emigration behavior. It seems that differences among groups in size and growth in streams were primarily due to fry size differences rather than to genetic differences among female parents or qualitative differences among their eggs.

In summary, the effect of initial size differences on subsequent growth varied widely among rearing environments and inferred levels of food availability, from no effect in the hatchery where food was abundant to a big effect at slow-growth sites in Twentymile Creek where limited food availability strongly favored initially large fish. Our results agree with those of Einum and Fleming (1999) who proposed and demonstrated that the effect of initial size differences is context specific.

Dispersal

Dispersal of sub-yearlings from release sites in the lower half of the Twentymile Creek study reach was not related to initial size or to growth during the first summer. Competitive displacement of small by larger fish was not apparent despite conditions of high density and depressed growth that promoted competition for food. Higher density of sub-yearlings in release than in non-release sites in the lower half of the study reach was presumably due to a tendency for fish to remain near where they were released. The pattern of higher age-0 density in release than in non-release sites was less apparent in the upper half of the study reach (Figure 7), possibly because the steep gradient (Figure 1) caused fry to be washed downstream.

Emigration of yearlings from Twentymile Creek was size dependent, but emigrants were larger on average than fish that chose not to emigrate, indicating that emigration was not caused by competitive displacement of small by larger fish. The emigrants likely were simply dispersing downstream since they were much smaller than naturally produced smolts in the Clearwater River drainage (Byrne 2001). Downstream movement of yearling pre-smolts during May-June has been observed previously for fry we released for other studies (Rubin et al. 2012b; 2012d) and for natural populations in the Clearwater River basin (Holubetz and Leth 1997; Byrne 2005).

In Twentymile Creek, a higher proportion of large than of small-group fish emigrated (age-1 migrants), leaving a deficit of large and a surfeit of small-group fish above the trap (age-1 residents; Tables 4 and 9). Size differences among groups were the same in Silver as in Twentymile Creek (large>medium>small), yet in Silver Creek the frequency of large relative to small-group fish did not decrease from age-0 to age-1 residents (Table 3). This suggests that the pattern of emigration in Silver Creek differed from that in Twentymile Creek. Possibilities for Silver Creek were: (1) a lower proportion of total yearlings emigrated so no change in relative frequency of the size groups between age-0 and age-1 residents was apparent, (2) emigration rate was similar among groups, or (3) differential mortality as well as differential emigration was involved (e.g., greater emigration of large than of small-group fish was counterbalanced by lower mortality for large than for small-group fish). Although there is no way to sort out which of these possibilities occurred, we suspect that higher temperature and faster growth in Silver Creek led to (1) or (2).

Size versus developmental stage of fry

In addition to being smaller, small-group fry were more developmentally advanced than large-group fry at release (Table 1); however, effects on juvenile survival and growth likely were primarily due to size rather than to developmental stage differences. Proportion yolk was $\geq$0.047 for all size groups of

fry (Table 1). A companion study evaluating the effect of developmental stage of fry at release on juvenile survival and growth found no effect for proportion yolk ≥0.044 (Rubin et al. 2012e). Results for percent of time to MAWW, our other index of developmental stage, were less clear cut since small-group fry were released at percentages that may have affected survival and growth. Small-group fry were released at 105% of time to MAWW in 1996 and 104% in 1999. The companion study found that survival and growth in a stream were lower for fry released at 108% of time to MAWW than at ≤103%. The difference in growth between large and medium-group fish in the current study was clearly due to fry size differences rather than to differences in development since proportion yolk and percent of time to MAWW for the medium and large groups were outside of ranges found to affect juvenile growth in the companion study.

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References

Abbott, J. C., R. L. Dunbrack, and C. D. Orr. 1985. The interaction of size and experience in dominance relationships of juvenile steelhead trout (*Salmo gairdneri*). Behaviour 92:241-253.

Bjorkstedt, E. P. 2000. DARR (Darroch Analysis with Rank-Reduction): A method for analysis of stratified mark-recapture data from small populations, with application to estimating abundance of smolts from outmigrant trap data. National Marine Fisheries Service, Administrative Report SC-00-02.

Busacker, G. P., I. R. Adelman, and E. M. Goolish. 1990. Growth. Pages 363-388 in C. B. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

Byrne, A. 2001. Idaho Fish and Game, fishery research - steelhead supplementation in Idaho rivers summary report 1 January 1993 - 31 December 1999. Bonneville Power Administration Report DOE/BP-01466-6. Portland, OR.

Byrne, A. 2005. Steelhead supplementation studies: Steelhead supplementation in Idaho Rivers, 2004 annual report. Project No. 199005500, 92 electronic pages, BPA Report DOE/BP-00020874-1.

Cone, R. S. 1989. The need to reconsider the use of condition indices in fisheries science. Transactions of the American Fisheries Society 118:510-514.

Cytel Software Corporation. 1997. StatXact-3, Version 3.1. Copyright 1989-1997.

Einum, S., and I. A. Fleming. 1999. Maternal effects of egg size in brown trout (Salmo trutta): norms of reaction to environmental quality. Proceedings of the Royal Society of London, Series B: Biological Sciences 266:2095-2100.

Elliott, J. M. 1975. The growth rate of brown trout (*Salmo trutta* L.) fed on maximum rations. Journal of Animal Ecology 44:805-821.

Elliott, J. M., M. A. Hurley, and R. J. Fryer. 1995. A new, improved growth model for brown trout, *Salmo trutta*. Functional Ecology 9:290-298.

Fausch, K. D. 1984. Profitable stream positions for salmonids: Relating specific growth rate to net energy gain. Canadian Journal of Zoology 62:441-451.

Hankin, D. G., and G. H. Reeves. 1988. Estimating total fish abundance and total habitat area in small streams based on visual estimation methods. Canadian Journal of Fisheries and Aquatic Sciences 45:834-844.

Holubetz, T. B., and B. D. Leth. 1997. Evaluation and monitoring of wild/natural steelhead trout production, annual progress report. Period covered: January 1, 1995-December 31, 1995. Report to Bonneville Power Administration, Department of energy, Portland, OR; contract number DE-BI79-91BP21182.

Jensen, J. O. T. 1988. A microcomputer program for predicting embryonic development in Pacific salmon and steelhead trout. World Aquaculture 19:80-81.

Peterson, J. T., R. F. Thurow, and J. W. Guzevich. 2004. An evaluation of multipass electrofishing for estimating the abundance of stream-dwelling salmonids. Transactions of the American Fisheries Society 133:462-475.

Rosenberger, A. E., and J. B. Dunham. 2005. Validation of abundance estimates from mark recapture and removal techniques for rainbow trout captured by electrofishing in small streams. North American Journal of Fisheries Management 25:1395-1410.

Rubin, S. P., R. R. Reisenbichler, J. E. Hensleigh, L. A. Wetzel, and B. M. Baker. 2012a. Genetic differences between hatchery and wild steelhead for growth and survival in the hatchery and seaward migration after release. Chapter 2 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, J. E. Hensleigh, L. A. Wetzel, B. M. Baker, F. E. Leonetti, K. D. Stenberg, and S. L. Slatton. 2012b. Genetic differences between hatchery and wild steelhead for survival, growth, dispersal, and male maturation in a natural stream. Chapter 3 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, and S. L. Slatton. 2012c. Predicted time from fertilization to maximum wet weight for steelhead alevins based on incubation temperature and egg size. Chapter 4 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, J. E. Hensleigh, F. E. Leonetti, B. M. Baker, S. L. Slatton, and K. D. Stenberg. 2012d. Differences in survival and growth in hatchery and stream environments, and in maturation of residuals in a stream, between progeny of hatchery and wild steelhead. Chapter 1 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Rubin, S. P., R. R. Reisenbichler, L. A. Wetzel, and K. D. Stenberg. 2012e. Effect of developmental stage of unfed fry on survival and growth of steelhead released in a stream and in hatchery ponds. Chapter 6 *in* S. P. Rubin, R. R. Reisenbichler, L. A. Wetzel, and M. C. Hayes, editors. Genetic Differences in Growth, Migration, and Survival between Hatchery and Wild Steelhead and Chinook Salmon. Final Report to the Bonneville Power Administration, Interagency Agreement No. DE-AI79-91BP17760, Project No. 90-052, Portland, Oregon.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry, 3rd edition. W. H. Freeman and Company, New York.

Thedinga, J. F., M. L. Murphy, S. W. Johnson, J. M. Lorenz, and K. V. Koski. 1994. Determination of salmonid smolt yield with rotary-screw traps in the Situk River, Alaska, to predict effects of glacial flooding. North American Journal of Fisheries Management 14:837-851.

Van Deventer, J. S., and W. S. Platts. 1985. Computer software system for entering, managing, and analyzing fish capture data from streams. Research Note, Intermountain Research Station, USDA Forest Service, Ogden, Utah.

Volk, E. C., S. L. Schroder, and K. L. Fresh. 1990. Inducement of unique banding patterns as a practical means to mass-mark juvenile pacific salmon. American Fisheries Society Symposium 7:203-215.

Tables

Table 1. Mean (SD) of preserved egg weights, and of fry lengths, weights, and proportion yolk ≤3 days before release. Also, the ratio between temperature model-predicted time to maximum alevin wet weight (MAWW) and egg size-specific time to MAWW, which is approximately the ratio of time between fertilization and release and time between fertilization and MAWW (see text). The ratio is expressed as a percentage.

		Eggs		Fry				
Year	Group	*n*[a]	Weight (g)[b]	*n*	Fork length (mm)	Weight (g)	Proportion yolk[c]	Percent of time to MAWW[d]
1996	Small	12	0.089 (0.008)	80	26.7 (0.7)	0.149 (0.016)	0.063 (0.032)	104.9
	Large	12	0.123 (0.006)	80	28.1 (0.8)	0.197 (0.011)	0.109 (0.030)	98.7
1999	Small	9	0.093 (0.005)	60	27.5 (0.7)	0.159 (0.013)	0.047 (0.029)	104.1
	Medium	9	0.110 (0.005)	60	28.2 (1.3)	0.190 (0.019)	0.117 (0.040)	101.0
	Large	9	0.124 (0.005)	60	28.9 (1.1)	0.201 (0.014)	0.108 (0.043)	98.5

[a]Number of females.
[b]Mean (SD) of mean egg weight per female.
[c]Yolk weight divided by whole body weight.
[d]This value was generated by entering mean egg weight from this table into the "mean" equation from Figure 4 of Rubin et al. (2012c).

Table 2. Relative survival, length, and condition of juveniles reared at Dworshak Hatchery. Juveniles were sampled in April, two weeks before release and about 10 months after ponding. Letters (x, y, z) indicate significance of paired comparisons; values without a letter in common are different (P<0.05).

Year-class	Date fry were ponded	Group	*N* or proportion fry ponded	*n* or proportion yearlings sampled	Relative survival	Mean (SD) fork length (mm)	Weight[a] (k[b]) at common length[c]
1996	4 Jun	Small	0.500	0.453	1.00 x	197 (18) x	74.5 (0.98) x
		Large	0.500	0.547	1.21 y	198 (16) x	75.0 (0.99) x
		Total	58,196	519			
1999	7 Jun	Small	0.331	0.342	1.00 x	180 (22) x	55.3 (0.96) x
		Medium	0.333	0.348	1.01 x	179 (19) x	55.2 (0.96) x
		Large	0.336	0.310	0.89 x	182 (23) x	54.7 (0.95) x
		Total	29,712	494			

[a]Weight (in grams) evaluated at common length with weight-length relations from AOCV; slopes always similar among groups (P>0.05).
[b]k=100,000*W/L^3; W=weight at common length; L=common length.
[c]Geometric mean of lengths pooled across groups.

Table 3. Apparent relative survival, length, and condition of juveniles in Silver Creek. Juveniles were sampled two months after release and again one year later (Table 5). Letters (x, y, z) indicate significance of paired comparisons; values without a letter in common are different (P<0.05).

Year-class	Release date	Group	*N* or proportion fry released	*n* or proportion juveniles sampled		Apparent relative survival		Mean (SD) fork length (mm)		Weight[a] (k[b]) at common length[c]	
				Age-0	Age-1	Age-0	Age-1	Age-0	Age-1	Age-0	Age-1
1996	5 Jun	Small	0.500	0.437	0.419	1.00 x	1.00 x	69.5 (5.5) x	114 (9) x	4.31 (1.23) x	[d]
		Large	0.500	0.563	0.581	1.29 y	1.39 y	72.4 (5.5) y	120 (10) y	4.31 (1.23) x	[d]
		Total	77,059	382	258						
1999	8 Jun	Small	0.341	0.340	0.341	1.00 x	1.00 x	60.3 (4.4) x	115 (10) x	2.53 (1.09) x	19.1 (1.19) x
		Medium	0.312	0.280	0.299	0.90 x	0.96 x	60.6 (3.9) x	117 (11) x	2.68 (1.15) x	19.3 (1.20) x
		Large	0.348	0.380	0.361	1.10 x	1.04 x	63.6 (3.8) y	121 (11) y	2.65 (1.14) x	19.3 (1.20) x
		Total	66,119	100	549						

[a]Weight (in grams) evaluated at common length with weight-length relations from AOCV; slopes always similar among groups (P>0.05).
[b]k=100,000*W/L^3; W=weight at common length; L=common length.
[c]Geometric mean of lengths pooled across groups.
[d]Weight not measured.

Table 4. Apparent relative survival, length, and condition of 1999 year-class juveniles in Twentymile Creek. Fry were released on 9-10 June. Residents were sampled two months after release and again one year later (Table 5). Migrants were sampled during April-July 2000 (Figure 6). Letters (x, y, z) indicate significance of paired comparisons; values without a letter in common are different (P<0.05).

			Group			
Variable/statistic	Age	Sub-population	Small	Medium	Large	Total
N or proportion released	0	Fry	0.331	0.333	0.336	39,155
Estimated number (SE) in sub-population	0	Residents				8801 (581)
	1	Migrants				8327 (351)[a]
						6130 (127)[b]
		Residents				4651 (266)
n or proportion juveniles sampled	0	Residents	0.323	0.334	0.343	674
	1	Migrants	0.254	0.327	0.420	796
		Residents	0.361	0.327	0.312	830
Apparent relative survival	0	Residents	1.00 x	1.03 x	1.04 x	
	1	Migrants	1.00 x	1.28 y	1.63 z	
		Residents	1.00 x	0.90 x	0.85 x	
Mean (SD) fork length (mm)	0	Residents	58.0 (5.1) x	60.3 (4.9) y	62.6 (4.5) z	
	1	Migrants	83.9 (8.7) x	84.6 (8.3) x	84.5 (7.5) x	
		Residents	100.6 (10.4) x	101.0 (10.1) x	106.8 (9.4) y	
Weight[c] (k[d]) at common length[e]	0	Residents	2.31 (1.06) x	2.31 (1.06) x	2.41 (1.11) y	
	1	Migrants	5.94 (1.00)[f]	5.94 (1.00)[f]	5.91 (0.99)[f]	
		Residents	12.1 (1.12) x	12.1 (1.13) x	12.1 (1.13) x	

[a]Trap efficiencies measured during 20 Jun-17 Jul were used in estimate.
[b]Estimate made by applying mean of trap efficiencies measured before 20 Jun to the 20 Jun-17 Jul time period; see text for details.
[c]Weight (in grams) evaluated at common length with weight-length relations from AOCV; slopes similar among groups (P>0.05) unless otherwise indicated.
[d]k=100,000*W/L^3; W=weight at common length; L=common length.
[e]Geometric mean of lengths pooled across groups.
[f]Slopes of weight-length relations differ among groups (P=0.038); paired comparisons not made.

Table 5. Dates of electrofishing at sites in study streams. Sites were numbered from downstream to upstream.

Stream	Year	Dates	Sites
Silver Cr	1996	20-24 Aug	All
	1997	6-11 Aug	All
	1999	3-8 Aug	All
	2000	11-16 Jul	All
Twentymile Cr	1999	18-24 Aug	1-22
		31 Aug-6 Sep	25-54
	2000	22-28 Aug	1-35
		6-8 Sep	38-54

Table 6. Statistics pertaining to the precision and accuracy of 3-pass removal estimates in Twentymile Creek, and the expansion factor used to estimate population size in sites where only one pass was made. *N*hat=removal estimate.

Statistic	Year	
	1999	2000
Number of 3-pass sites	8	8
Mean probability of capture[a]	0.450	0.514
Mean ratio of total 3-pass catch to *N*hat	0.832	0.883
Mean ratio of *N*hat to first pass catch	2.13	1.95
SE(ratio)	0.11	0.15

[a]Computed by removal estimate software.

Table 7. Comparison of mean length in Twentymile Creek between migrants released upstream and recaptured in the trap or between residents captured on pass 1 (at 3-pass sites) and those captured on passes 2 and 3 combined. *P* = *P*-value of AOCV (migrants, covariate capture date) or 2-way AOV (residents, second factor capture site) test for difference in mean length; values <0.05 are bolded. The interaction between group and date (migrants) or between group and site (residents) was never significant (*P*>0.1)

Sub-population	Age	Mean (SD) [*n*] of fork length (mm)		Percent difference in means[a]	*P*
		Released or pass 1	Recaptured or passes 2-3		
Migrants	1	85.09[b] (8.30) [2208]	84.77[b] (8.20) [936]	-0.4	0.32
Residents	0	62.47[b] (4.10) [269]	61.59[b] (4.39) [264]	-1.4	**0.03**
	1	101.3[b] (13.0) [332]	98.7[b] (12.3) [230]	-2.6	**0.03**

[a]100*(recaptured-released)/released, or 100*(passes 2-3-pass 1)/pass 1.
[b]Least square mean.

Table 8. The proportion of fish from the large group captured on pass 1 and on passes 2 and 3 combined at 3-pass sites in Twentymile Creek. *P* = *P*-value of 2x2 *G*-test of independence for a difference in the proportion of large fish between pass 1 and passes 2-3; values <0.05 are bolded.

	Proportion large fish[a] (*n*)		*P*
Age	Pass 1	Passes 2-3	
0	0.573 (96)	0.457 (92)	0.14
1	0.540 (124)	0.442 (86)	0.21
Total	0.555 (220)	0.449 (178)	**0.04**

[a]Large/(large + small).

Table 9. Apparent relative survival in Twentymile Creek unadjusted (i.e., from Table 4) and adjusted for size selectivity of electrofishing. See text for explanation of adjustment method.

			n or proportion sampled				Apparent relative survival		
Age	Site type	Pass	Small	Medium	Large	Total	Small	Medium	Large
0	1-pass	1	0.308	0.368	0.324	413			
	3-pass	1	0.325	0.238	0.437	126			
		2-3	0.370	0.319	0.311	135			
		Total	0.349	0.280	0.372	261			
	Total	1	0.312	0.338	0.351	539			
		2-3	0.370	0.319	0.311	135			
		Total	0.323	0.334	0.343	674	1.00	1.03	1.04
		Adjusted total	0.330	0.364	0.307		1.00	1.10	0.92
1	1-pass	1	0.363	0.350	0.287	537			
	3-pass	1	0.339	0.262	0.399	168			
		2-3	0.384	0.312	0.304	125			
		Total	0.358	0.283	0.358	293			
	Total	1	0.357	0.329	0.313	705			
		2-3	0.384	0.312	0.304	125			
		Total	0.361	0.327	0.312	830	1.00	0.90	0.85
		Adjusted total	0.370	0.340	0.290		1.00	0.91	0.77

Table 10. Relative survival to age-1 in Twentymile Creek computed from migrants and residents combined or from residents alone, using resident estimates that were either unadjusted or adjusted for size selectivity of electrofishing. True relative survival was between combined and residents-only values (see text).

Resident adjustment	Computational method	Relative survival		
		Small	Medium	Large
None	Combined	1.00	1.08	1.22
	Residents only	1.00	0.90	0.85
For size selection	Combined	1.00	1.09	1.18
	Residents only	1.00	0.91	0.77

Table 11. Proportion and length for each group in release and non-release sites in the lower and upper sub-reach in Twentymile Creek (Figure 7). Within a row, mean lengths without a letter in common differ (*P*<0.05; 1-way AOVs with sequential Bonferonni adjustments for paired comparisons).

	Sub-	Release		Proportion			Mean (SD) fork length (mm)			
Age	reach	site	*n*	Small	Medium	Large	Small	Medium	Large	Total
0	Lower	Yes	290	0.338	0.341	0.321	55.4 (4.4) x	57.5 (4.2) y	60.4 (4.3) z	57.7 (4.7)
		No	164	0.335	0.311	0.354	60.4 (4.7) x	62.8 (3.9) y	63.9 (3.7) y	62.4 (4.3)
		Total	454	0.337	0.330	0.333	57.2 (5.1)	59.3 (4.8)	61.7 (4.4)	59.4 (5.1)
	Upper	Yes	62	0.194	0.419	0.387	61.8 (5.4) x	64.5 (3.3) xy	65.4 (4.3) y	64.3 (4.3)
		No	158	0.335	0.310	0.354	59.4 (4.2) x	61.0 (4.6) x	63.8 (4.2) y	61.5 (4.7)
		Total	220	0.295	0.341	0.364	59.8 (4.5)	62.2 (4.5)	64.3 (4.2)	62.3 (4.7)
1	Lower	Yes	209	0.311	0.368	0.321	97.5 (9.5) x	99.4 (10.3) x	104.5 (8.9) y	100.4 (10.0)
		No	360[a]	0.433	0.300	0.267	105.1 (9.7) x	104.8 (9.6) x	108.5 (8.4) y	105.9 (9.5)
		Total	569[a]	0.388	0.325	0.286	102.9 (10.2)	102.5 (10.2)	106.8 (8.8)	103.9 (10.0)
	Upper	Yes	52	0.308	0.288	0.404	92.2 (5.9) x	97.4 (8.3) xy	104.4 (12.1) y	98.6 (10.7)
		No	209[b]	0.301	0.340	0.359	94.6 (8.1) x	97.9 (9.2) x	107.5 (9.8) y	100.4 (10.6)
		Total	261[b]	0.303	0.330	0.368	94.1 (7.8)	97.8 (9.0)	106.8 (10.4)	100.0 (10.6)

[a]1 less small, 1 less medium, and 1 less large-group fish for lengths.
[b]1 less small-group fish for lengths.

Table 12. Growth rate in streams from release to age-0 sampling. Rates were computed from mean length at release (Table 1) and at age-0 (Tables 3 and 4), and from release dates and mean capture dates using standard formulas (Busacker et al. 1990).

			Growth rate	
Stream	Year	Group	Absolute (mm/d)	Specific (%/d)
Silver Cr	1996	Small	0.548	1.226
		Large	0.566	1.211
	1999	Small	0.558	1.335
		Medium	0.552	1.305
		Large	0.589	1.339
Twentymile Cr	1999	Small	0.394	0.965
		Medium	0.410	0.971
		Large	0.431	0.989

Figures

Figure legends

Figure 1. Characteristics of the Twentymile Creek study reach. Wetted width was measured at 20 m intervals, depth and substrate at 0.25, 0.5, and 0.75 of width at each transect, and gradient between each pair of transects. Substrate particle size in a 1 m^2 area was indexed as 1 (sand, <2 mm), 2 (gravel, 2-64 mm), 3 (cobble 64-256 mm), or 4 (boulders, >256 mm) according to which size class covered the most area. Means were computed for each of 54 approximately 100 m long contiguous sites and plotted against the midpoint of the site. Also plotted are counts of boulders (>0.5 m diameter) and pieces of large woody debris (LWD; length>1 m and diameter>0.1 m) at least partially submerged in each site. All measurements were made in July 1998.

Figure 2. Frequency of females contributing eggs to the experiments by mean egg weight (from measurements of fresh eggs). Small, medium, and large refer to fry size groups. Text in upper right of panels indicates return year.

Figure 3. Mean egg weight (from measurements of preserved eggs) and fecundity versus length of females contributing eggs to the experiments. Vertical dashed lines are divisions between age-3, age-4, and age-5 females based on length of known age returns to Dworshak Hatchery

Figure 4. Length and weight distributions of fry $\leq$3 days before release. Top panels are for 1996 and bottom panels 1999. Within a size group, relative frequency sums to 1.

Figure 5. Length frequency of juveniles reared at Dworshak Hatchery. Juveniles were sampled in April, two weeks before release and about 10 months after being ponded and started on feed. Text in upper right of panels indicates year-class. Within a size group, bar heights sum to 1.

Figure 6. Weekly catch of age-1 migrants in the Twentymile Creek screw trap. Trap efficiency and water level near the trap are included. Total trapped=total number of fish trapped; small sampled=number of small-group fish (multiplied by four to improve figure appearance) in the sample of fish collected for otolith analysis (note that we only sampled ¼ of the total number of fish trapped); same for medium sampled and large sampled.

Figure 7. Density and mean length at sites where fry were released (Yes) or not (No) in Twentymile Creek. Left panels are for sub-yearlings and right for yearlings. The dotted line in upper panels divides the reach into lower and upper sub-reaches. The lowest panels depict mean length versus density by site type and sub-reach.

Figure 8. Length difference between large-group and small-group age-0 parr versus mean (across all size groups) growth rate at study stream sites. Plotted values were computed from mean lengths; sites were included only if >2 individuals from each size group were sampled.

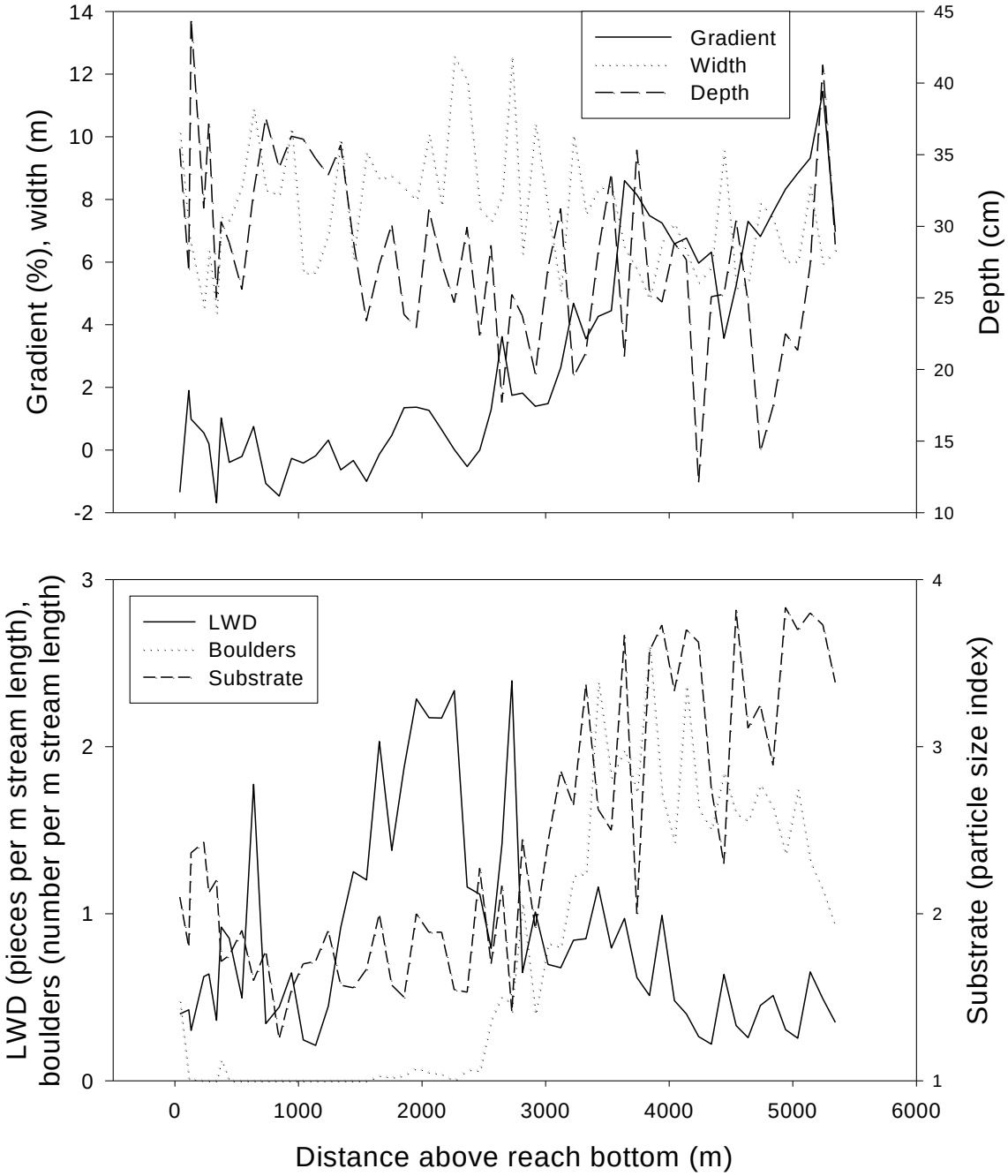
Gradient
Width
Depth
Gradient (%), width (m)
Depth (cm)
LWD
Boulders
Substrate
LWD (pieces per m stream length), boulders (number per m stream length)
Substrate (particle size index)
Distance above reach bottom (m)

Figure 1.

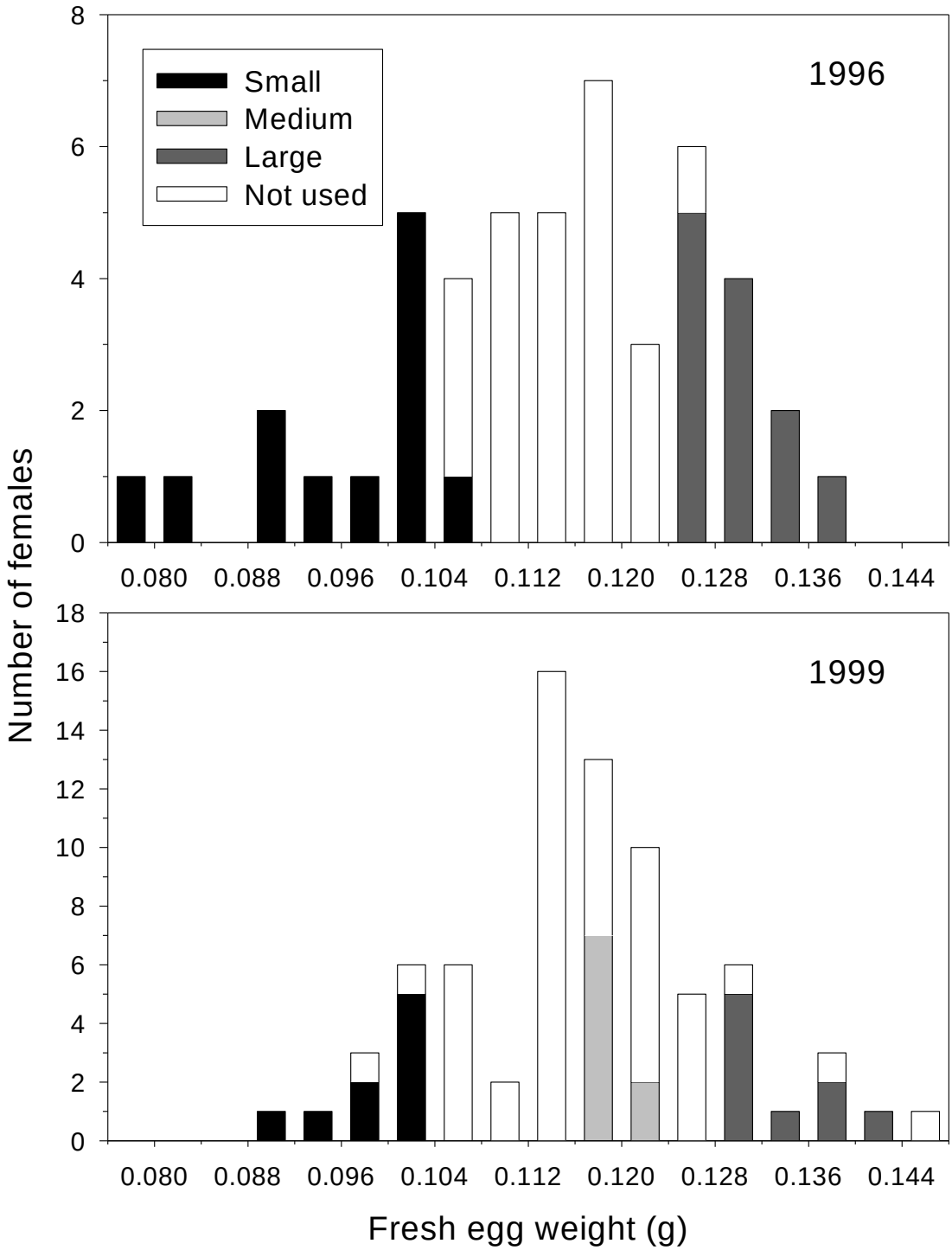
Small
Medium
Large
Not used
1996
1999
Number of females
Fresh egg weight (g)
0
2
4
6
8
10
12
14
16
18
0.080
0.088
0.096
0.104
0.112
0.120
0.128
0.136
0.144

Figure 2.

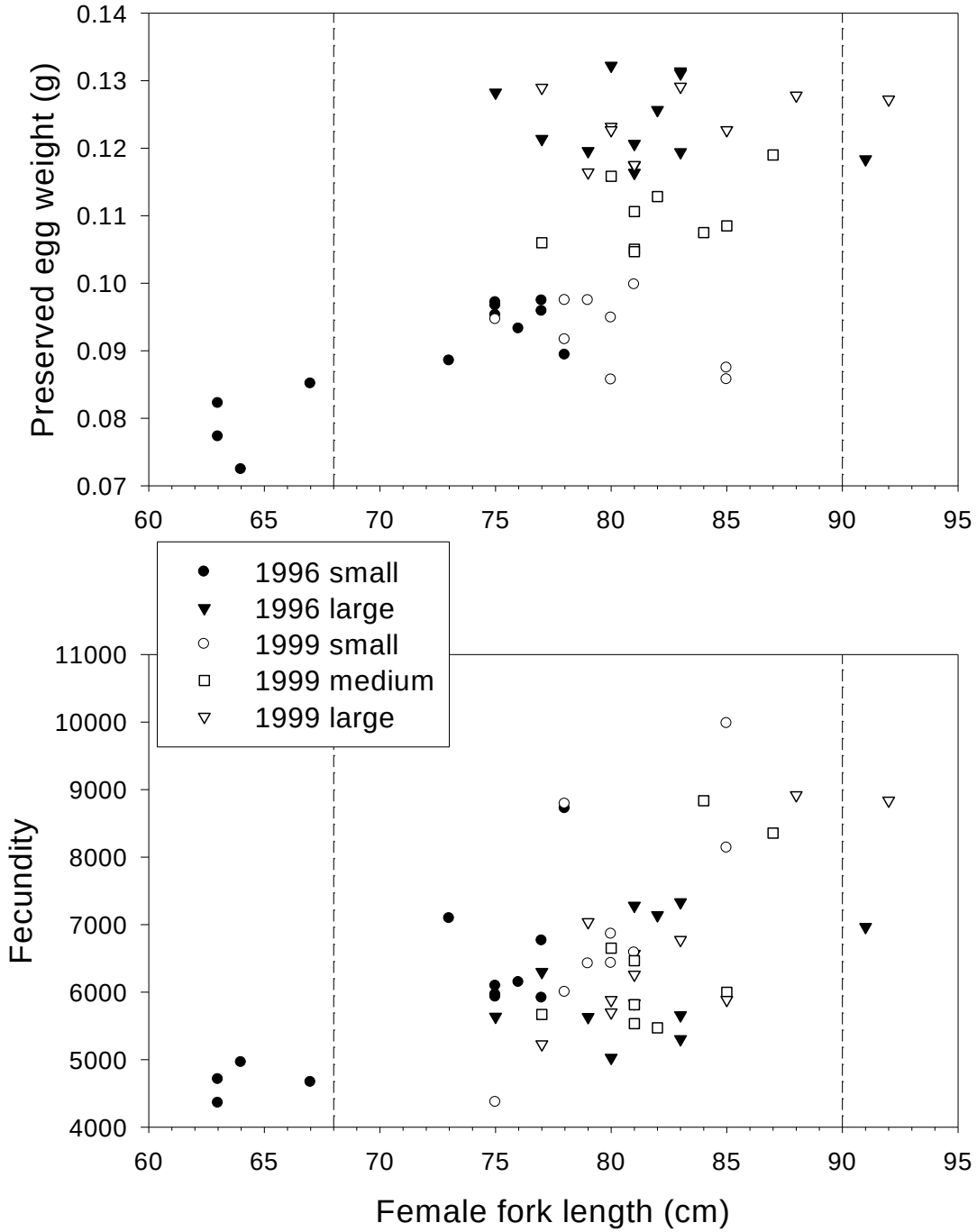
Preserved egg weight (g)
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
60
65
70
75
80
85
90
95
1996 small
1996 large
1999 small
1999 medium
1999 large
Fecundity
11000
10000
9000
8000
7000
6000
5000
4000
Female fork length (cm)

Figure 3.

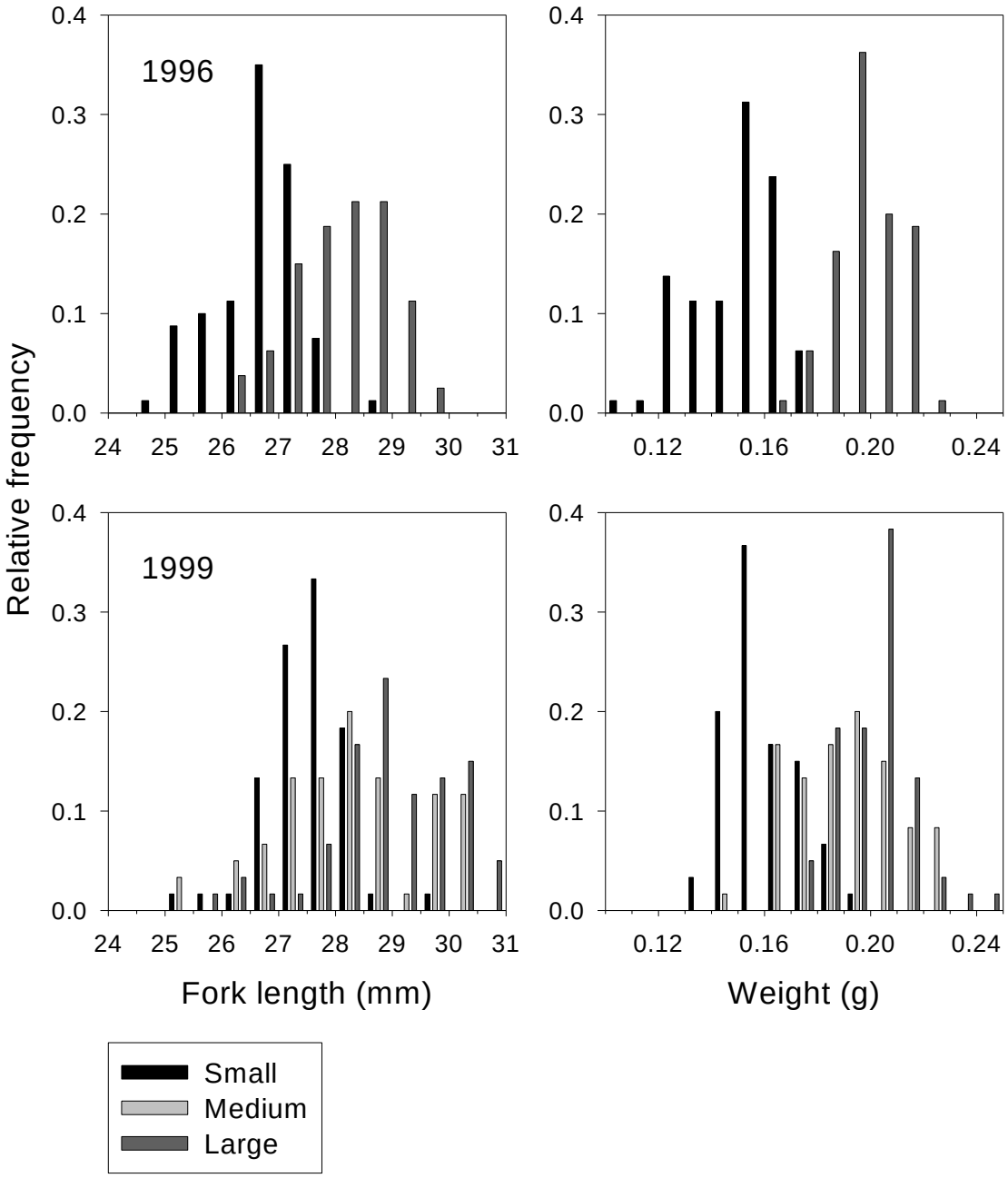
1996
1999
Relative frequency
0.0
0.1
0.2
0.3
0.4
24
25
26
27
28
29
30
31
0.12
0.16
0.20
0.24
Fork length (mm)
Weight (g)
Small
Medium
Large

Figure 4.

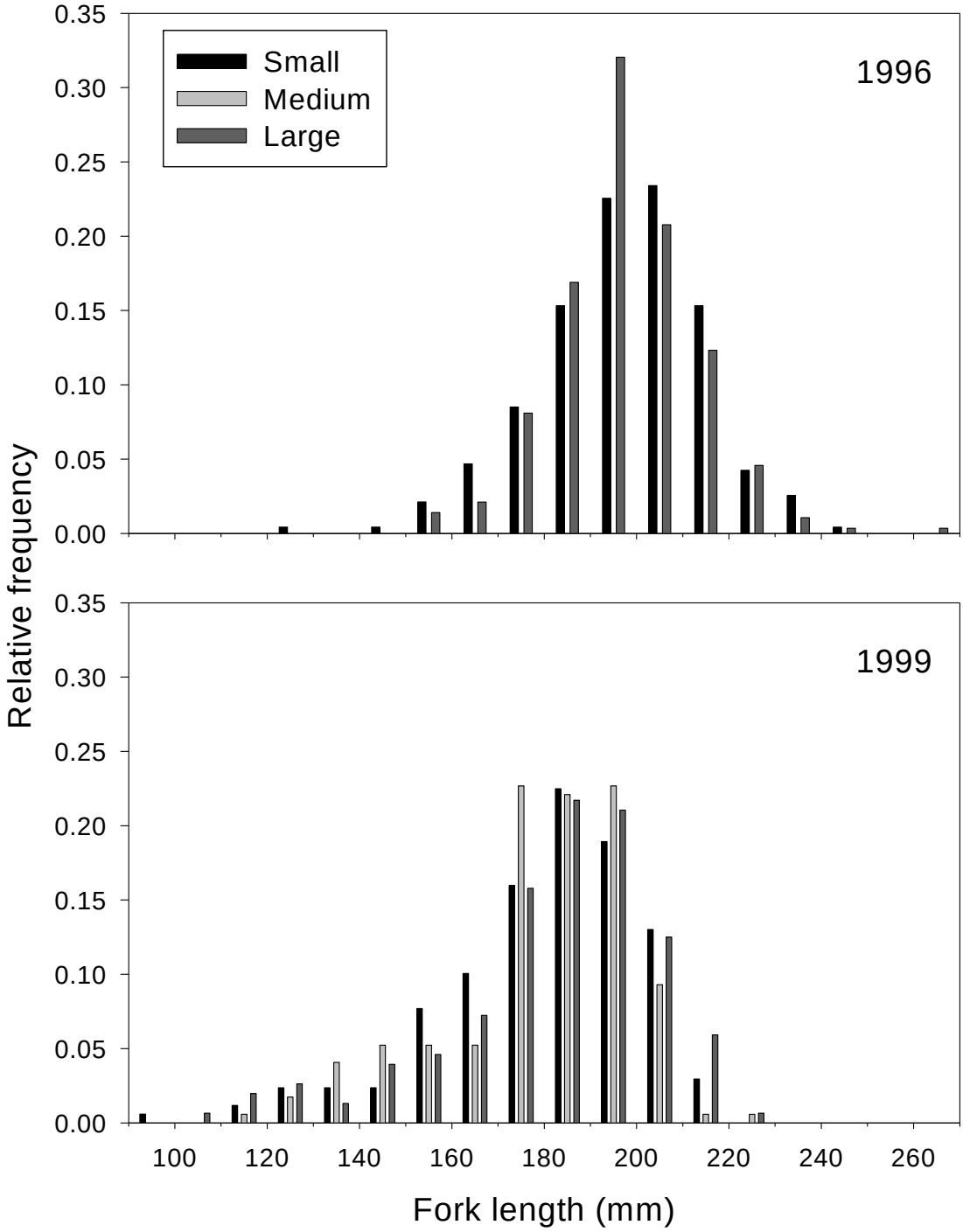
Small
Medium
Large
1996
1999
Relative frequency
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
100
120
140
160
180
200
220
240
260
Fork length (mm)

Figure 5.

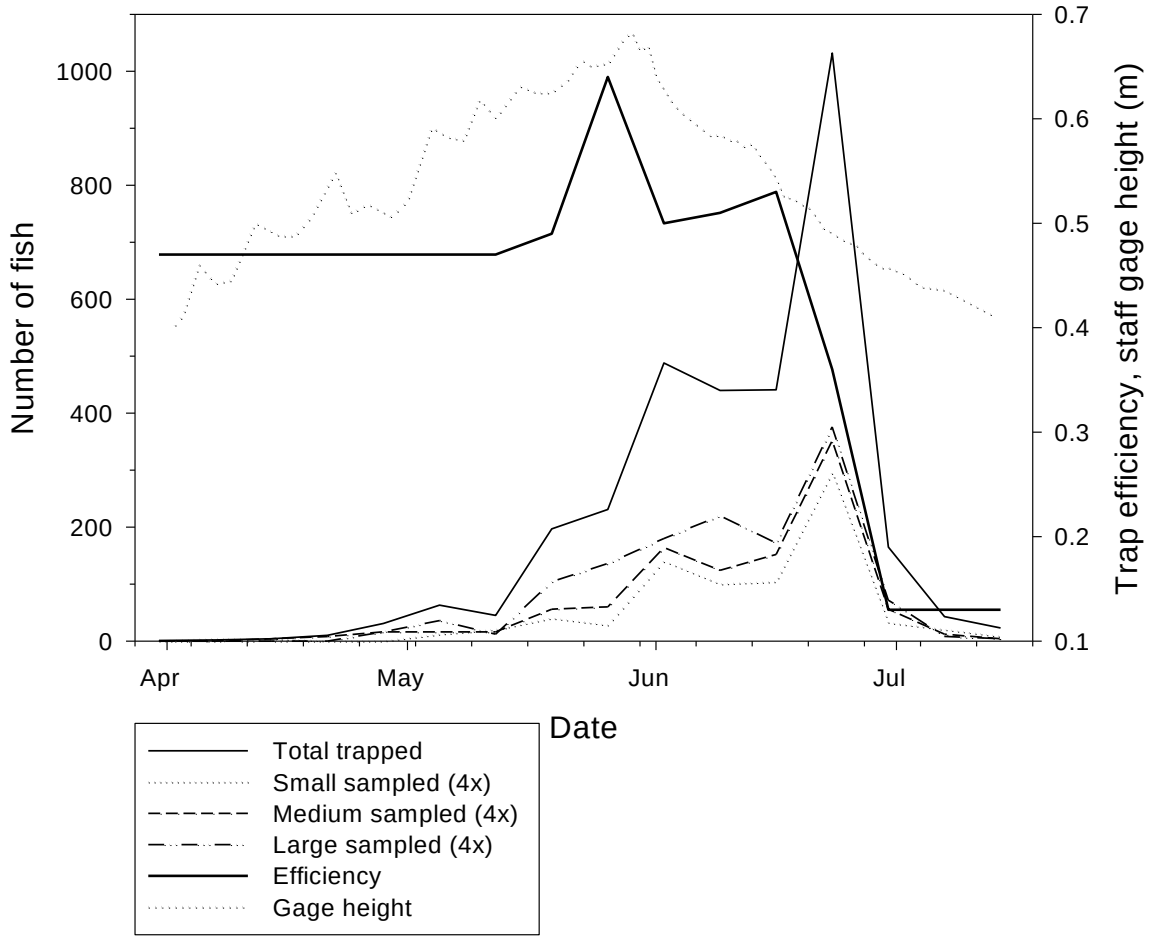
Number of fish
0
200
400
600
800
1000
Trap efficiency, staff gage height (m)
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Apr
May
Jun
Jul
Date
Total trapped
Small sampled (4x)
Medium sampled (4x)
Large sampled (4x)
Efficiency
Gage height

Figure 6.

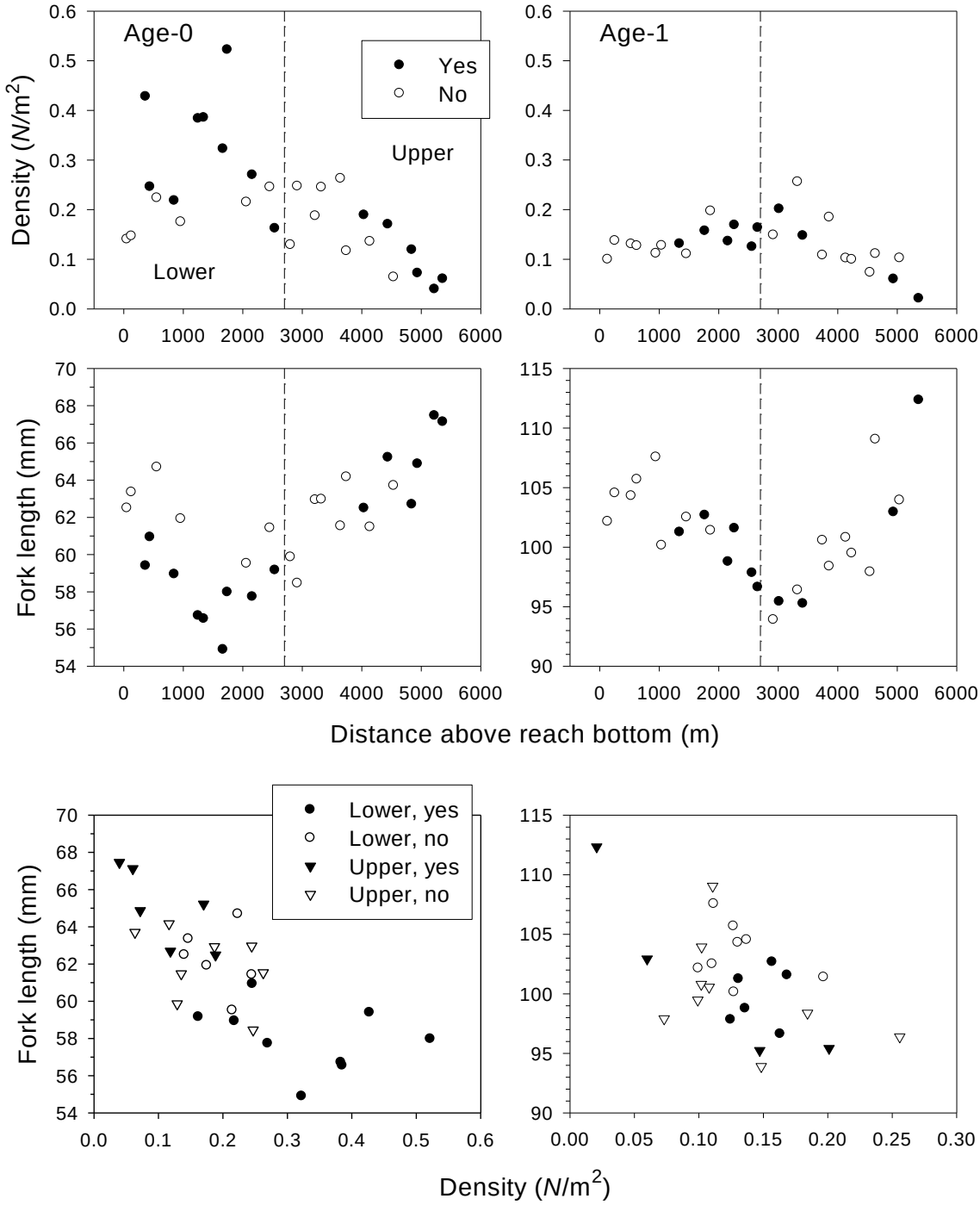
Age-0
Age-1
Yes
No
Upper
Lower
Density (N/m2)
Fork length (mm)
Distance above reach bottom (m)
Lower, yes
Lower, no
Upper, yes
Upper, no
Density (N/m2)

Figure 7.

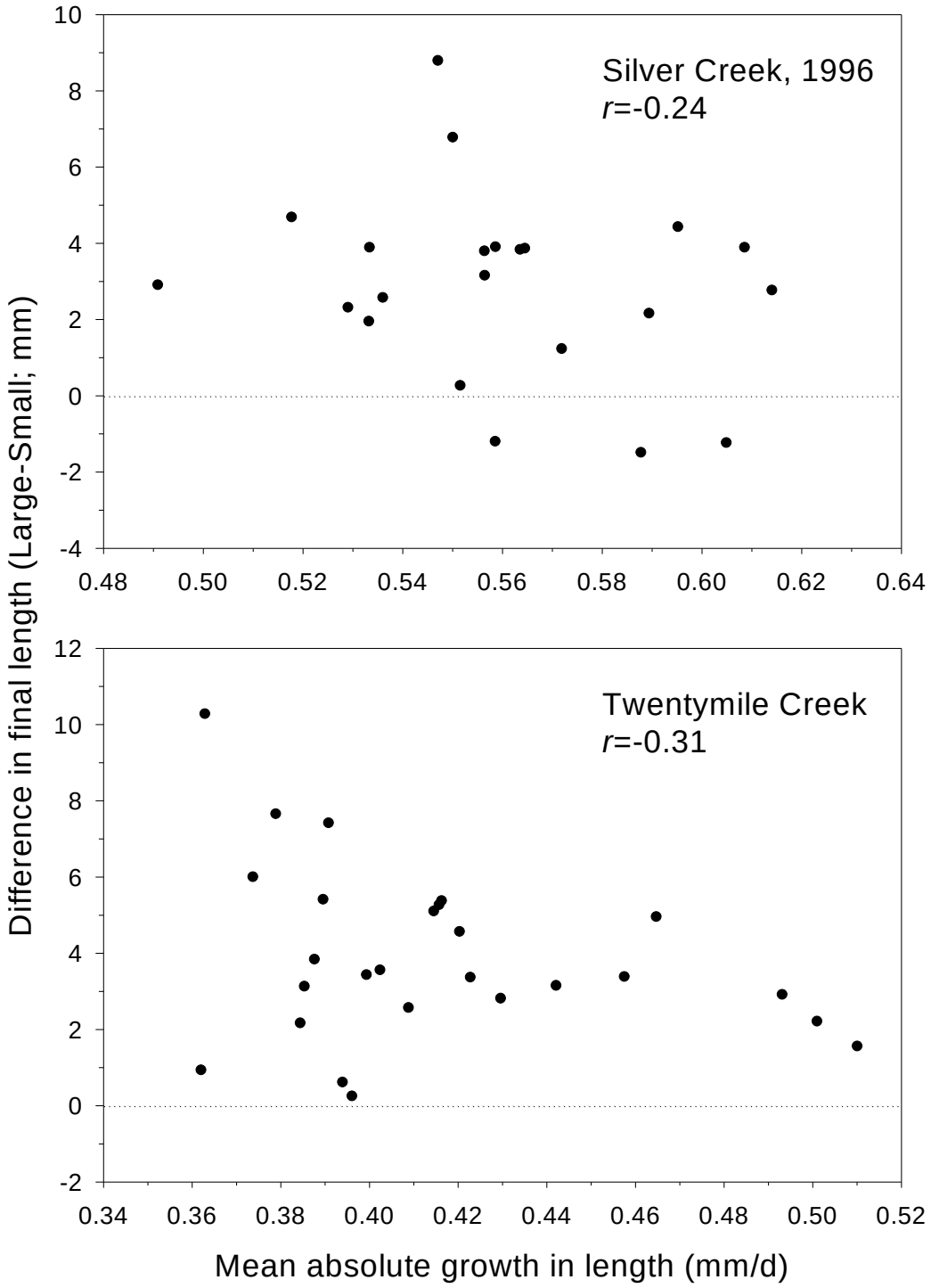
Silver Creek, 1996
r=-0.24
Twentymile Creek
r=-0.31
Difference in final length (Large-Small; mm)
Mean absolute growth in length (mm/d)
10
8
6
4
2
0
-2
-4
12
0.48
0.50
0.52
0.54
0.56
0.58
0.60
0.62
0.64
0.34
0.36
0.38
0.40
0.42
0.44
0.46
0.48
0.50
0.52

Figure 8.

Chapter 8: Testing for genetic differences in survival and growth between hatchery and wild Chinook salmon from Warm Springs River, Oregon *(Study sites: Warm Springs Hatchery and Little White Salmon River; Stocks: Warm Springs hatchery and Warm Springs River wild; Year classes: 1992 and 1996)*

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Abstract

The program at Warm Springs National Fish Hatchery in north-central Oregon was initiated with spring Chinook salmon *Oncorhynchus tshawytscha* from the Warm Springs River. Managers included wild fish in the broodstock most years and avoided artificial selection to minimize genetic divergence from the wild founder population. We tested for genetic differences in survival and growth between the hatchery and wild populations to ascertain whether this goal has been achieved. Progeny of hatchery x hatchery (HH), hatchery female x wild male (HW), and wild x wild (WW) crosses were genetically marked at the *sSOD*-1* allozyme locus and released together as unfed fry in hatchery ponds in 1992 and 1996 and in the Little White Salmon River, in south-central Washington, in 1996. Fish were evaluated to returning adult at the hatchery and over their freshwater residence of 16 months in the stream. The three crosses differed on several measures including survival to outmigration in the stream (WW>HH>HW) and juvenile growth in the hatchery (1992 year-class; WW>HW>HH); however, results may have been confounded. The genetic marks were found to differentially effect survival in a companion study (HH mark favored over WW mark; HW mark intermediate). Furthermore, HW survival in the current study was neither intermediate, as would be expected from additive genetic effects, nor similar to that of HH fish as would be expected from maternal effects since HW and HH fish were maternal half-siblings. Finally, the unexpected performance of HW fish precludes ruling out maternal differences between hatchery and wild mothers as the cause of differences between HH and WW fish. The key finding that survival of HH fish in a stream was 0.91 that for WW fish, indicating a small loss of fitness for natural rearing in the hatchery population, is valid only if three conditions hold: (1) any selection on the genetic marks was in the same direction as in the companion study, (2) lower survival in the stream for HW than for HH fish resulted because some HW families were genetically atypical, not from problems with either pure type, and (3) lower survival for HH than for WW fish was not due to maternal effects. Although all three conditions had support, none of it was conclusive. This study provides only suggestions, not definitive answers for the primary question of whether the hatchery population has diverged genetically from its wild founder population in fitness-related traits.

Introduction

Hatchery propagation of anadromous salmonids can effect genetic change that substantially reduces fitness for natural spawning and rearing (Reisenbichler and Rubin 1999). Domestication (natural selection for fish adapted to the hatchery program; Reisenbichler and Rubin 1999) is considered a predominant cause of such genetic change, and happens even in sea-ranched populations where most mortality occurs in the natural environment after release (Reisenbichler et al. 2004). Wild populations are harmed when hatchery fish interbreed with wild fish because reduced fitness for natural rearing can be passed to the next generation. The magnitude of effect, however, is uncertain.

The program for spring Chinook salmon *Oncorhynchus tshawytscha* at Warm Springs National Fish Hatchery (Figure 1) was designed to protect the wild population in the Warm Springs River from adverse genetic change (Olson et al. 1995; Olson et al. 2004). Hatchery personnel initiated the hatchery population with wild adults returning to the Warm Springs River in 1978-1981, included wild adults in

the hatchery broodstock in most years (mean for 1982-2002 = 18%; Olson et al. 2004), and avoided selective breeding for traits such as time of spawning and body size. Hatchery juveniles were marked so that they could be recognized as returning adults. An artificial barrier at the hatchery directed all returning adults into the hatchery where marked fish were separated and prevented from passing upstream. Even so, a small but unknown number of hatchery fish were released above the dam each year and presumably spawned with wild fish because not all hatchery fish were successfully marked as juveniles (mark retention usually >90%; Olson et al. 1995).

Our study evaluated whether the practices at Warm Springs Hatchery prevented the hatchery population from diverging genetically from the wild population in traits associated with fitness. Our goal was to test for genetic differences in survival and growth between progeny of these hatchery (H) and wild (W) spring Chinook salmon reared together at Warm Springs Hatchery and in the Little White Salmon River (LWSR) in South Central Washington (Figure 1). We created HH, HW, and WW crosses, each with a distinct genotype or "mark" at *sSOD-1** (a locus of superoxide dismutase; Shaklee et al. 1990). The genetic marks allowed us to recognize the crosses even though the experimental fish were released into a common rearing environment as button-up fry, too small for most other marks. Because the offspring were reared together any observed differences among cross types should result from genetic differences, or possibly maternal effects (e.g., vertically transmitted disease, egg size, or egg quality), rather than from environmental differences. Hybrid crosses of hatchery females and wild males were included for comparison with maternally similar crosses of hatchery females and hatchery males to assess maternal effects. Performance in the hatchery program was evaluated by sampling juveniles at the end of the hatchery rearing period and adults returning to the hatchery. Additionally, some juveniles were not released from the hatchery but instead were transported to a seawater facility and monitored for survival and growth during 3-6 months of seawater rearing. Performance in the LWSR was evaluated by sampling fish residing in the stream and emigrating from it during the 16 months following fry release.

Domestication is expected to decrease fitness for natural rearing but increase fitness for hatchery rearing (Reisenbichler and Rubin 1999). We therefore tested whether survival of HH fish relative to that for WW fish differed between rearing environments in this manner. Relaxed selection in the hatchery population can also cause genetic divergence from the original wild population and loss of fitness for natural rearing (Lynch and O'Hely 2001) but would not result in better performance by hatchery progeny than by wild progeny under hatchery rearing (Reisenbichler and Rubin 1999).

Methods

Experimental crosses

Adult salmon were collected as they entered WSNFH in spring and summer of 1992 and 1996. Hatchery adults were recognized by a clipped adipose fin and the presence of a coded wire tag (CWT; Jefferts et al. 1963) and wild adults by the lack of those marks. The *sSOD-1** genotype was determined for each adult by taking a small piece of fin tissue and analyzing it electrophoretically following Aebersold et al. (1987). Hatchery adults homozygous for the common (**-100*) allele and wild adults homozygous for the alternate (**-260*) allele were subsequently spawned, thus creating the three genetically marked offspring groups: HH = *-100/-100*, HW = *-100/-260*, and WW = *-260/-260*.

Spawning took place in late summer (Table 1). Gametes were taken from mature fish and held on ice until fertilization (<8 hours). In 1996, wild males were returned alive to holding ponds to provide milt on subsequent spawning days. In both years, families were produced in partial diallel crosses where milt from one male was used to fertilize one-half of the eggs from each of two females and milt from a second male fertilized the other half of the eggs from each female (Table 1). In 1996, each hatchery female was crossed with one hatchery and one wild male, thus creating maternally identical HH and HW families, whereas in 1992 some hatchery females were crossed exclusively with hatchery or with wild males (Table

2). Similarly, contribution of wild males was more similar between HW and WW families in 1996 than in 1992 (Table 2). In 1992, some females had one of their two progeny families discarded because one of the sires had been assigned an incorrect *sSOD-1** genotype initially. The error was found by assaying a second tissue sample collected after spawning. In 1996 all families were confirmed to have the correct genetic marks. In both years, a sample of ovarian fluid was taken from each female and tested for bacterial kidney disease (BKD, caused by *Renibacterum salmoninarum*) infection level using the Enzyme-Linked Immunosorbant Assay (ELISA; Pascho and Mulcahy 1987). BKD is vertically transmitted from females to progeny, as well as horizontally transmitted among progeny, and can cause significant mortality in Columbia River basin spring Chinook salmon (Pascho et al. 1991). Progeny of females with ELISA absorbance >0.5 were excluded from the study. In both years, a sample of $\geq$30 eggs from each female was preserved in 10% buffered formalin and after 90 days weighed to calculate mean egg weight per female.

Each full-sib family was placed in a separate colander independently supplied with upwelling water. In 1992, all experimental progeny were retained for rearing at Warm Springs Hatchery; none were released in the LWSR. At the eyed stage, the embryos were transferred to vertical incubators, one family per tray. In 1996, each family was divided proportionately, using an electronic counter (manufactured by Jensorter^{R}), to provide eyed embryos for the hatchery and for the LWSR; thus family composition was identical between hatchery and stream release groups. Eyed embryos destined for stream release were transported to Carson Depot Springs (Figure 1) for the remainder of incubation. At each incubation site, families from the same spawning date and cross were mixed within an incubator tray; crosses were placed in separate trays and spawning dates in separate stacks of trays. Mortalities and abnormal alevins were periodically counted and removed from the trays for the remainder of incubation.

In both years, water temperature in vertical incubators was manipulated to remove differences in development among embryos from different spawning dates. At the hatchery, water was heated 3-5°C in November and December when ambient temperatures were below 7°C. At Carson Depot Springs, water was chilled $\leq$3.6^{o}C below the constant ambient temperature of 9.5^{o}C. At both locations, embryos from later spawning dates were held for more days in warm and fewer days in cool water than were those from early dates. At Carson Depot Springs, embryos from the first spawning date remained slightly more developed than the others despite our manipulations, and fry from the first date were therefore released in LWSR a week earlier than the others (see below). A model that predicts developmental stage from incubation duration and temperature (Jensen 1988) assisted with synchronizing embryo development. Mean incubation temperature differed among crosses by $\leq$0.3^{o}C (Table 3).

Fry were sampled for length and weight as they reached the button-up stage, 0-1 days before release in hatchery tanks or the study stream. Individual fork lengths and a collective weight were measured for a sample collected from each incubator tray. Developmental index (K_D; Bams 1970) was computed as:

$K_D = 10*W^{1/3}*L^{-1}$;

W = mean weight (mg); L = mean fork length (mm). Developmental index declines continuously from hatching to complete yolk absorption; i.e., it is inversely related to developmental stage.

Hatchery rearing

1992 year-class juveniles.—Fry from each 1992 year-class family (tray) were divided equally between two indoor nursery tanks, using an electronic counter (Applebee and Tipping 1991; number of fry given in Table 4), on 22 January 1993 when food was first offered. On 30 March, fish from one tank were moved to an outdoor Burrows pond and fish from the other to a second pond, and on 10-11 May each individual was marked with an adipose fin clip and a CWT (one code per pond). Production (non-

experimental) juveniles received the same marks (but distinct CWT codes) so that identity as our experimental fish could only be determined from the CWT code when the fish returned as adults. On 15-16 February 1994 a sample of 300 juveniles was collected from each pond with a dip net after crowding fish to one end of the pond. Individuals were measured, weighed, examined for milt expression, and a ventral fin was excised and frozen for subsequent electrophoretic analysis to determine *sSOD-1** genotype (i.e., cross). Additionally, half of the fish in each sample were injected with an individually coded passive integrated transponder (PIT) tag (Prentice et al. 1990) and on 16 February were transported to a facility with seawater rearing capability (see below). The fish without PIT tags were returned to the ponds, and on 22 April all fish remaining in the ponds were released into the Warm Springs River.

1996 year-class juveniles.—Fry were released into a single nursery tank on 14 January 1997 (Table 4) and were moved to a Burrows pond and marked similarly to the 1992 year-class on 28 April. Standard procedures at the hatchery had changed, so unlike the 1992 year-class, juveniles were fed feed containing erythromycin in May and September, and their pond was shaded from mid-July to mid-October. Another change was that we set up a mock outflow during mid-October to mid-November by siphoning water into an adjacent holding pond through a 10 cm diameter plastic tube. The purpose was to simulate the fall volitional release opportunity typical for production fish at the hatchery (Olson et al. 2004). On 19 November, we counted all of the fish that had swum through the siphon, sampled a portion of them, and then released all of them into the Warm Springs River. We also sampled fish (non-migrants) remaining in the original raceway. Non-migrants were retained in the hatchery through the winter and sampled again on 14-16 April 1998. Fall and spring samples were collected by crowding the fish, dip netting them, and running each netful of fish through a quarter sampler (a net with four equally sized compartments only one of which was sewn at the bottom). Thus we handled four times the number of fish actually needed, presumably improving randomization. Individuals were measured, weighed, examined for milt expression, and sampled for fin tissue. Additionally, all individuals in the spring sample (n=666) were measured for skin reflectance (Haner et al. 1995), one third of them were non-lethally sampled for gill tissue and returned to the pond, and the remaining two thirds were PIT-tagged and held separately. All fish in the pond were released into the Warm Springs River on 15-16 April and the PIT tagged fish were transported to the seawater facility on 17 April. The gill tissue samples were subsequently assayed for ATPase (Schrock et al. 1994).

Seawater rearing

The PIT tagged juveniles were transported to the U.S. Geological Survey's Marrowstone Marine Station, Nordland, WA. In 1994 (1992 year-class), samples from the two ponds at the hatchery were kept separate; each (n=150 fish) was placed in a 1.2 m diameter circular tank. In 1998 (1996 year-class), tissue samples were analyzed and cross was determined before fish arrived at Marrowstone. Fish were distributed among 10 0.6 m diameter circular tanks (n=44$\pm$2 fish per tank) such that each tank contained 39% HH, 30% HW, and 31% WW fish. Furthermore, the shortest fish from each cross (the 10% of the cross with the shortest lengths) were placed in the first tank, the next shortest fish (the next 10%) in the second tank, and so forth.

For both year-classes, dead fish were removed daily, measured, weighed, and frozen; kidney samples were taken later and processed by ELISA to screen for BKD infection level. Tanks were gradually switched from freshwater to seawater during 18-22 April 1994 and 20-24 April 1998. In 1994, all fish had died by 8 July. Growth rate was calculated as the difference between length at death and length at tagging divided by the number of days between death and tagging. In 1998, survivors were measured, weighed, and returned to their tanks on 8 June and 20 July. On 13 July, nearly all of the fish in three of the 10 tanks died from an accident creating gas super-saturation and on 20 July the fish remaining in these tanks were euthanized and sampled as above. The experiment was terminated on 23 September when survivors in the other tanks were euthanized and sampled. Growth rate was computed only for

mortalities resulting from euthanization or super-saturation; length and weight measurements from other mortalities were highly variable and were not used in analyses.

Adult returns

Adults returned in 1995-1997 (1992 year-class) and 1999-2001 (1996 year-class). Sampling efficiency (proportion of total returns sampled) and location (Warm Springs Hatchery versus Deschutes River fishery) varied among return years. Data collected from sampled individuals always included length, tissue (for cross determination), and CWT, but additional data varied among years. In 1995-1997, all returns to the hatchery were retained for broodstock, and we sampled most of them (Table 5) at spawning or as pre-spawning mortalities; sex and spawning date were recorded. In 1999 we again sampled most returns to the hatchery (Table 5), mostly upon arrival when they were killed and distributed for human consumption. Arrival date was recorded but sex usually was unknown because most of these fish were distributed whole. In 2000, the largest return year on record, and 2001 we sampled only 33% and 51%, respectively, of adult returns to the hatchery, again mostly upon their arrival. The Deschutes River fishery was sampled in 2000 and 2001, when capture date and sex were recorded for individuals, but not in 1996. Few or no fish were legally harvested in 1995, 1997, and 1999 (Table 5).

Natural rearing

A waterfall near the mouth of the LWSR was impassible to anadromous adults (Figure 1). Rainbow trout *O. mykiss* and brook trout *Salvelinus fontinalis* were the only fish species above the falls. The 11.9 km study reach extended from the Oklahoma Campground bridge downstream to Moss Creek (Figure 1). Mean wetted width was 10.3 m in July 1997. The reach contained 306 pools (Bisson et al. 1982), accounting for 40% of total reach surface area; average maximum pool depth was 1.1 m. Elevation at the Oklahoma Campground bridge was 500 m above sea level and mean gradient of the study reach was 0.8%. Mean monthly temperature upstream from Moss Creek ranged from 13°C in August 1997 to 4°C in January 1998. Downstream from Moss Creek summer temperatures were much lower (7°C in August) due to inputs of cold water from Moss Creek.

Fry from the first spawning date (16% of the total fry; Table 6) were released on 8 January 1997, 50 m upstream from the mouth of Berry Creek (Figure 1). Fry from the last three spawning dates were released on 15-16 January throughout the stream between Okalahoma Campground bridge and the previous release site (Figure 1, sections 1 and 2) at densities of 18 fry/m above and 22 fry/m below the mouth of Lusk Creek. Proportion of each cross (Table 6) was the same on both release dates.

Outmigrants were monitored continuously from 29 April 1997 to 2 June 1998 with one or two 1.5 m diameter screw traps (Thedinga et al. 1994). The traps, located at Elbow and Big Cedars (Figure 1), were checked three days each week during the summer and daily the rest of the year. Details of trap operation are given in the Appendix. All unmarked fish caught in the traps were measured, weighed, and sampled for tissue to determine *sSOD-1** genotype. Initially (29 April-3 June) all fish were euthanized and frozen whole. During 4-30 June the left ventral (LV) fin was removed to provide tissue for analysis and fish were released upstream to measure trap efficiency. The *sSOD-1** analyses were successful for less than one-half of these samples because of insufficient tissue quantity so we reverted to euthanizing all trapped fish on 1 July. In August the fish had grown sufficiently and we resumed fin clipping and efficiency tests.

Recapture rate of marked fish released upstream was used to estimate the number of outmigrants each week (Appendix). The relative frequency of each cross in a week was computed from the total number of fish of each cross caught in both traps during the week. We estimated the relative frequency of each cross for the entire migration period in two ways. First, we pooled the weekly samples. Second, we

took a weighted average of the weekly cross proportions where the weights were the estimated number of outmigrants each week.

Fish were captured with beach seines and backpack electrofishers from sections 1-7 (Figure 1) during 5-26 August 1997. Captured fish were measured, weighed, and given a right ventral (RV) clip to provide tissue for *sSOD-1** analysis. This mark was distinct from the LV clip for fish caught in the screw traps. The fish were released where they had been captured. RV clipped fish subsequently recaptured in the screw traps were counted as unmarked fish (i.e., as first time captures) but were not released upstream for efficiency tests. Seining was the most effective capture method in sections 2-7. Bait (salmon eggs) was frequently used to attract fish out from cover and into the seine. Electrofishing was used in sections 2-7 in areas where seining was not effective and exclusively in section 1 where pools were shallower. In sections where both methods were used, cross proportions were independent of method (P = 0.342, 2x3 G-test of independence; n_{seined} = 1513, $n_{electrofished}$ = 170), and neither length (P = 0.291) nor weight (P = 0.963) differed between methods (two-way AOV with section as the second factor). Therefore, fish captured by different methods were pooled. Data from different dates were pooled because we found no difference in mean fish size between early and late August in sections where fish were captured at both of those times. Inadvertent mortalities (mostly from electrofishing in section 1), and a sub-sample of the fish captured in section 3, were examined for precociously mature males.

The number of fish in each section during July-August was estimated by snorkeling in all pools and then calibrating the counts with multiple pass removal or mark-recapture estimates from a subset of pools (Appendix). Cross proportions were computed for each section from the fish captured in the section. Cross proportions for the entire reach were estimated by pooling the samples collected from each section, and also by taking a weighted average of section-specific proportions where the weights were the estimated number of fish in each section.

Snorkeling or electrofishing upstream from section 1 and in the tributaries large enough to provide fish habitat (Lusk, Berry, and Cabbage creeks) revealed few fish in the pools closest to the study reach and none farther upstream. Snorkeling from section 7 downstream to the Elbow site (Figure 1) revealed no fish. Therefore, we concluded that sampling within the study reach was sufficient to characterize the population upstream from the screw traps.

Beginning in August, recaptures in the screw traps of fish previously caught in the traps (marked LV) or by electrofishing or seining (RV) were euthanized and examined for maturity, and beginning in March 1998 recaptures were additionally sampled for gill tissue to assess ATPase activity (Schrock et al. 1994). During July 1998 we snorkeled most of the study reach to census residuals.

Analyses

The survival of HH relative to WW fish ($RS_{HH/WW}$) was computed as:

$(P_{HH2}*P_{WW1})/(P_{HH1}*P_{WW2})$;

P_{HH1} = proportion of HH fish at release, P_{HH2} = proportion of HH fish at time 2 (e.g., juvenile sampling), P_{WW1} = proportion of WW fish at release, and P_{WW2} = proportion of WW fish at time 2. Survival of HW relative to WW fish and survival of HW relative to HH fish were computed similarly. Relative survival was termed "apparent" (and abbreviated ARS) for naturally reared juveniles because estimates reflected emigration as well as mortality. Significance of relative survival (i.e., whether different from 1) was tested with G-tests for goodness of fit (Sokal and Rohlf 1995); expected frequencies were the number of fish sampled times the proportion of each cross from the original number of fry released (Tables 5 and 7). Difference in relative survival between rearing environments (stream and hatchery) was tested with replicated G-tests for goodness of fit (Sokal and Rohlf 1995). Expected frequencies for the pooled (over

rearing environments) sample were the sum of the expected frequencies in each rearing environment. The statistic of interest ($G_{heterogenity}$) was obtained by subtraction (G_{total}-G_{pooled}). Differences in relative frequency between samples collected at different times or places (e.g., migrants and residents) were tested with contingency table *G*-tests of independence (Sokal and Rohlf 1995). Exact rather than asymptotic *P*-values for goodness of fit and independence tests were used in cases of small sample size.

Differences in temporal or spatial distribution among crosses coupled with temporal or spatial differences in capture efficiency can result in biased estimates of relative survival when samples are pooled over temporal or spatial strata. Our statistical tests for ARS of naturally reared juveniles used such pooled samples; however, we also estimated ARS using stratum-specific population estimates as weighting factors (see above). These estimates corrected for differences in capture efficiency but were subject to error in the population estimates. We provide both pooled and weighted ARS estimates and argue that similar estimates lend credence to the statistical test based on the pooled sample.

Size and condition factor ($K=10^5*W/L^3$; W=weight (g), L=fork length (mm)) were tested with analysis of variance (AOV) or analogous non-parametric tests when AOV assumptions were violated. Juveniles emigrated from the LWSR over a several month period. Their size and condition were tested with analysis of covariance (AOCV) to account for the effect of capture date. Linear and non-linear (polynomial) time trends were explored for significance ($P<0.05$). If no time trend was significant, data were tested by AOV. Sequential Bonferroni adjustments (Sokal and Rohlf 1995) were made to account for multiple comparisons of frequency and of size data.

Results

Maternal, embryo, and fry measures

ELISA absorbance and individual egg weight were similar between hatchery and wild females, and embryo survival during incubation was similar among crosses, in 1992 and in 1996 (Table 3). Final size and condition of fry were similar among crosses in both years except that length of HW fry was greater than that of WW fry in 1992 (Table 3).

In 1996, frequency of HW abnormal alevins was $\geq$3 times that of the other crosses (Figure 2, upper panel). The most common abnormality was a permanently round shape due to the spine not straightening after hatch. Abnormal alevins apparently resulted primarily from $\leq$5 of the 28 HW families (families 6-10), because the occurrence of abnormal alevins in an incubation tray was proportional to the representation of families 6-10 in the tray (Figure 2, lower panel). It was not possible to pinpoint which of families 6-10 were responsible for the abnormalities.

These abnormal alevins were removed from the study and therefore did not directly affect our other results. Even in the HW group there were only 294 of them, about half the number of dead HW embryos removed between eye-up and button-up and far too few to affect our conclusion of equal survival among crosses during incubation. We focus on abnormal alevins because they may indicate other problems with the HW group. We expected HW performance to be either intermediate to that of the other crosses (additive genetic effect) or similar to HH performance (maternal effect); however, HW performance was often neither (see below).

High incidence of abnormal alevins may indicate other irregularities with the respective family or families. If all abnormal alevins came from only one of families 6-10, those alevins would have composed 23-27% of the initial eyed embryos from that family, meaning that the rest of the embryos from that family, accounting for 2.3-2.8% of total HW embryos, would have remained in the experiment. One wild male sired three of families 6-10. If the abnormal alevins were due to that male, then normal embryos from that male would have accounted for 9.4% of the total HW embryos remaining in the study.

The male did not sire any other families on the same day (i.e., from the same milt collection), but he did on other days, and those families did not exhibit high numbers of abnormal alevins. Two other males each sired one of families 6-10 and also sired, on different days, other families without abnormal alevins. The mothers of families 6-10 were also mothers of HH families spawned on the same day and which lacked abnormal alevins. Abnormal alevins were not recorded in 1992.

Hatchery rearing

Juveniles.—Survival of juveniles from release of fry in nursery tanks at the hatchery to release of juveniles from the hatchery into the Warm Springs River was high (93% for the 1992 year-class; 97% for 1996) and similar among crosses (Table 4). About 10% of 1996 year-class juveniles exited their raceway via the mock outflow (siphon tube) during the fall volitional release period (Table 4). Frequency of HH relative to WW fall migrants was greater than expected; HW migrants seemed intermediate but were not significantly different from either HH or WW (Table 4).

Length (Figure 3) and weight of juveniles were bimodally distributed. Mean length and weight were least for HH, intermediate for HW, and greatest for WW juveniles of the 1992 year-class; condition factor was similar among crosses of that year-class (Table 7). The size differences were consistent between ponds and remarkably large (WW 12% longer and 52% heavier, on average, than HH). For the 1996 year-class, frequency in the upper length mode (Figure 3) was greater for HW juveniles than for either HH or WW juveniles sampled at spring release (age=20 months; Table 7); mean length and weight of juveniles sampled then and five months previously (fall migrants and non-migrants) showed the same trend (HW bigger than HH or WW) but the differences were not significant. Condition at 15 and 20 months was lower for WW than for HH non-migrants (the trend was similar but non-significant for fall migrants); condition of HW fish was either intermediate (at 15 months) or similar to HH (at 20 months; Table 7). Size differences among crosses were much smaller for the 1996 than for the 1992 year-class (Table 7).

Mature males composed 0.8% of 1992 year-class juveniles. They composed 1.8% of 1996 year-class fall migrants (percentage similar among crosses; Table 7) but were not found in samples of non-migrants collected in the fall or the following spring. Skin reflectance and gill ATPase were similar among crosses (Table 7).

Seawater rearing.—All fish of the 1992 year-class had died by 8 July (within 142 days of tagging; Figure 4). HW fish died earliest and WW fish latest (as indicated by the difference in mean number of days between tagging and death); mean growth rate was positive and similar among crosses (Table 8).

For the 1996 year-class, there seemed to be a trend of higher mortality for HxH than for WxW fish during most of the seawater rearing period, but that trend may have reversed (lower mortality for HxH than for WxW fish) near the end of seawater rearing. Survival of 1996 year-class fish through 12 July (day 89), the day before three tanks experienced high mortality from gas super-saturation, appeared lowest for HH and highest for WW fish; however, the difference was not significant (Figure 4; Table 8). Cumulative mortality of fish in the seven tanks not experiencing gas super-saturation seemed higher for HH than for WW fish through about 1 August (day 109), but during August (through day 139) mortality rate appeared higher for WW than for the other two crosses with the result that cumulative mortality was slightly higher for HH than for WxW fish by the end of seawater rearing (Figure 4). Considering only fish that died before 13 July (from all tanks), mean days to death differed among crosses (P=0.03) and was fewest for HH fish and most for WW fish; however, the paired comparison between HxH and WxW fish was only marginally significant (Table 8).

Growth rate of the 1996 year-class from tagging to 8 June (day 55) was similar among crosses, but from 8 June to 20 July (day 67) it was higher for WW than for HW fish and marginally higher for WW than for HH fish (Table 8). For the latter time period, differences in growth among crosses were inconsistent among tanks ($P_{cross*tank}$=0.08) in a manner related to differences in initial fish length (Figure 5). Mean growth rate was highest for WW fish (i.e., higher than for HH or HW) in six of the seven tanks containing the smallest fish (tanks 236-242) whereas it was highest in none of the remaining three tanks (243-245; Figure 5). Only 14 fish survived to 23 September when the experiment was terminated. Growth rate was not computed for the time period between 20 July and 23 September due to small sample size.

The distribution of ELISA absorbance was bimodal for 1996 year-class mortalities (Figure 6, lower left panel) and was also bimodal for 1996 year-class survivors (Figure 6, lower right panel); in both cases lower modal absorbance values clustered near 0 and upper modal values clustered near 3. ELISA absorbance for mortalities was positively correlated with days to death (r=0.30; P<0.01). Fish that died early had low absorbance values; then 43 days after tagging mortalities with high absorbance (i.e., in the upper mode) began to occur. About 106 days after tagging, mortalities with low absorbance mostly stopped occurring but those with high absorbance began to occur at a higher rate (more deaths per day; Figure 6). Fish that died on 13 July (day 90) from gas super-saturation were more frequently in the low mode for ELISA and less frequently in the high mode relative to fish that survived to 23 September (day 162; Figure 6). The distribution and time series of ELISA absorbance suggest that mortalities with high absorbance died from BKD whereas those with low absorbance died from other causes, and that an extended period of seawater rearing was required for BKD to progress sufficiently to cause mortality. ELISA absorbance of 1992 year-class fish was positively correlated with days to death (r=0.37; P<0.01) but was not bimodally distributed (Figure 6).

Mean ELISA absorbance was similar among crosses for both year-classes (Table 8). For 1996 year-class mortalities, the relation between ELISA absorbance and days to death differed among crosses. Frequency of HH mortalities relative to mortalities from the other crosses was higher before day 43 (Figure 7; upper panel), when ELISA absorbance of all mortalities was near 0 (Figure 6; lower left panel), than after day 43 (Figure 7; upper panel) when ELISA absorbance of most mortalities was near 3 (Figure 6; lower left panel). Considering only mortalities with high ELISA values (i.e., near 3), frequency of WW mortalities relative to mortalities from the other crosses seemed higher after day 106 (Figure 7; lower panel), when rate of mortalities with high ELISA values increased (Figure 6; lower left panel), than before day 106; however the difference was not significant (Figure 7; lower panel). The latter finding suggests that lower mortality through July but higher mortality during August for WW relative to HH and HW fish (Figure 4) may have resulted from differences among crosses in BKD incubation rate. Perhaps lethal BKD infection levels were delayed but not avoided in WW fish compared to the other two crosses.

Adults.—Adult return rate (survival of juveniles released from the hatchery to adults returning to the Deschutes River [ages 3, 4 and 5 combined]) was 0.05% for 1992 year-class experimental fish. No difference in survival among crosses was shown by the 18 adults sampled (Table 4). Adult return rate of the 1996 year-class was 28 times higher (1.4%). In the pooled sample (all ages and recovery sites combined), survival was marginally higher for HW than for WW fish (0.05<P<0.10) and intermediate for HH fish (Table 4). For age-4 adults, there may have been relatively less HW and more WW fish caught in the fishery than returning to the hatchery (P=0.06; 2x3 G-test for independence of recovery site [fishery or hatchery] from cross; Table 4). Nonetheless, pooling fishery and hatchery samples is justified because sampling efficiency was similar between the fishery (31%) and the hatchery (33%; Table 5).

Age-composition of adults was similar among crosses for both year-classes ($P \geq 0.26$; 3x3 G-tests of independence; Table 4). Length of age-4 returns from both year-classes appeared least for HH and greatest for WW fish (Figure 8); the difference was marginally significant for the 1996 year-class

(P=0.06). In contrast, length of 1996 year-class age-3 returns may have been least for WW and greatest for HW (P=0.06; Figure 8). Arrival date at the hatchery of 1996 year-class age-3 returns differed marginally among crosses (P=0.08; Kruskal-Wallis test) and was earliest for HH fish (Figure 9). Capture date in the fishery and arrival date at the hatchery for age-4 returns of that year-class (Figure 9) were similar among crosses (P>0.10).

Sex ratio was similar among crosses for the 1992 year-class but may have been higher for HH than for WW returns of the 1996 year-class (P=0.10; Table 9). Hatchery returns of both year-classes were checked for ripeness at 6-7 day intervals starting in late August. Spawning date of age-4 females was similar among crosses (P=0.40 for data pooled over year-classes; Table 10); nearly all males were ripe on the first spawning date.

Natural rearing

Juveniles emigrated during May-July 1997 ("pre-summer") and late August 1997-May 1998 ("post-summer") (Figure 10). Fish residing in the study reach above the traps were sampled between these periods (in August 1997) and will henceforth be called summer residents. In the middle of the pre-summer period (4-30 June), when fin tissue rather than muscle was sampled from emigrants, 57% of the fish could not be assigned to cross due to unsuccessful *sSOD-1** analysis (Table 11). The large percentage of unassigned fish was worrisome so we omitted from formal analyses all data from 4-30 June. Nevertheless, we note that $ARS_{HH/WW}$ from fish assigned during 4-30 June was low relative to $ARS_{HH/WW}$ early and late in pre-summer (Table 11), suggesting that $ARS_{HH/WW}$ for the total pre-summer period was no higher than indicated by pooled and weighted estimates made without data from 4-30 June (Table 6). Similarly, $ARS_{HW/WW}$ and $ARS_{HW/HH}$ during 4-30 June were also low relative to those estimates early and late in pre-summer (Table 11), so $ARS_{HW/WW}$ and $ARS_{HW/HH}$ for the total pre-summer period were likely no higher than indicated by pooled and weighted estimates made without data from 4-30 June (Table 6).

Pooled estimates of $ARS_{HW/WW}$ and of $ARS_{HW/HH}$ for pre-summer migrants were somewhat higher than weighted estimates (Table 6), but the weighted estimates may have been more accurate because the tail end of the pre-summer run, when few fish were emigrating (Figure 10) and when the frequency of HW relative to the other two crosses was particularly high (Table 11), was over-represented in the pooled sample. Pooled and weighted estimates were more similar to each other for the other comparisons ($ARS_{HH/WW}$ of pre-summer migrants and ARS for all three cross pairs of summer residents and post-summer migrants; Table 6).

$ARS_{HH/WW}$ was similar between summer residents and post-summer migrants (Table 6). When summer residents were pooled with post-summer migrants to increase sample size, $ARS_{HH/WW}$ showed a significant decrease of 9% in the frequency of HH relative to WW fish since fry release (Table 6). The decrease was due to higher mortality for HH than for WW fish during the pre-summer period, not to greater emigration for HH than for WW fish since $ARS_{HH/WW}$ for pre-summer migrants was similar to that for summer residents and post-summer migrants (Table 6). We conclude that survival after release was significantly lower for HH than for WW fish and that the time period of higher mortality for HH than for WW fish was between release and summer.

$ARS_{HW/WW}$ and $ARS_{HW/HH}$ were significantly lower for post-summer migrants than for summer residents (P<0.05; 2x2 G-tests of independence; Table 6). This indicates that survival between August and subsequent outmigration was significantly lower for HW fish than for the other two crosses. ARS estimates for post-summer migrants showed significant decreases of $\geq$27% following fry release in the frequency of HW fish relative to the other two crosses (Table 6); however, some of the decrease could have been accounted for by greater emigration for HW than for HH or WW fish during pre-summer. We considered total migrants to evaluate whether differential emigration during pre-summer (HW>HH,

HW>WW) was great enough to counteract opposite differential emigration during post-summer (HW<HH; HW<WW). $ARS_{HW/WW}$ and $ARS_{HW/HH}$ differed significantly between pre-and post-summer (P<0.05; 2x2 G-tests of independence), and sampling efficiency (proportion of the total subpopulation that was sampled) was much lower for pre- than for post-summer migrants (Table 6); thus pooling pre- and post-summer samples would have resulted in biased estimates of $ARS_{HW/WW}$ and $ARS_{HW/HH}$ for total migrants. Weighted estimates for total migrants corrected for unequal sampling efficiency but may suffer from variance associated with the subpopulation size estimates, particularly the pre-summer estimate (Table 6). Despite their shortcomings, the weighted estimates for total migrants suggest that survival from release to outmigration was lowest for HW fish, intermediate for HH fish, and highest for WW fish (Table 6). Apparently, the surfeit of HW pre-summer migrants was not sufficient to offset the deficit of HW post-summer migrants.

Abundance of summer residents varied among study sections with peak numbers in sections 2-4 (Figure 11). Distribution was similar between HH and WW fish, but HW fish were more frequent in sections 1-3 and less so in sections 4-7 relative to HH or to WW fish (P<0.05 [Bonferroni adjusted *a posteriori* comparisons]; 2x2 G-tests of independence).

Precocious males were common in the post-summer migration until 7 October (89% in a sub-sample examined for sexual maturity; n=35), but after 7 October only one precocious male was encountered (n=396). The frequency of WW relative to HH or to HW post-summer migrants was higher before 7 October than afterward (P<0.05; 2x2 G-tests of independence) (Figure 12). Emigration timing was similar between WW and the other two crosses after 7 October, and between HW and HH fish throughout the post-summer period (Figure 12).

Length and weight increased steadily during the study (Figure 13). Condition factor increased from the pre-summer migration to a peak in early fall, then decreased to a minimum in mid-winter and increased again through the spring (Figure 13). Because early fall (25 August-7 October) migrants exhibited a high incidence of precocious males, their size and condition were analyzed separately from the rest of the post-summer migrants. In the subsample of early fall migrants examined for maturity, precocious males had higher condition factors (P<0.01) but were similar to immature fish in length and weight ($P \geq 0.4$; unequal variance t-tests). A subsample of summer residents was also examined for maturity, and 7.5% were found to be precocious males (n=308). These precocious males were longer, heavier, and had higher condition factors than immature residents (P<0.01). Length (Figure 14) and weight of summer residents increased from the upper to the lower end of the study reach.

Length and weight of pre-summer migrants were similar among crosses; condition was higher for HH than for HW or WW migrants late in the pre-summer period (Table 12). Length and perhaps weight were greater for WW than for HH or HW summer residents (P_{cross}=0.03 for length and 0.08 for weight; 0.05<P<0.10 for paired comparisons of length; Table 12) but the length differences were somewhat inconsistent among stream sections ($P_{cross*section}$=0.08; Figure 14). Condition of summer residents was similar among crosses (Table 12).

Length and weight were consistently greater for WW than for HW post-summer migrants (Figure 13; differences were significant after 7 October but not before; Table 12). Length and weight of HH post-summer migrants were usually intermediate but closer to HW than to WW (Figure 13; Table 12). After 7 October, condition decreased more during fall and increased more the following spring for WW than for HW or HH migrants (highly significant interactions between cross and both the linear and quadratic terms of the time trend; Table 12) with the result that condition was lower for WW than for the other crosses in December-January but higher the following April (Figure 13). The size differences among crosses for summer residents and for post-summer migrants, although consistent and usually significant, were

relatively small (e.g., WW fish were 1.6% longer and 3.8% heavier, on average, than HW post-summer migrants after 7 October; Table 12).

In the sub-sample of summer residents examined for maturity, 11.2% of the WW fish were precocious males compared to 5.4% of HH and 6.0% of HW fish (Table 12). This difference was not significant statistically (P=0.29), but it suggests that incidence of precocious males was higher for WW than for the other crosses when considered along with the higher frequency of WW relative to HH or to HW migrants in the early fall period (Figure 12), a time when most migrants were mature males.

Almost no fish remained in the stream after spring 1998. We were able to find only three residuals over the entire study reach in July 1998.

Comparison of relative survival between hatchery and natural rearing

Domestication is expected to increase fitness of the hatchery population for hatchery rearing but decrease fitness of the hatchery population for natural rearing compared to its wild founder population. We tested for domestication of the hatchery population by comparing $RS_{HH/WW}$ between hatchery and natural rearing. Domestication would be indicated if $RS_{HH/WW}$ under hatchery rearing was >1, $RS_{HH/WW}$ under natural rearing was <1, and $RS_{HH/WW}$ differed significantly between hatchery and natural rearing. Ideally, RS should be to returning adult from hatchery reared juveniles or from naturally reared juveniles. For our study, RS under natural rearing was only available to emigration of juveniles from LWSR. Nevertheless, comparing naturally reared juvenile outmigrants to hatchery reared returning adults is valid as long as egg-to-adult $RS_{HH/WW}$ is no greater than egg-to-outmigrant $RS_{HH/WW}$ under natural rearing, and other studies indicate that this is usually the case (e.g., Leider et al. 1990). There was no point in comparing naturally reared juveniles to hatchery reared juveniles because mortality of juveniles in the hatchery was negligible.

$RS_{HH/WW}$ of the 1996 year-class was marginally higher under hatchery rearing (based on adult returns) than under natural rearing (based on pooled summer residents and post-summer migrants) (P=0.07; Figure 15). Neither $RS_{HW/WW}$ nor $RS_{HW/HH}$ could be statistically compared between rearing environments because for natural rearing, temporal changes in the frequency of HW relative to the other crosses and in sampling efficiency precluded pooling samples over subpopulations (see above). However, weighted estimates of ARS for total migrants suggested that the relative survival difference between rearing environments was greater for the HW/WW pair than for HH/WW, and less for HW/HH than for HH/WW (Figure 15).

Discussion

We found differences among crosses on a number of measures (summarized in Table 13); however, several issues complicate interpretation.

Potentially confounding factors

Non-neutrality of genetic marks.—The *sSOD-1** genotypes used to mark crosses were shown not to be selectively neutral in a separate study (Hayes 2011). Differences among genotypes were found in egg-to-adult survival in the Warm Springs Hatchery program and in egg-to-summer resident survival in south-central Washington streams. When survival differences occurred, the *-100/-100* genotype (the HH mark) was favored over *-260/-260* (the WW mark); *-100/-260* (the HW mark) was intermediate. Differences in growth were less common than in survival, but when they occurred *-100/-100* was again favored over *-260/-260*; *-100/-260* was similar to *-260/-260* rather than intermediate.

Despite superior survival and growth, the *-*100* allele shows no evidence of moving toward fixation in the salmon populations so it appears that the selection actually occurred at another locus, temporarily in linkage disequilibrium with *sSOD-1*, or that selection differentials change under different environmental conditions, sometimes favoring the *-*260* allele. Regardless of the cause, differential performance of the marks poses an obvious problem for interpretation of experimental results.

The current study and the mark evaluation study were conducted under similar conditions and we might therefore hypothesize that the marks performed similarly between the two studies. Egg-to-adult survival of 1992 and 1996 year-class fish at Warm Springs Hatchery were assessed concurrently by both studies. The mark evaluation study assessed fry-to-outmigrant survival of the 1999 year-class in the same stream (LWSR) as the current study, although not with the same stock (Chinook salmon from LWSR Hatchery rather than Warm Springs).

Higher fry-to-outmigrant survival for WW than for HH fish in a stream was a key result of our study. This result probably was not confounded by the genetic marks if the hypothesis of similar mark performance between the two studies is true because the mark evaluation study showed the WW mark to be either disfavored or neutral in streams. Survival comparisons between HH and WW fish at the hatchery would be confounded because WW fish may have done better, relative to HH fish, had they not carried the disfavored mark. Growth differences probably were not confounded because growth was sometimes slower but never faster for HH than for WW fish, whereas growth in the mark evaluation study was sometimes faster but never slower for fish with HH mark compared to those with the WW mark.

HW neither intermediate nor similar to HH.—Common ancestry and almost continuous gene flow between hatchery and wild populations (see above) should prevent genetic differences that result in hybrid vigor or severe outbreeding depression in hybirds. Because 1996 year-class HW families had the same mothers as HH families and the same fathers as WW families, performance of HW fish should have been either intermediate to that of the other crosses, indicating additive genetic differences (from domestication) between the hatchery and wild parents, or similar to that of HH fish, indicating that a maternal effect, not a genetic difference, caused observed differences between HH and WW. Performance of HW fish often was neither.

Survival in the stream from summer resident to post-summer migrant was lower for HW than for HH or for WW fish. HW fish also grew faster at the hatchery than either of the pure types (higher proportion in upper length mode at 20 months of age; Table 7) and had a different summer distribution in the LWSR (Figure 11). Our key result that survival was higher for WW than for HH fish in a stream is contradicted by the lower survival for HW than for HH fish in the stream. The former suggests that wild fish are more fit for natural rearing than are hatchery fish whereas the latter suggests the opposite. We note that HW performance for the 1992 year-class was intermediate for the trait that differed most among crosses, growth at the hatchery (Table 7) and was extreme for only one measure, days to death during seawater rearing (fewest for HW; Table 8).

Other than heterosis or outbreeding depression which were unlikely as discussed above, possible explanations for extreme performance of HW fish include: (1) a subset of HW families was unrepresentative of the "hybrid population", for example from atypically high genetic compatibility or incompatibility between particular pairs of parents, and (2) novel environmental conditions favored the HW cross over either pure type. In support of the former was the higher incidence of HW abnormal alevins compared to those from the other crosses and the fact the abnormalities resulted from a small number of families ($\leq$5). In opposition to the latter, HW performance was poor in the LWSR, which was novel to both HH and WW fish (i.e., to both the hatchery and wild populations), whereas HW performance may have been superior at the hatchery, an environment that was not novel to HH fish. But regardless of the cause(s), extreme HW performance seriously impairs interpretation of our results,

including the key finding of higher survival for WW than for HH fish in a stream. We cannot with certainty resolve the seeming contradiction that survival in the stream was higher for WW than for HH fish yet lower for HW than for HH fish.

Maternal effects.—Only a portion of 1992 year-class HH and HW fish were maternal half-siblings, but growth of that year-class was slowest for HH, intermediate for HW, and fastest for WW fish (Table 7) indicating that the differences were due to additive genetic effects. All 1996 year-class HH and HW fish were maternal half-siblings so any differences between them could not have been due to maternal effects. Growth in the hatchery and survival in a stream differed between HH and HW fish of that year-class, but HW performance was not intermediate to that HH and WW fish as discussed above. This raises the question of whether differences between 1996 year-class HH and WW fish could have been due to maternal effects even though differences between HH and HW fish were not. We note that egg size, which determines fry size and can affect survival and growth in streams (Einum and Fleming 2000), and ELISA absorbance of mothers, a measure of vertically transmissible BKD that can affect offspring survival (Pascho et al. 1991), were both similar between hatchery and wild females in 1996 (Table 3), suggesting that differences between HH and WW fish were not due to either of these maternal effects. Of all the factors that could have confounded our results, maternal effect seems the least likely .

Comparison between year-classes

Six measures are comparable between year-classes: size and condition of juveniles near the end of hatchery rearing, days to death and ELISA level for juveniles in seawater, fry-to-adult survival in the hatchery program, and size of age-4 adults. Fry-to-release survival of juveniles at the hatchery is not included since mortality at the hatchery was negligible. If we only consider differences among crosses that are at least marginally significant, there was little consistency between year-classes on these six measures. The only agreement was for size of HW relative to HH juveniles at the hatchery (HW>HH; Table 13). If, on the other hand, the direction of differences between means is considered regardless of whether the differences were significant, there was more consistency between year-classes, particularly when only HH and WW fish were compared (Table 14). When looked at this way, performance of HH relative to WW fish was consistent between year-classes for three of the four juvenile measures and one of the two adult measures (Table 14). Similar to findings for juveniles in the hatchery, mean size of 1996 year-class juveniles in a stream was also greater for WW than for HH fish, although not significantly so (Table 12). Note that 1992 year-class adult measures were based on very small sample sizes ($n_{survival}$=18; n_{size}=14). Treatment of juveniles at the hatchery was similar between year-classes except that the 1996 year-class received medicated feed, shaded raceways, and a fall volitional release whereas the 1992 year-class did not. Also, the hatchery broodstock included 40% wild fish in 1988, when most hatchery parents to our 1992 year-class offspring were conceived, but only 10% wild fish in 1992 when most hatchery parents to our 1996 year-class offspring were conceived (Olson et al. 2004). Thus hatchery parents may have been more similar to wild parents in 1992 than in 1996.

Conclusions

Interpretation of our results is seriously impaired by several potentially confounding factors discussed above. Nevertheless, we note that (1) *if* extreme performance by HW fish was due to anomalous HW families as we suspect (rather than to "problems" with either HH or WW fish), and (2) *if* any selection on the genetic marks acted in the same direction as observed in our mark evaluation study under similar conditions (Hayes et al., 2011), and (3) *if* differences between HH and WW fish were not due to maternal effects, then fry-to-outmigrant survival in a stream was higher for WW fish than for HH fish. This result would in turn indicate genetic divergence of the hatchery population from the wild founder population and a resultant, albeit small, loss of fitness for natural rearing ($RS_{HH/WW}$=0.91). Such a result would agree with those of many other studies that have tested for genetic differences between

hatchery and wild populations (reviewed by Reisenbichler and Rubin 1999 and Araki et al. 2008). Our results also suggest genetic differences between the hatchery and wild populations in several other traits (Table 13). Our conditional conclusion suggests that practices at Warm Springs Hatchery have not been sufficient to prevent genetic divergence between the hatchery and wild populations in traits associated with fitness. Nevertheless, the three conditions for this conclusion remain unsubstantiated, and it is unclear whether domestication has occurred in this hatchery population.

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References

Aebersold, P. B., G. A. Winans, D. J. Teel, G. B. Milner, and F. M. Utter. 1987. Manual for starch gel electrophoresis – a method for the detection of genetic variation. NOAA Technical Report NMFS 61.

Applebee, A. E., and J. M. Tipping. 1991. Use of electronic counters for coho and Chinook salmon, steelhead, and cutthroat trout smolts. The Progressive Fish Culturist 53:195-198.

Araki, H., Berejikian, B. A., Ford, M. J. & Blouin, M. S. 2008. Fitness of hatchery-reared salmonids in the wild. Evol. Appl. 1, 342–355.

Bams R. A. 1970. Evaluation of a revised hatchery method tested on pink and chum salmon fry. Journal of the Fisheries Research Board of Canada 27:1429-1452.

Bisson, P. A., J. L. Nielson, R. Palmason, and L. E. Grove. 1982. A system of naming habitat types in small streams, with examples of habitat utilization by salmonids during low stream flows. Pages 62-73 *in* N. B. Armantrout, editor. Acquisition and utilization of aquatic habitat inventory information. American Fisheries Society, Western Division, Bethesda, Maryland.

Bjorkstedt, E. P. 2000. DARR (Darroch Analysis with Rank-Reduction): A method for analysis of stratified mark-recapture data from small populations, with application to estimating abundance of smolts from outmigrant trap data. National Marine Fisheries Service, Administrative Report SC-00-02.

Einum, S., and I. A. Fleming. 2000. Highly fecund mothers sacrifice offspring survival to maximize fitness. Nature 405:565-567.

Haner, P. V., J. C. Faler, R. M. Schrock, D. W. Rondorf, and A. G. Maule. 1995. Skin reflectance as a non-lethal measure of smoltification for juvenile salmonids. North American Journal of Fisheries Managements 15:814-822.

Hankin, D. G. 1984. Multistage sampling designs in fisheries research: applications in small streams. Canadian Journal of Fisheries and Aquatic Sciences 41:1575-1591.

Hayes, M. C., R. R. Reisenbichler, S. P. Rubin, L.A. Wetzel, and A. R. Marshall. 2011. Differential survival among *sSOD-1** genotypes in Chinook salmon. Transactions of the American Fisheries Society 140:1305-1316.

Jefferts, K. B., P. K. Bergman, and H. F. Fiscus. 1963. A coded wire identification system for macro-organisms. Nature 198:460-462.

Jensen, J.O.T. 1988. A microcomputer program for predicting embryonic development on Pacific salmon and steelhead trout. World Aquaculture 19(4):80-81.

Leider, S. A., P. L. Hulett, J. J. Loch, and M. W. Chilcote. 1990. Electrophoretic comparison of the reproductive success of naturally spawning transplanted and wild steelhead trout through the returning adult stage. Aquaculture 88:239-252.

Lynch, M., and M. O'Hely. 2001. Captive breeding and the genetic fitness of natural populations. Conservation Genetics 2:363-378.

Olson, D. E., B. C. Cates, and D. H. Diggs. 1995. Use of a national fish hatchery to complement wild salmon and steelhead production in an Oregon stream. American Fisheries Society Symposium 15:317-328.

Olson, D. E., B. Spateholts, M. Paiya, and D. E. Campton. 2004. Salmon hatcheries for the 21st century: a model at Warm Springs National Fish Hatchery. Pages 585-602 *in* M. J. Nickum, P. M. Mazik, J. G. Nickum, and D. D. MacKinlay, editors. Propagated fish in resource management. American Fisheries Society, Symposium 44. Bethesda, Maryland.

Pascho, R. J., D. G. Elliott, and J. M. Streufert (1991). Brood stock segregation of spring Chinook salmon *Oncorhynchus tshawytscha* by use of the enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody technique (FAT) affects the prevalence and levels of *Renibacterium salmoninarum* infection in progeny. Diseases of Aquatic Organisms, 12(1):25-40.

Pascho, R.J. and D. Mulcahy. 1987. Enzyme-linked immunosorbent assay for a soluble antigen of *Renibacterium salmoninarum*, the causative agent of salmonid bacterial kidney disease. Canadian Journal of Fisheries and Aquatic Sciences 44:183-191.

Prentice, E.F., T.A. Flagg, C.S. McCutcheon, and D.F. Brastow. 1990. PIT-tag monitoring systems for hydroelectric dams and fish hatcheries. In: N.C. Parker, A.E. Giorgi, R.C. Heidinger, D.B. Jester, Jr., E.D. Prince, & G.A. Winans (editors), pp. 323-334. Fish-marking techniques. American Fisheries Society Symposium 7, Bethesda, MD.

Reisenbichler, R.R., and S.P. Rubin. 1999. Genetic changes from artificial propagation of Pacific salmon affect the productivity and viability of supplemented populations. ICES Journal of Marine Science 56:459-466.

Reisenbichler, R. R., S. P. Rubin, L.A. Wetzel, and S. R. Phelps. 2004. Natural selection after release from a hatchery leads to domestication in steelhead, *Oncorhynchus mykiss*. Pages 371-383 *in* K.M. Leber, H.L. Blankenship, S. Kitada, and T. Svåsand [editors] Stock Enhancement and Sea Ranching: developments, pitfalls, and opportunities. 2nd edition. Blackwell Science Ltd, Oxford.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Fisheries Research Board of Canada Bulletin No. 191.

Scheaffer, R. L., W. Mendenhall, and L. Ott. 1986. Elementary survey sampling, third edition. Duxbury Press, Boston.

Schrock, R. M. J. W. Beeman, D. W. Rondorf, and P. V. Haner. 1994. A microassay for gill sodium, potassium-activated ATPase in juvenile Pacific salmonids. Transactions of the American Fisheries Society 123:223-229.

Shaklee, J. B. F. W. Allendorf, D. C. Morizot, and G. S. Whitt. 1990. Gene nomenclature for protein-coding loci in fish. Transactions of the American Fisheries Society 119:2-15.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry, 3rd edition. W. H. Freeman and Company, New York.

Thedinga, J. F., M. L. Murphy, S. W. Johnson, J. M. Lorenz, and K. V. Koski. 1994. Determination of salmonid smolt yield with rotary-screw traps in the Situk River, Alaska, to predict effects of glacial flooding. North American Journal of Fisheries Management 14:837-851.

Van Deventer, J. S., and W. S. Platts. 1985. Computer software system for entering, managing, and analyzing fish capture data from streams. Research Note, Intermountain Research Station, USDA Forest Service, Ogden, Utah.

APPENDIX: Methods used to estimate number of outmigrants each week and number of summer residents in each stream section

Outmigrants

A screw trap was deployed at Elbow (Figure 1) on 29 April 1997. A second trap was deployed on 18 June at Elbow about 25m upstream from the first. On 23 September, one of the traps was moved to Big Cedars (Figure 1). Big Cedars seemed the better location after flows increased in the fall but provided space for only one trap. The traps were operated in this configuration (one at Big Cedars and the other at Elbow) through 2 June 1998.

Fish were released upstream for efficiency tests. These fish were given a caudal clip to specify trap of origin and release date in addition to the ventral fin clip for sSOD-1* analysis. We used four different caudal marks, two of which specified release date. The marks were alternated daily so we could tell whether a recapture had been released an odd or an even number of days previously. Over 90% of the recaptures had the odd-day clip suggesting that most fish were recaptured one day after release.

Fish caught at Elbow were released 1 km upstream but downstream from Big Cedars. Fish caught at Big Cedars were released 0.5 km upstream. Trap efficiency was estimated as the proportion of marked fish from a trap site that were recaptured at that trap site. Estimates were temporally stratified with one week set as the smallest time increment. When assigning recaptures to week of release, we assumed that recaptures with the odd-day clip had been released one day previously and those with the even-day clip two days previously. We used the DARR software package (Bjorkstedt 2000) to decide when to enlarge a stratum by pooling across two or more consecutive weeks to allow valid efficiency estimates while retaining as much information as possible about temporal variation. Trap efficiency did not differ among crosses at Big Cedars (P=0.43) or Elbow (P=0.96; G-tests of independence between recapture status [recaptured or not] and cross), so efficiency data were pooled over crosses.

While one trap was at Big Cedars and the other at Elbow (24 September to end of study), efficiencies were independently estimated for each trap and then summed. The combination represented the total efficiency of trapping for the two traps. Before combining, Elbow efficiency had to be adjusted (decreased) to account for fish that would have been caught at Big Cedars had the marked fish been released upstream from Big Cedars. The adjustment was accomplished by (1) expanding the Elbow catch of unmarked fish with unadjusted Elbow efficiency, (2) adding the Big Cedars catch of unmarked fish to the expansion, and (3) dividing the Elbow trap catch of unmarked fish by the sum (Table A1).

Recaptures at Elbow of fish actually released upstream from Big Cedars (i.e., of fish originally trapped at Big Cedars) were excluded to achieve comparable efficiency estimates for the two traps. The catch of unmarked fish indicated that efficiency of the Elbow trap was approximately one-half that of the Big Cedars trap because the former caught about half as many fish as the latter (Table A1). Excluding recaptures of Big Cedars fish yielded an Elbow efficiency approximately one-half that for Big Cedars, whereas Elbow efficiency estimated from recaptures of Big Cedars fish alone was only 0.18 that for Big Cedars (Table A1). Thus, excluding Big Cedars fish caught at Elbow gave an accurate estimate of Elbow efficiency relative to the estimated efficiency at Big Cedars. Estimated efficiency at both traps may have been too high; however, this bias did not affect our analysis because our primary purpose was to characterize temporal changes in efficiency rather than absolute efficiency.

During periods when we trapped at Big Cedars and at Elbow, efficiencies were independently estimated for each trap for temporal strata selected by the software (strata not identical between traps). The number of outmigrants was estimated on a weekly basis as the total number of unmarked fish captured in both traps divided by the sum of Big Cedars efficiency and adjusted Elbow efficiency (see above for explanation of adjustment). Similar procedures were used to estimate weekly outmigrants during the time when both traps were at Elbow, except that efficiency was estimated as the sum of the recaptures in both traps divided by the total number of marked fish released upstream. Weekly estimates of the number of outmigrants and associated variance were summed to obtain a total for the entire migration period.

Estimated trap efficiency during the first two weeks of efficiency tests (5-18 June 1997; trapping only at Elbow) was only 2.9% (69 marked fish released). We felt this estimate was unrealistically low, possibly because marked fish early in the run did not resume their downstream migration. Efficiency estimated the following week for the same trap was 6.3% (64 marked fish released). We applied an efficiency of 6.3% to the first two weeks (5-18 June) and to 16 May-4 June when efficiency tests were not conducted because all trapped fish were euthanized (Figure 10). Efficiency estimated during 26 June-1 July was extrapolated to the following three weeks, also because all trapped fish were euthanized during that period (Figure 10).

Summer residents

The number of fish in each of sections 1-7 (Figure 1) during summer 1997 was estimated by conducting snorkel counts in all pools and calibrating the counts with multiple pass removal or mark-recapture estimates in a subset of pools. Snorkel counts and removal/mark-recapture estimates were accomplished during 12 July-21 August. Counts were made by one snorkeler in small and medium sized pools and by two snorkelers in large pools so that the entire width could be examined simultaneously. Removal and mark-recapture pools were blocknetted during fish capture, and the nets were left in place overnight in the mark-recapture pools, to prevent fish from leaving or entering the pools. After the nets were placed but before otherwise disturbing the fish, a snorkel count was made. Three or four-pass removal estimates were made in two pools in section 1. Fishing upstream with two backpack electrofishers constituted a pass. Mark-recapture estimates were made in six pools throughout the remainder of the study reach. Fish were captured by seining, marked with a ventral fin clip, and released back into the pool. The following day, the pool was seined again, and captured fish were examined for marks. The Microfish computer program (Van Deventer and Platts 1986) was used to calculate population estimates and 95% confidence intervals from the removal data. Mark-recapture population estimates and 95% confidence intervals were calculated using Ricker's (1975) formulas for the adjusted Peterson estimate. Removal and mark-recapture estimates are given in Table A2 along with their associated snorkel counts.

The snorkel count in pools lacking removal or mark-recapture estimates was multiplied by 4.4, the ratio of the sum of the population estimates to the sum of the snorkel counts for the eight pools with both population estimates and snorkel counts (Table A2; Hankin 1984). The number of fish in each section was obtained by summing the expanded counts for the pools in the section. The total number of fish in the reach and its standard error were also estimated. This "two-stage estimation" accounted for variation among pools in the ratio of population estimates to snorkel counts (first stage) and the within-pool variance of the population estimates themselves (second stage; Hankin 1984).

Ratios of population estimate to snorkel count generally increased from upstream to downstream areas (Table A2), suggesting a corresponding decrease in the efficiency of our

snorkel counts. We suspect that the decrease was real but not as extreme as indicated. Snorkel counts conducted under similar conditions in a later year, but with more thorough calibration, gave mean ratios of 1.5 for section 1, 2.7 for sections 2-4, and 3.7 for sections 4-7 (U.S. Geological Survey, unpublished data). The use of a single expansion factor for the entire reach may have overestimated the number of fish in upstream sections and underestimated the number in downstream sections.

Because we snorkeled in pools only, fish occupying riffles were not included in our estimates; however, few fish occurred in riffle habitat. Number of fish was correlated with mean pool depth (r=0.54; P<0.001; n=78), and pools with mean depths <0.33 m contained few fish (mean=7; n=9). Although we did not measure depth in riffles, most appeared to be shallower than the shallowest pools.

Tables

Table 1. Number of adult spring Chinook salmon spawned and resulting number of full-sib families. The eggs from each female were divided into two portions, each fertilized by a different male. In 1992, each hatchery female was crossed with either two hatchery males, one hatchery and one wild male, or two wild males, whereas in 1996 each hatchery female was crossed with one hatchery and one wild male (Table 2). In 1992 but not in 1996, one of the two families produced by some females was discarded because a second assay indicated that a sire had originally been scored incorrectly for *sSOD-1** genotype. Males were crossed with a variable number of females (Table 2). The number of males not used on a previous date are given in parentheses (in 1992 males were used on only one date).

	Date	Females		Males		Families		
Year	spawned	Hatchery	Wild	Hatchery	Wild	HH	HW	WW
1992	27 Aug	7	2	6 (6)	3 (3)	10	4	4
	2 Sep	9	4	6 (6)	6 (6)	8	7	6
	8 Sep	2	6	4 (4)	3 (3)	4	0	9
	Total	18	12	(16)	(12)	22	11	19
1996	22 Aug	5	1[a]	5 (5)	3 (3)	5	5	3
	29 Aug	15	3	9 (9)	9 (6)	15	15	6
	5 Sep	8	4	8 (8)	8 (1)	8	8	8
	12 Sep	0	1	0 (0)	2 (1)	0	0	2
	Total	28	9	(22)	(11)	28	28	19

[a]Eggs were divided into three portions.

Table 2. Number of adult males or females producing various combinations of full-sib families.

Year	Sex	Stock	Number of males or females	Number of families		
				HH	HW	WW
1992	Females	Hatchery	1	0	1	
			2	0	2	
			2	1	0	
			6	1	1	
			7	2	0	
		Wild	5			1
			7			2
	Males	Hatchery	6	2		
			10	1		
		Wild	1		1	0
			1		1	1
			1		1	2
			1		2	2
			2		0	1
			3		0	3
			3		2	1
1996	Females	Hatchery	28	1	1	
		Wild	1			3
			8			2
	Males	Hatchery	6	2		
			16	1		
		Wild	1		0	1
			1		1	1
			1		2	1
			1		3	1
			1		4	1
			1		4	3
			1		6	3
			4		2	2

Table 3. ELISA absorbance and egg size of female parents; temperature, embryo density, and survival during incubation; and size and developmental index (k_D) of fry at release. Paired comparison results: HH v WW in HH column; HW v HH precedes comma and HW v WW succeeds comma in HW column; ** = P<0.05; * = 0.05<P<0.10; ns = not significant.

			Mean (SD)			
Year	Incubation site	Variable	HH	HW	WW	P_{cross}[a]
1992	Warm Springs	ELISA absorbance[b]	0.134 (0.071) ns	--	0.135 (0.051)	0.93
		Egg weight (mg)[bc]	269 (21) ns	--	260 (33)	0.46
		N eggs per colander[d]	1216 (260) ****	1279 (258) ns, ns	1472 (395)	**0.04**
		Survival to eye-up (%)[d]	86.9 (22.3) ns	88.1 (14.7) ns, ns	90.9 (18.4)	0.71
		N eyed embryos per tray[d]	1050 (350) *	1141 (329) ns, ns	1318 (417)	**0.08**
		Eye-to-fry survival (%)	96.1	96.4	97.3	--
		Fry length (mm)[ef]	35.2 (0.9) ns	35.4 (1.0) *, **	35.1 (0.9)	**0.01**
		Fry weight (mg)	391	381	383	--
		K_D	2.08	2.04	2.07	--
		Incubation temperature	6.9	6.8	7.1	--
1996	Warm Springs	ELISA absorbance[b]	0.182 (0.110) ns	--	0.213 (0.132)	0.54
		Egg weight (mg)[bg]	240 (32) ns	--	230 (26)	0.35
		N eggs per colander[d]	1400 (304) ns	1426 (312) ns, ns	1567 (199)	0.12
		Survival to eye-up (%)[d]	93.6 (9.4) ns	91.4 (11.1) ns, ns	93.2 (6.7)	0.62
		N eyed embryos per tray[hi]	1500 (363) ns	1484 (357) ns, ns	1167 (644)	0.31
		Eye-to-fry survival (%)	98.2	97.5	98.9	--
		Fry length (mm)[ej]	36.1 (0.3) ns	36.2 (0.4) ns, ns	35.9 (0.4)	--
		Fry weight (mg)	372	368	350	--
		K_D	1.99	1.98	1.96	--
		Incubation temperature	7.4	7.4	7.5	--
	Carson Depot Springs	*N* eyed embryos per tray[hk]	1940 (576) ns	1920 (576) ns, ns	2006 (400)	0.93
		Eye-to-fry survival (%)	98.3	97.4	98.9	--
		Fry length (mm)[ej]	35.3 (0.3) ns	35.3 (0.3) ns, ns	34.9 (0.2) ns	--
		Fry weight (mg)	382	365	365	--

K_D	2.06	2.02	2.04	--
Incubation temperature	7.8	7.8	8.0	--

[a]H_o: No difference among the three crosses.
[b]Standard deviation among females; *n* from Table 1.
[c]Water-hardened eggs.
[d]Standard deviation among families; *n* from Table 1.
[e]Standard deviation among individuals.
[f]n = 10 per family.
[g]Unfertilized eggs.
[h]Standard deviation among incubator trays; each tray contained a mix of families.
[i]n = 8 per cross.
[j]$4 \leq n \leq 10$ per incubation tray. The number of fry differed considerably among trays (mean range = 1000-2200) and was not proportional to number of fry measured, therefore mean and SD length in each tray were weighted by the number of fry in the tray to compute mean and SD length (and SE mean length; Scheaffer et al. 1986) for each cross. Mean fry weight and K_D were computed similarly. We compared mean length between pairs of crosses using SEs of mean length but did not conduct an overall test for differences among the three crosses.
[k]n = 12, 12, and 9 for HH, HW, and WW, respectively.

Table 4. Relative survivals under hatchery rearing. ** = $P<0.05$; * = $0.05<P<0.10$; ns = not significant.

Year-class	Life stage	Age[a]	N[b]	n	Proportion HH	Proportion HW	Proportion WW	Relative survival HH/WW	Relative survival HW/WW	Relative survival HW/HH	P_{cross}[c]
1992	Fry	5 months									
		Pond 1	30,236		0.375	0.207	0.418				
		Pond 2	29,251		0.385	0.203	0.413				
		Total	59,487		0.380	0.205	0.415				
	Juveniles	18 months									
		Pond 1	27,976	299	0.348	0.187	0.465	0.83 ns	0.81 ns	0.97 ns	0.26
		Pond 2	27,549	300	0.417	0.213	0.370	1.21 ns	1.17 ns	0.97 ns	0.32
		Total	55,525	599	0.382	0.200	0.417	1.00 ns	0.97 ns	0.97 ns	0.96
	Adults	3 years	6	3	0.000	0.667	0.333	0.00	4.05	--	--
		4 years	21	14	0.429	0.143	0.429	1.09	0.68	0.62	--
		5 years	1	1	0.000	0.000	1.000	0.00	0.00	--	--
		Total	28	18	0.333	0.222	0.444	0.82 ns	1.01 ns	1.24 ns	0.92
1996	Fry	5 months	32,454		0.362	0.355	0.283				
	Juveniles	15 months									
		Migrants	3,098	439	0.410	0.362	0.228	**1.41 ****	1.27 ns	0.90 ns	**0.02**
		Non	28,463	458	0.373	0.328	0.299	0.98 ns	0.87 ns	0.89 ns	0.46
		Total	31,561								
		20 months	28,426	665	0.358	0.326	0.316	0.89 ns	0.82 ns	0.93 ns	0.14

Adults	3 years	43	35	0.371	0.371	0.257	1.13	1.15	1.02	--
	4 years									
	Fishery		44	0.386	0.318	0.295	1.02	0.86	0.84	--
	Hatchery		62	0.323	0.532	0.145	1.74	2.93	1.68	--
	Total	375	106	0.349	0.443	0.208	1.32	1.71	1.29	--
	5 years	15	6[d]	0.667	0.333	0.000	--	--	0.51	--
	Total	433	147	0.367	0.422	0.211	1.37 ns	**1.60 ***	1.17 ns	**0.09**

[a]From conception.
[b]Fry: number ponded. Juveniles: number marked minus subsequent mortalities. Adults: estimated total return to the Deschutes River; based on CWT recovery rates (our experimental fish relative to all fish tagged at Warm Springs Hatchery) and estimated total return to the Deschutes River of Warm Springs hatchery stock.
[c]H_o: No difference in survival among the three crosses.
[d]All were recovered at the hatchery. Although the fishery was sampled (see methods), no age-5 adults were recovered

Table 5. Harvest rate for Warm Springs hatchery fish in the Deschutes River, and sampling efficiency in the fishery and the hatchery (the percentage of fish caught in the Deschutes River fishery and returning to the hatchery that were sampled). Dashes indicate no sampling.

Return			Number of returns			Harvest	Number sampled		Sampling efficiency (%)	
year	Life stage	Age(s)	To fishery	To hatchery	Total	rate (%)	From fishery	At hatchery	From fishery	At hatchery
1995	Jack/Jill	3	0	146	146	0.0	--	121	--	82.9
1996	Adult	4 and 5	108	624	732	14.8	0	520	0.0	83.3
1997	Adult	4 and 5	0	1,072	1,072	0.0	--	746	--	69.6
1999	Jack/Jill	3	5	889	894	0.6	0	742	0.0	83.5
2000	Adult	4 and 5	2,642	6,567	9,209	28.7	819	2,142	31.0	32.6
2001	Adult	4 and 5	1,024	3,659	4,683	21.9	293	1,865	28.6	51.0

Table 6. Apparent relative survival (ARS) of naturally reared fish. Pooled estimates for total migrants were precluded by differences in sampling efficiency (proportion of the total subpopulation sampled) between pre- and post-summer migrants; weighted estimates for total migrants corrected for these sampling efficiency differences. ** = $P<0.05$; * = $0.05<P<0.10$; ns = not significant.

		Sub-population					
Estimate type	Estimate	Released fry	Pre-summer migrants	Summer residents (SR)	Post-summer migrants (PoSM)	SR+PoSM	Total migrants
Pooled[a]	*N*	62,958[b]	97	1,970	1,242	3,046[c]	1339
	Proportion						
	HH	0.362	0.289	0.354	0.389	0.364	0.382
	HW	0.356	0.443	0.343	0.279	0.322	0.291
	WW	0.283	0.268	0.303	0.332	0.314	0.327
	ARS						
	HH/WW		0.84 ns	0.91 ns	0.92 ns	**0.91 ****	0.91 ns
	HW/WW		1.31 ns	0.90 ns	**0.67 ****		
	HW/HH		1.56 ns	0.99 ns	**0.73 ****		
	P_{cross}[d]		0.17	0.14	**0.00**		
Weighted[e]	*N*hat[f] (SE)		3,130 (≥981[g])	8,432 (1,457)	4,569 (421)		7,699 (≥1067[g])
	Proportion						
	HH		0.321	0.363	0.388		0.361
	HW		0.408	0.330	0.277		0.330
	WW		0.271	0.307	0.335		0.309
	ARS						
	HH/WW		0.93	0.92	0.90		0.91
	HW/WW		1.20	0.85	0.66		0.85
	HW/HH		1.29	0.93	0.73		0.93

[a]Samples were pooled across strata (weeks for migrants; sections for residents).
[b]Total number released.
[c]Post-summer migrants that had been sampled as summer residents were excluded.
[d]H_o: No difference in apparent survival among the three crosses.
[e]Samples were weighted by the estimated number of fish in each stratum.
[f]Estimated total number of fish in the sub-population.
[g]This is a minimum because of uncertainties associated with extrapolating trap efficiencies (Figure 10).

Table 7. Size, condition factor (*K*), maturity, and smoltification indices of hatchery-reared juveniles. Upper length mode (%) = percent of individuals in upper length mode (Figure 3). Paired comparison results: HH v WW in HH column; HW v HH precedes comma and HW v WW succeeds comma in HW column; ** = $P<0.05$; * = $0.05<P<0.10$; ns = not significant.

Year-class	Age[a] (months)	Variable	Mean (SD) [*n*] HH	HW	WW	Analysis[b]	P_{cross}[c]
1992	18						
	Pond 1	Fork length (mm)	120 (13) [103]	127 (23) [56]	135 (27) [139]		
		Upper length mode (%)	1.9 [103]	8.9 [56]	19.4 [139]		
		Weight (g)	18.6 (8.9) [103]	23.6 (16.5) [56]	28.7 (19.6) [139]		
		K	1.028 (0.055) [103]	1.033 (0.046) [56]	1.031 (0.048) [139]		
	Pond 2	Fork length (mm)	123 (16) [125]	130 (23) [64]	139 (27) [109]		
		Upper length mode (%)	3.2 [125]	10.9 [64]	21.1 [109]		
		Weight (g)	20.4 (11.1) [125]	24.9 (16.2) [64]	31.0 (20.4) [109]		
		K	1.029 (0.050) [125]	1.039 (0.060) [64]	1.043 (0.042) [109]		
	Pooled	Fork length (mm)	122 (15) [228] ****	129 (23) [120] **, **	137 (27) [248]	Kruskal	**0.00**
		Upper length mode (%)	2.6 [228] ****	10.0 [120] **, **	20.2 [248]	Indep	**0.00**
		Weight (g)	19.6 (10.2) [228] ****	24.3 (16.3) [120] **, **	29.7 (19.9) [248]	Kruskal	**0.00**
		K	1.028 (0.052) [228] ns	1.036 (0.054) [120] ns, ns	1.037 (0.046) [248]	AOV	0.17
1996	15						
	Migrant	Fork length (mm)	120 (17) [179] ns	124 (21) [158] ns, ns	122 (21) [100]	Kruskal	0.20
		Upper length mode (%)	8.4 [179] ns	13.9 [158] ns, ns	11.0 [100]	Indep	0.28
		Weight (g)	20.4 (10.2) [179] ns	23.1 (13.6) [158] ns, ns	21.8 (14.8) [100]	Kruskal	0.20
		K	1.114 (0.060) [179] ns	1.110 (0.066) [158] ns, ns	1.106 (0.057) [100]	AOV	0.59
		Mature males (%)	1.7 [180] ns	0.6 [160] ns, ns	4.0 [101]	Indep	0.24

Non	Fork length (mm)	122 (19) [169] ns	125 (22) [150] ns, ns	123 (22) [136]	Kruskal	0.30
	Upper length mode (%)	11.2 [169] ns	14.0 [150] ns, ns	14.0 [136]	Indep	0.69
	Weight (g)	22.5 (11.0) [169] ns	24.0 (14.4) [150] ns, ns	22.4 (13.6) [136]	Kruskal	0.14
	K	1.154 (0.056) [169] ****	1.140 (0.058) [150] ****, ****	1.122 (0.071) [136]	AOV	**0.00**
	Mature males (%)	0.0 [171]	0.0 [151]	0.0 [137]		
20	Fork length (mm)	138 (13) [236] ns	144 (19) [212] ns, ns	141 (14) [206]	Kruskal	**0.09**
	Upper length mode (%)	5.9 [236] ns	18.4 [212] ****, ****	8.3 [206]	Indep	**0.00**
	Weight (g)	27.8 (9.1) [236] ns	32.1 (14.9) [212] ns, ns	28.8 (10.4) [206]	Kruskal	0.14
	K	1.022 (0.042) [236] ****	1.025 (0.048) [212] ns, ****	1.001 (0.039) [206]	AOV	**0.00**
	ATPase	12.4 (4.9) [52] ns	13.1 (4.8) [72] ns, ns	14.2 (4.5) [51]	AOV[d]	0.11
	Skin reflectance	5.22 (0.71) [237] ns	5.21 (0.69) [213] ns, ns	5.35 (0.68) [207]	AOV	**0.07**

[a]From conception.
[b]Kruskal = Kruskal-Wallis; Indep = *G*-test of independence; AOV = analysis of variance.
[c]H_o: No difference among the three crosses.
[d]On square root transformed ATPase.

Table 8. Survival, growth, and ELISA absorbance of juveniles reared in seawater. Second factor in analyses was tank. Paired comparison results: HH v WW in HH column; HW v HH precedes comma and HW v WW succeeds comma in HW column; ** = $P<0.05$; * = $0.05<P<0.10$; ns = not significant.

Year-class	Variable	Mean (SD[a]) [n[b]] HH	HW	WW	Analysis[c]	P_{cross}[d]	$P_{cross*tank}$[e]
1992	Days to death[f]	77.9 (16.6) [113] ns	71.5 (20.4) [57] *****, ******	80.5 (17.7) [111]	2-way AOV	**0.01**	0.79
	Growth rate (mm/d)[g]	0.049 (0.068) [113] ns	0.064 (0.061) [56] ns, ns	0.058 (0.064) [111]	2-way AOCV[h]	0.90	0.82
	ELISA absorbance	1.74 (0.48) [113] ns	1.69 (0.57) [57] ns, ns	1.81 (0.48) [111]	2-way AOV	0.37	0.69
1996	Survival to 13 Jul (all tanks)	0.651 [166] ns	0.695 [128] ns, ns	0.757 [136]	Indep	0.13	
	Survival to 23 Sep (tanks without super-sat. problem)	0.017 [115] ns	0.068 [88] ns, ns	0.063 [95]	Indep	0.16	
	Days to death for fish that died before 13 Jul (all tanks)	64.7 (16.7) [58] *****	72.8 (10.5) [39] *****, ns	73.5 (11.4) [33]	2-way AOV	**0.03**	0.61
	Days to death for fish that died before 23 Sep (tanks without problem)	100.8 (28.9) [113] ns	108.0 (27.6) [82] ns, ns	108.1 (25.6) [89]	2-way AOV	**0.10**	**0.10**
	Growth rate (mm/d), 15 Apr-8 Jun[i]	0.164 (0.057) [147] ns	0.153 (0.059) [121] ns, ns	0.152 (0.062) [129]	2-way AOV	0.18	0.89
	Growth rate (mm/d), 8 Jun-20 Jul[j]	0.120 (0.072) [103] *****	0.111 (0.079) [86] ns, ******	0.146 (0.077) [92]	2-way AOV	**0.01**	**0.08**
	ELISA absorbance	1.87 (1.11) [166] ns	1.96 (0.98) [126] ns, ns	1.90 (1.10) [134]	2-way AOV	0.77	0.54
	Percent in high ELISA mode[k]	63.9 [166] ns	67.5 [126] ns, ns	64.2 [134]	Indep	0.79	

[a]Least square mean (root MSE) if 2-way AOV or AOCV; value from data pooled over tanks if independence test.
[b]For survival estimates, *n* is the number of individuals at the start of seawater rearing.
[c]AOV = analysis of variance; AOCV = analysis of covariance; indep = *G*-test of independence.
[d]H_o: No difference among crosses.
[e]H_o: No interaction between cross and tank.
[f]Number of days between tagging and death.
[g]Difference between length at death and length at tagging divided by days to death.
[h]Covariate was length at tagging; it was inversely related to growth rate ($P<0.01$) but did not interact with cross or tank ($P>0.10$).
[i]Mortalities were excluded.
[j]Mortalities from causes other than the super-saturation problem were excluded; growth rate for super-saturation mortalities was for 8 Jun-13 Jul.
[k]Boundary between modes given in Figure 3.

Table 9. Sex ratio ($n_{females}/(n_{females}+n_{males})$) of adult returns.

Year-class	Collection site	Age(s)	Sex ratio [*n*] HH	HW	WW	*P*-value[a]
1992	Hatchery	3-5 pooled	0.667 [6]	0.750 [4]	0.750 [8]	1.00
1996	Hatchery	3-5 pooled	0.571 [7]	0.500 [10]	0.250 [4]	0.72
	Fishery	4	0.688 [16]	0.643 [14]	0.333 [12]	0.19
	Pooled	Pooled	0.652 [23]	0.583 [24]	0.313 [16]	0.10

[a]H_o: No difference in sex ratio among crosses.

Table 10. Spawning date of age-4 females.

Year-class	Date spawned	Number spawned HH	HW	WW
1992	29 Aug	3	0	4
	5 Sep	1	2	1
1996	31 Aug	1	2	1
	12 Sep	0	1	0

Table 11. Success of assigning pre-summer migrants to cross from *sSOD-1** analysis, and apparent relative survival estimated from successful assignments. ** = $P<0.05$; * = $0.05<P<0.10$; ns = not significant.

		n		Proportion of total assigned			Apparent relative survival			
Time period	Tissue	Un-assigned	Assigned	HH	HW	WW	HH/WW	HW/WW	HW/HH	P_{cross}[a]
16 May-3 Jun	Muscle	0	44	0.341	0.386	0.273	0.98 ns	1.13 ns	1.15 ns	0.91
4-30 Jun	Fin	124	95	0.316	0.368	0.316	0.78 ns	0.93 ns	1.19 ns	0.61
1-21 Jul	Muscle	0	53	0.245	0.491	0.264	0.73 ns	1.48 ns	2.03 *	0.10
1 and 3 pooled	Muscle	0	97	0.289	0.443	0.268	0.84 ns	1.31 ns	1.56 ns	0.17

[a]H_o: No difference in apparent survival among the three crosses.

Table 12. Size, condition factor (*K*), maturity, and gill ATPase of naturally reared juveniles. Paired comparisons: HH v WW in HH column; HW v HH precedes comma and HW v WW succeeds comma in HW column; ** = $P<0.05$; * = $0.05<P<0.10$; ns = not significant.

Sub-population	Capture dates	Variable	Mean (SD)[a] [*n*] HH	HW	WW	Analysis[b]	Time trend[c]	P_{cross}	$P_{interaction}$ Fact 2 or lin[d]	Quad[e]
Pre-summer migrants	16 May-3 Jun 1997	Fork length (mm)	67.5 (5.4) [15] ns	70.5 (5.5) [17] ns, ns	69.8 (8.0) [12]	1-way AOV		0.40		
		Weight (g)	3.37 (0.79) [15] ns	3.82 (0.97) [17] ns, ns	3.71 (1.46) [12]	1-way AOV		0.47		
		K	1.072 (0.043) [15] ns	1.071 (0.037) [17] ns, ns	1.047 (0.045) [12]	1-way AOV		0.21		
	1-21 Jul 1997	Fork length (mm)	78.4 (3.6) [13] ns	78.5 (5.2) [26] ns, ns	76.6 (7.4) [14]	1-way AOV		0.56		
		Weight (g)	5.66 (1.05) [13] ns	5.11 (1.02) [26] ns, ns	4.96 (1.45) [14]	1-way AOV		0.25		
		K	1.167 (0.132) [13] *	1.051 (0.117) [26] **, ns	1.075 (0.091) [14]	1-way AOV		**0.02**		
Summer residents	5-26 Aug 1997	Fork length (mm)	83.6 (5.3) [698] *	83.6 (5.6) [673] ns, *	84.5 (5.6) [595]	2-way AOV[f]		**0.03**	0.08	
		Weight (g)	7.03 (1.52) [698] ns	7.00 (1.53) [672] ns, ns	7.23 (1.72) [595]	2-way AOV[f]		**0.08**	0.23	
		K	1.183 (0.093) [698] ns	1.180 (0.092) [672] ns, ns	1.176 (0.096) [594]	2-way AOV[f]		0.48	0.28	
		Mature males (%)	5.4 [92] ns	6.0 [116] ns, ns	11.2 [98]	Indep		0.29		
Post-summer migrants	25 Aug-7 Oct 1997	Fork length (mm)	93.3 (5.9) [29]	93.7 (5.0) [19] ns, ns	94.6 (6.4) [45]	1-way AOV		0.66		
		Weight (g)	9.91 (1.78) [29]	9.87 (1.55) [19] ns, ns	10.30 (2.02) [45]	1-way AOV		0.58		
		K	1.211 (0.074) [29]	1.191 (0.082) [19] ns, ns	1.206 (0.077) [45]	1-way AOV		0.69		
		Mature males (%)	85.7 [7] ns	77.8 [9] ns, ns	94.7 [19]	Indep		0.79		
	8 Oct 1997-4 May 1998	Fork length (mm)	103.1 (6.4) [454] ns	102.3 (7.1) [328] ns, **	103.9 (6.6) [366]	AOCV	Linear	**0.01**	0.44	
		Weight (g)	11.89 (2.28) [453] ns	11.73 (2.44) [327] ns, **	12.17 (2.30) [367]	AOCV	Quad	**0.04**	0.54	0.50
		K	1.064 (0.051) [452]	1.072 (0.049) [327]	1.064 (0.053) [366]	AOCV	Quad	[g]	**0.01**[h]	**0.00**[h]
		Mature	0.0 [164] ns	0.0 [103] ns, ns	0.8 [123]	Indep		0.58		

	males (%)							
2 Mar-5 May 1998	ATPase	14.6 (7.6) [57] ns	13.9 (5.2) [30] ns, ns	13.4 (4.9) [40]	AOCV	Linear	0.66	0.71

[a]Least square mean (root MSE) if 2-way AOV or AOCV.
[b]AOV = analysis of variance; Indep = *G*-test of independence; AOCV = analysis of covariance.
[c]If AOCV, is the time trend linear or quadratic?
[d]Interaction of cross with factor 2 if 2-way AOV or with linear term of time trend if AOCV.
[e]Interaction of cross with quadratic term of time trend.
[f]Second factor is stream section (Figure 1).
[g]Main effects comparison invalid due to significant cross*time interaction.
[h]Cross*time interaction significant ($P<0.05$) for comparison between HH and WW, and between HW and WW, but not between HW and HH.

Table 13. Metrics that differed among crosses.

Environ-ment	Life stage	Year-class	Metric	Difference[a]	Signif-icance[b]
Incubation	Alevin	1992	Fry length	HW>HH=WW	High
		1996	Incidence of abnormalities	HW>WW=HH	High
Hatchery	Juvenile	1992	Length and weight at 18 months	WW>HW>HH	High
		1996	Number of fall migrants (relative to expectations)	HH>WW	High
			Condition of non-migrants at 15 months	HH>HW>WW	High
			Length at 20 months (mean)	HW>HH	Marginal
			Length at 20 months (percent in upper length mode)	HW>WW=HH	High
			Condition at 20 months	HW=HH>WW	High
			Skin reflectance	WW>HW	Marginal
	Seawater	1992	Days to death	WW=HH>HW	High
		1996	Days to death	WW>HH	Marginal[c]
			Growth (8 Jun-20 Jul)	WW>HH=HW	High
	Adult	1996	Survival	HW>WW	Marginal
			Harvest rate of age-4s	WW>HW	Marginal
			Length at age-3	HW>WW	Marginal
			Length at age-4	WW>HH	Marginal
			Run timing of age-3s	WW>HH	Marginal
			Sex ratio (females:males)	HH>WW	Marginal
Stream	Juvenile	1996		HW>HH	Marginal
			Number of late pre-summer (July) migrants (relative to expectations)		
			Post-summer survival of summer residents	HH=WW>HW	High
			Overall survival	WW>HH, HW lowest	High, ?[d]
			Distribution of summer residents	HW≠WW=HH	High
			Timing of post-summer migrants	HW=HH>WW	High
			Condition of late pre-summer (July) migrants	HH>WW=HW	High
			Length of summer residents	WW>HH=HW	High
			Weight of summer residents	WW>HW	Marginal
			Length and weight of migrants after 7 Oct	WW>HW	High
			Condition of migrants after 7 Oct	WW≠HH=HW	High
			Incidence of precocious males	WW>HW=HH	?[e]

Hatchery vs stream	Adult (hatchery), juvenile (stream)	1996	Survival of HH relative to WW[f]	Hatch>stream	Marginal

[a]If two crosses are shown, the third is intermediate but not different from either of the other two (P>0.10). If three crosses are shown, the cross in the middle is intermediate and different from at least one of the two by Bonferroni adjusted paired comparisons (P<0.10). $\neq$ means not equal.
[b]Based on the overall test (H_o: no difference among crosses): high = P<0.05; marginal = 0.05<P<0.10; ? = difference not testable but may be significant anyway (see footnotes).
[c]For fish that died before end of experiment; fish from tanks experiencing an O_2 problem were excluded.
[d]Comparisons between HW and the other crosses were from weighted estimates and were therefore not testable.
[e]The difference was not significant, but was strengthened by the fact that WW fish were more frequent than the other crosses in September (Figure 12) when most migrants were precocious.
[f]Neither $RS_{HW/WW}$ nor $RS_{HW/HH}$ were tested.

Table 14. Qualitative consistency of relative performance of the crosses between year-classes without regard to whether the differences were statistically significant.

Life stage	Site	Measure	Year-class 1992	Year-class 1996	HH v WW, agreement[a]
Juvenile	Hatchery	Size[b]	WW>HW>HH	HW>WW>HH	Yes
		Condition[c]	WW>HW>HH	HW>HH>WW	No
	Seawater	Days to death	WW>HH>HW	WW>HW>HH	Yes
		ELISA[d]	WW>HH>HW	HW>WW>HH	Yes
Adult	Hatchery	Survival[e]	HW>WW>HH	HW>HH>WW	No
		Age-4 length	WW>HW>HH	WW>HW>HH	Yes

[a]Yes=The direction of the difference between HH and WW means was consistent between year-classes; No=the direction of the difference between HH and WW means was inconsistent between year-classes.
[b]Length and weight.
[c]Condition factor.
[d]ELISA absorbance indicating BKD infection level.
[e]Fry-to-adult survival.

Table A1. Trap efficiency (*E*hat), number of outmigrants (*N*hat), and relative efficiency of the Elbow trap to the Big Cedars trap. Estimates for Elbow were made from recaptures of marked fish released (1) downstream from Big Cedars, and (2) upstream from Big Cedars. U = number of unmarked fish captured, M = number of marked fish released, and R = number of recaptures. The subscript "adj" indicates that an estimate was adjusted (see text). Data were pooled over 25 September 1997-5 May 1998.

		Elbow	
Sample or estimate	Big Cedars	Method 1	Method 2
U	806	384	384
M	669	345	669
R	153	51	27
*E*hat = R/M	0.23	0.15	0.04
*N*hat = U/*E*hat	3524	2598	9515
$N\text{hat}_{\text{adj}} = N\text{hat} + U_{\text{Big Cedars}}$		3404	
$E\text{hat}_{\text{adj}} = U/N\text{hat}_{\text{adj}}$		0.11	
Relative efficiency of Elbow to Big Cedars			
$E\text{hat}_{\text{adj}}/E\text{hat}_{\text{Big Cedars}}$ (method 1), or $E\text{hat}/E\text{hat}_{\text{Big Cedars}}$ (method 2)		0.49	0.18
$U/U_{\text{Big Cedars}}$		0.48	0.48

Table A2. Number of Chinook salmon estimated by multiple pass removal or by mark-recapture, number counted in snorkel surveys, and the ratio of the estimate to the count, for eight pools. CI = confidence interval.

Method	Section	Population estimate	Lower limit of 95% CI	Upper limit of 95% CI	Snorkel count	Ratio[a]
Removal	1	36	31[b]	47	23	1.6
		12	12[b]	13	13	0.9
Mark-recapture	2	90	68[b]	115	30	3.0
		91	71[b]	113	34	2.7
	3	439	409	469	76	5.8
	4	94	53[b]	137	27	3.5
	6	70	47[b]	106	7	10.0
	7	151	99	202	15	10.1
Total	Total	982			225	4.4

[a]Equals (population estimate)/(snorkel count).
[b]Equals the total number of fish captured.

Figures

Figure legends

Figure 1. Study area. The right panel shows the study sections in the Little White Salmon River. Screw traps were located at Big Cedars and Elbow.

Figure 2. Abnormal alevins expressed as a proportion of the initial number of eyed embryos. The upper panel shows proportion abnormalities by incubation site (WSH=Warm Springs Hatchery; CDS=Carson Depot Springs) and cross. Within a site, crosses without a letter in common differ ($P<0.05$). The lower panel shows proportion abnormalities in an incubation tray in relation to the frequency of eyed embryos from five of 28 HW families (families 6-10) in the tray. Frequency of those five families is expressed as a proportion of the initial number of eyed embryos; each data point represents a tray. Note that each of families 6-10 is equally represented in trays containing embryos from any of those families.

Figure 3. Length frequency of juveniles sampled at Warm Springs Hatchery. Text in panels indicates year-class, age, and whether fish migrated from the rearing pond during the fall volitional release period. Vertical dashed lines show the boundary between lower and upper length modes used in analyses (Table 7); boundaries were selected by eye.

Figure 4. Cumulative mortality of juveniles at the Marrowstone seawater facility. Fish were tagged on 16 February (1992 year-class) and 14-15 April (1996) and were gradually switched to seawater starting on 18 April (1992) and 20 April (1996). Nearly all fish in three of 10 1996 year-class tanks (237, 243, and 245) died from gas super-saturation on 13 July (day 90). The middle panel shows cumulative mortality in all tanks through 12 July (day 89). The lower panel shows cumulative mortality to 23 September (day 162), when the experiment was terminated, for the seven tanks not experiencing super-saturation.

Figure 5. Mean length (+SD) at tagging and growth rate of 1996 year-class juveniles in seawater tanks.

Figure 6. ELISA absorbance of mortalities (plotted against number of days between tagging and death) and survivors from seawater rearing for data pooled over crosses. Vertical dashed lines indicate transition to seawater. The horizontal dashed line in the lower panels shows the boundary (selected by eye) between lower and upper ELISA modes used in analyses. Vertical dotted lines in the lower left panel separate an early period when all mortalities were in the lower ELISA mode, a middle period when mortalities were common in both the lower and upper modes, and a late period when most mortalities were in the upper mode. Period boundaries also were selected by eye. High-ELISA mortality rate (deaths in the upper mode per day) increased from 1.5 in the middle period to 2.5 in the late period. Fish surviving "to 13 July" (day 90) are those that died on that date from the super-saturation problem.

Figure 7. Proportions of total mortality occurring in time periods with different ELISA profiles. In the upper panel, only mortalities occurring before 13 July (day 90) are considered (these mortalities are from all tanks). The first and second time periods shown in the lower left panel of Figure 6 (before and after day 43) are compared. Of the nine mortalities occurring in the first period, eight were HH fish and one was a WW fish. In the lower panel, only mortalities with high ELISA absorbance (i.e., in the upper mode) but occurring anytime during the study are considered (these mortalities are from the tanks not experiencing super-saturation); the second and third periods shown in Figure 6 (before and after day 106) are compared. *P*-values are from *G*-tests for independence between cross and time period.

Figure 8. Box plots for length of returning adults. The darker line within a box designates the mean and the lighter line the median. Box edges are 25th and 75th percentiles; whiskers 10th and 90th percentiles; and filled circles show individuals outside the 10th and 90th percentiles.

Figure 9. Cumulative frequency of arrival date at the hatchery and capture date in the fishery for 1996 year-class age-3 and age-4 returns. Data series labels indicate mean rank of date [and sample size].

Figure 10. Number of unmarked fish captured in the screw traps (U), estimated number of emigrants (*N*hat), and estimated trap efficiency (*E* meas and *E* extrap) each week in the Little White Salmon River. *E* meas = trap efficiency estimated from recaptures of marked fish released during the week; *E* extrap = trap efficiency extrapolated from estimates made in preceding or succeeding weeks.

Figure 11. Distribution of experimental fish among study sections in the Little White Salmon River (Figure 1) during August 1997. Top panel: *n* = number of fish captured, *N*hat = estimated number of fish, and *E*hat = capture efficiency (= *n*/*N*hat). Middle panel: Relative frequencies computed from *n*; relative frequencies sum to 1 within a cross. Bottom panel: relative frequencies computed from *N*hat.

Figure 12. Emigration timing during the post-summer period. Top panel: Relative frequencies of the number of migrants trapped each week in the Little White Salmon River. Relative frequencies sum to 1 within each cross. Bottom panel: same as top except relative frequencies were computed from the estimated total number of migrants each week.

Figure 13. Length, weight, and condition factor (*K*) of migrants and summer residents in the Little White Salmon River. The regression of size or condition on capture date is plotted if date had a significant effect, otherwise the mean of size or condition is plotted. Post-summer migrants before 8 October are shown separately because of the high percentage of precocious males (see text). Curves are second order polynomials ($y = a + bx + cx^2$). The "crosses pooled" line indicates trends when cross was unknown because *sSOD-1** analysis failed.

Figure 14. Mean length (+SD) of summer residents, by study section, in the Little White Salmon River (Figure 1).

Figure 15. Relative survivals for 1996 year-class fish reared in the hatchery or in the stream. Values are calculated as the survival of the first cross in a pair relative to survival for the second cross. Values for hatchery rearing are from total adult returns (Table 4) and for stream rearing are from pooled summer residents and post-summer migrants for the HH/WW cross pair and from weighted estimates of total migrants for the HW/WW and HW/HH pairs (Table 6). Statistical comparison was made only for the HH/WW pair because weighted estimates were not testable. The reported *P*-value (0.07) was not Bonferroni adjusted since only one comparison was made.

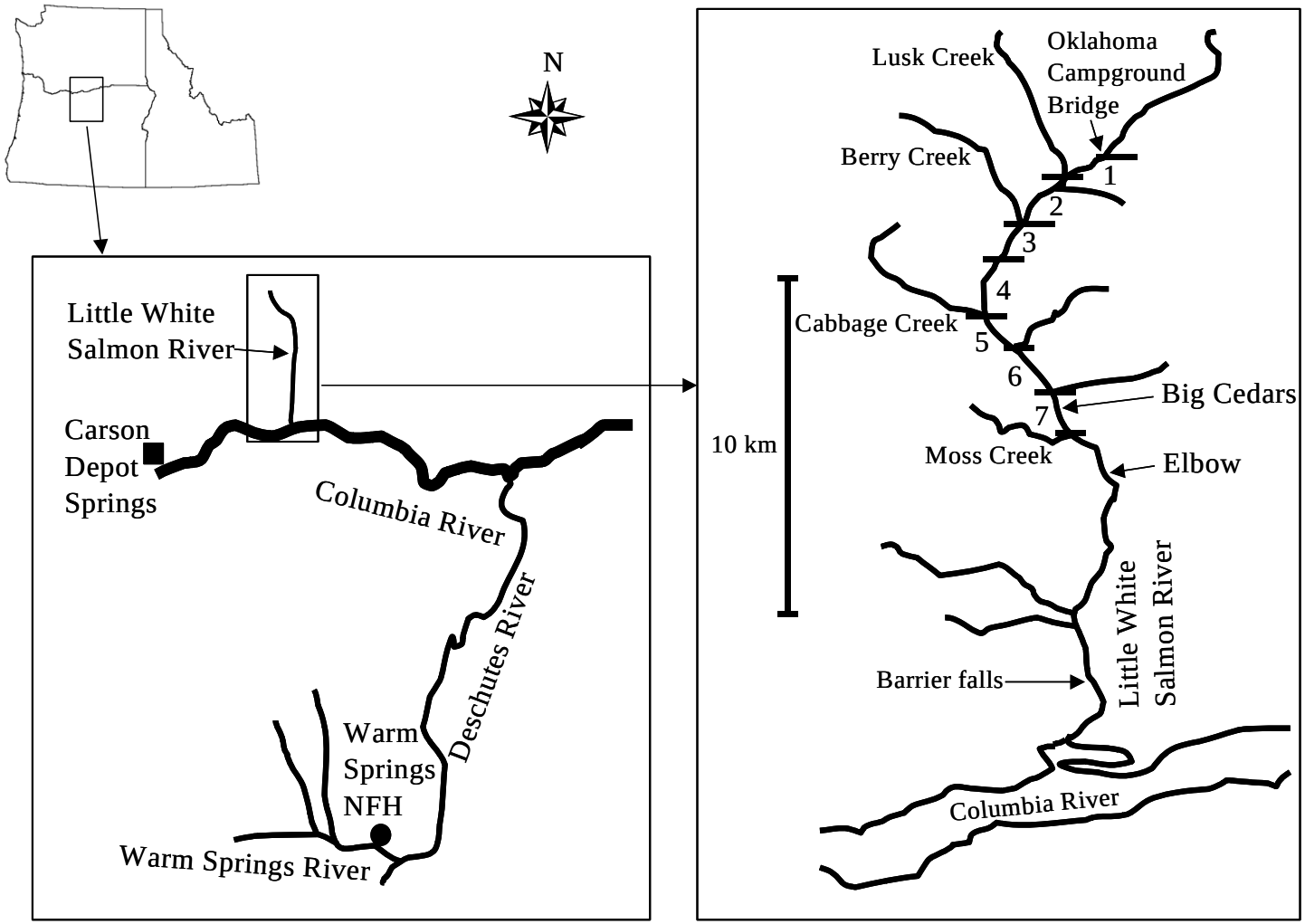
N
Little White
Salmon River
Carson
Depot
Springs
Columbia River
Deschutes River
Warm
Springs
NFH
Warm Springs River
Lusk Creek
Oklahoma
Campground
Bridge
Berry Creek
1
2
3
4
Cabbage Creek
5
6
Big Cedars
7
10 km
Moss Creek
Elbow
Little White
Salmon River
Barrier falls
Columbia River

Figure 1.

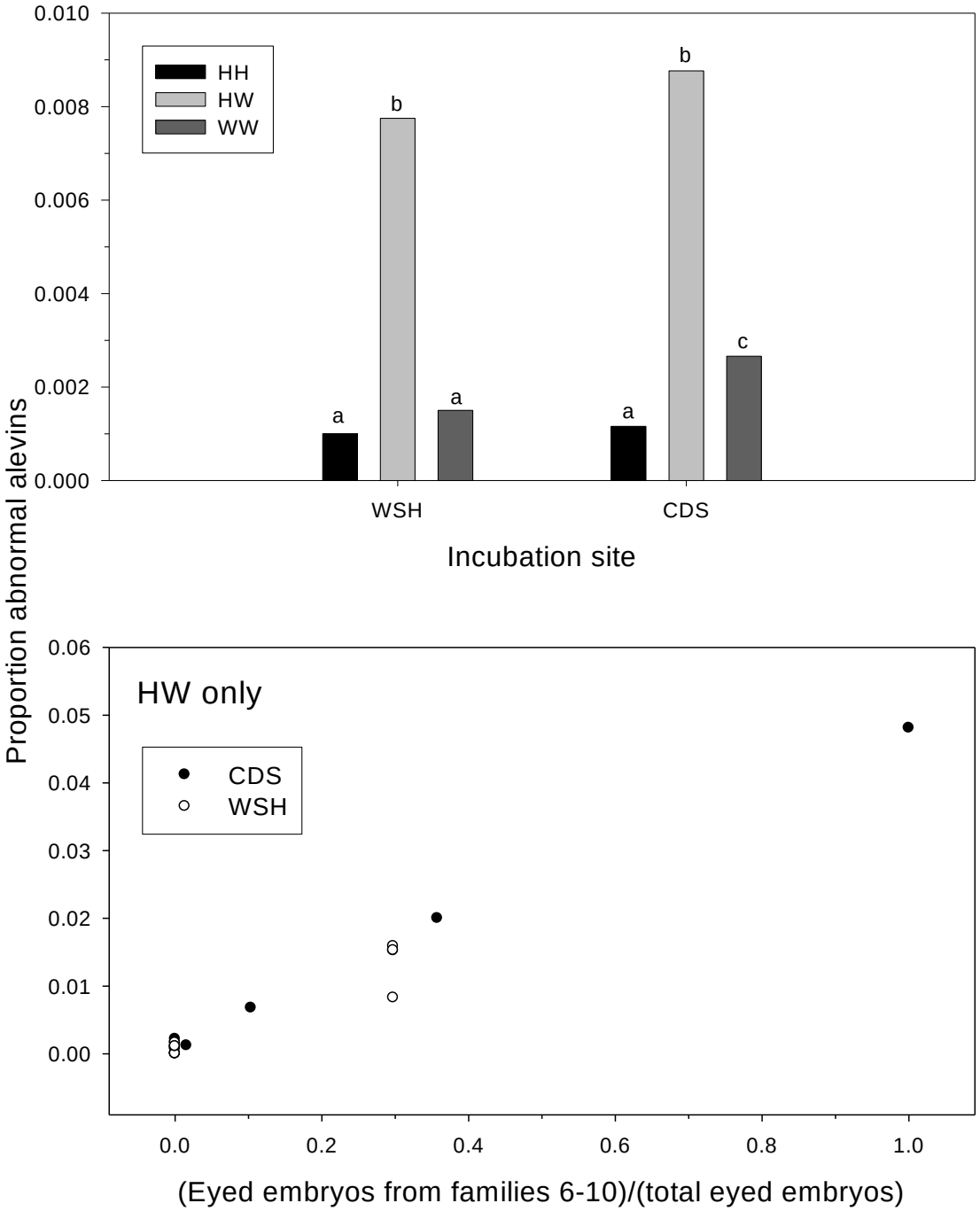
0.010
0.008
0.006
0.004
0.002
0.000
HH
HW
WW
a
b
a
a
b
c
WSH
CDS
Incubation site
Proportion abnormal alevins
0.06
0.05
0.04
0.03
0.02
0.01
0.00
HW only
CDS
WSH
0.0
0.2
0.4
0.6
0.8
1.0
(Eyed embryos from families 6-10)/(total eyed embryos)

Figure 2.

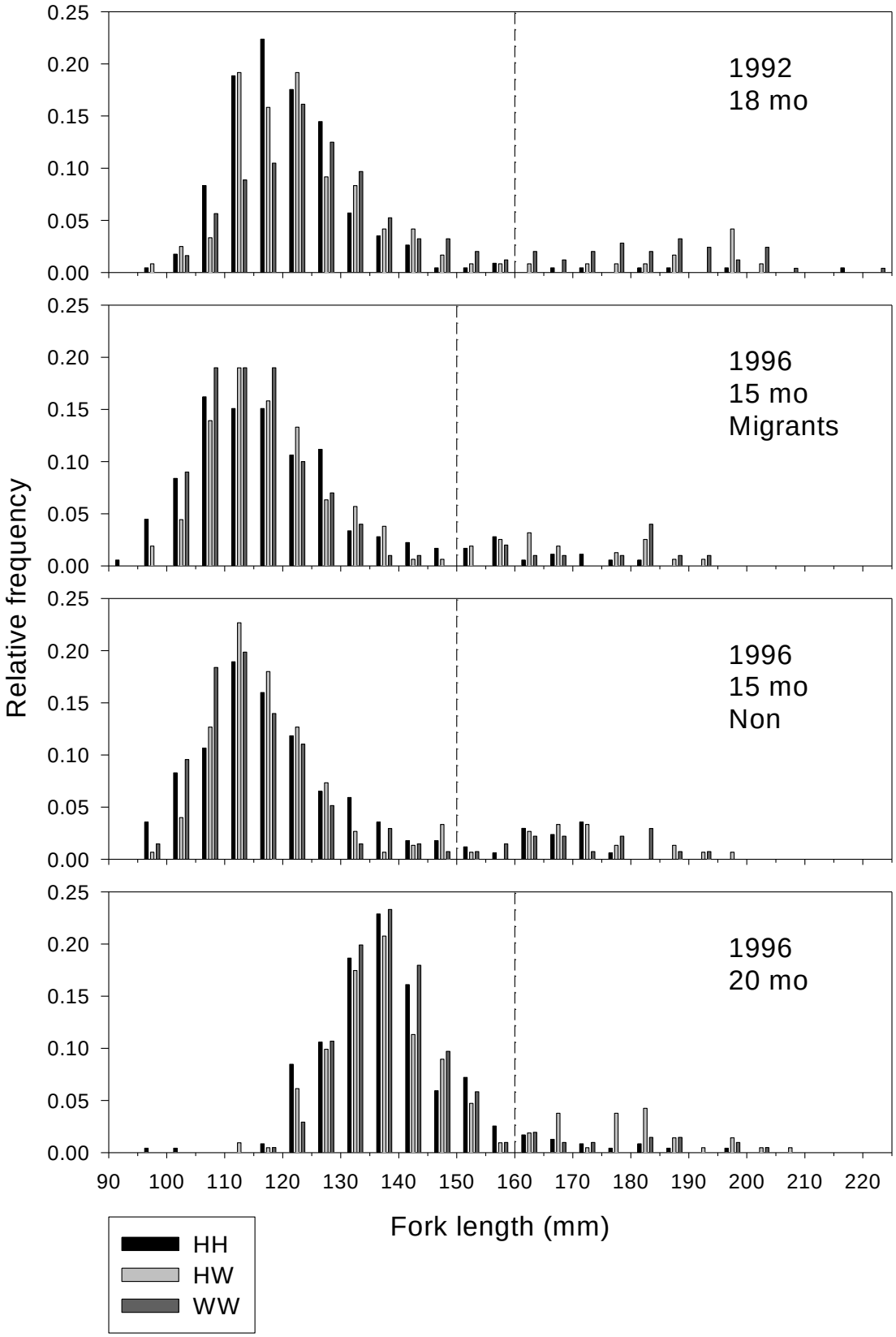
1992
18 mo
1996
15 mo
Migrants
1996
15 mo
Non
1996
20 mo
Relative frequency
Fork length (mm)
HH
HW
WW

Figure 3

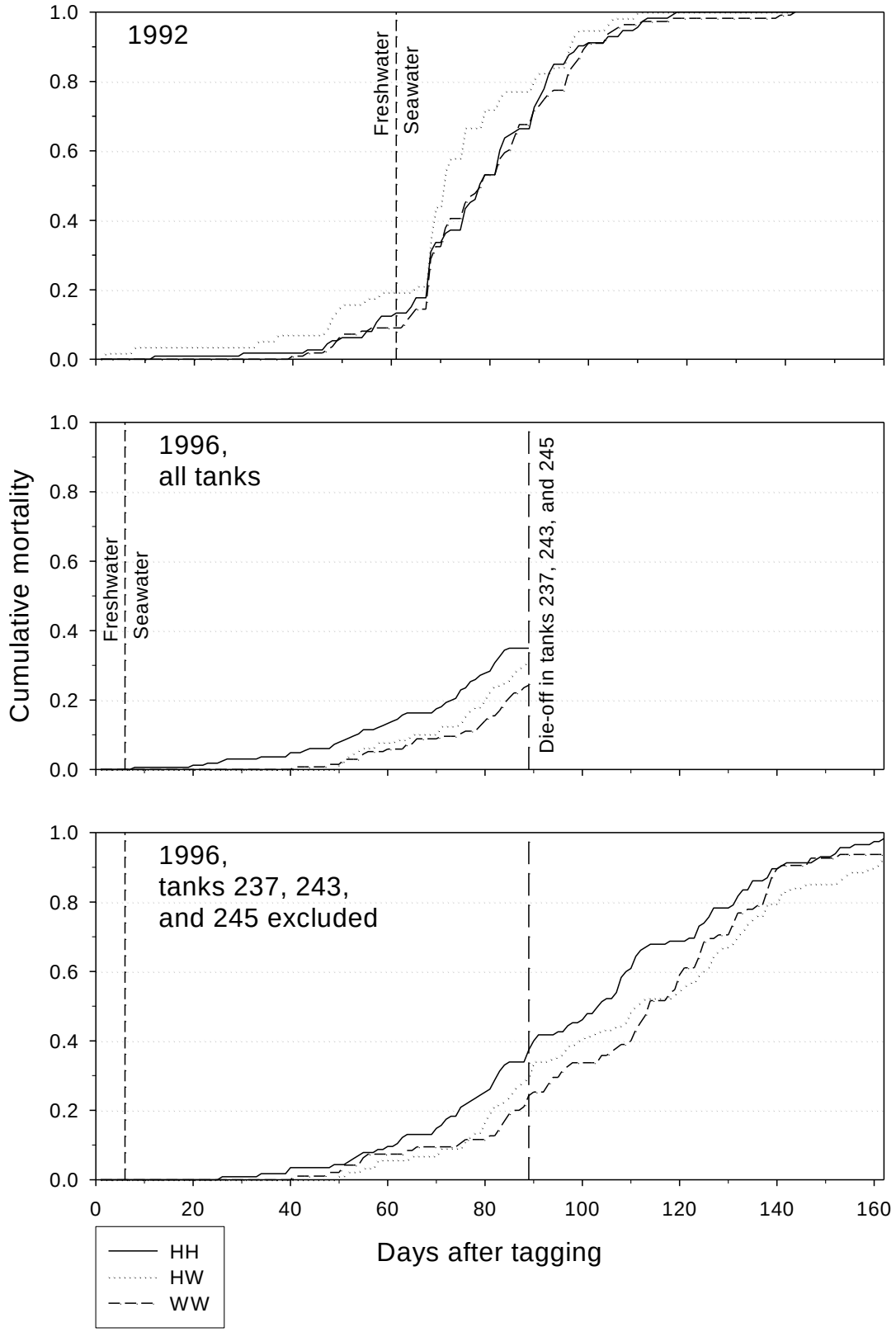
1992
Freshwater
Seawater
1996,
all tanks
Freshwater
Seawater
Die-off in tanks 237, 243, and 245
1996,
tanks 237, 243,
and 245 excluded
Cumulative mortality
Days after tagging
HH
HW
WW

Figure 4.

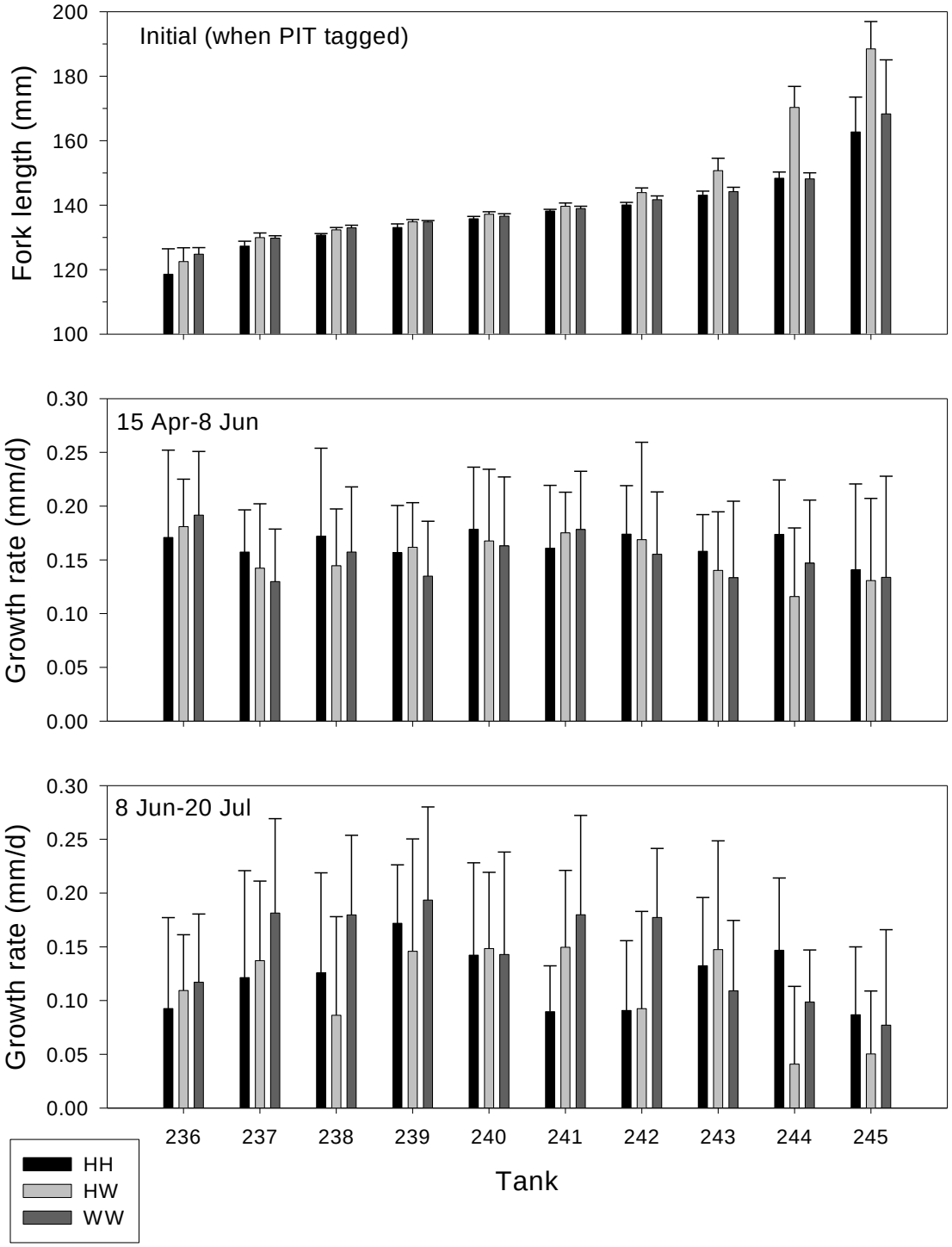
Initial (when PIT tagged)
Fork length (mm)
200
180
160
140
120
100
15 Apr-8 Jun
Growth rate (mm/d)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
8 Jun-20 Jul
Growth rate (mm/d)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
236
237
238
239
240
241
242
243
244
245
Tank
HH
HW
WW

Figure 5.

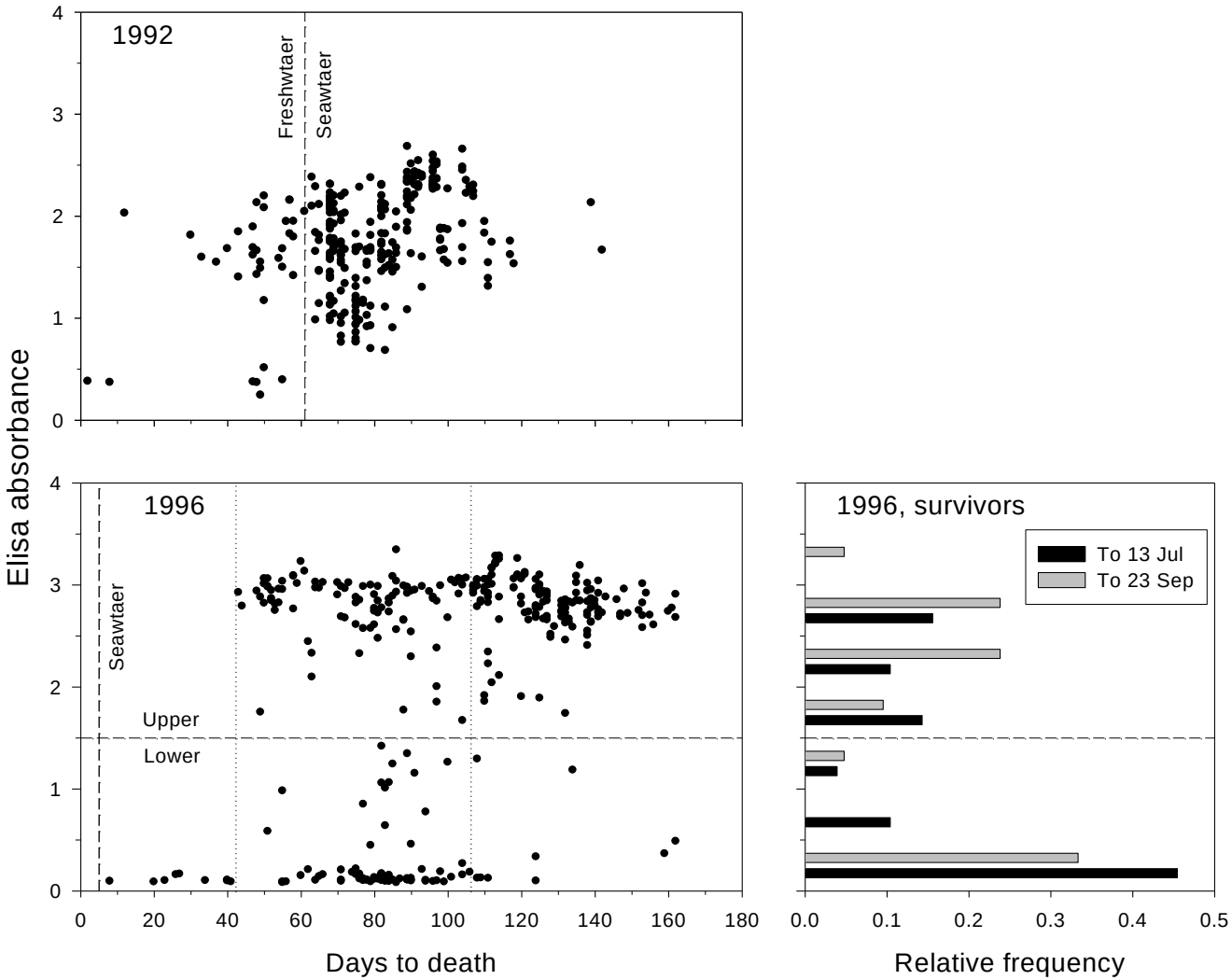
1992
Freshwtaer
Seawtaer
1996
Seawtaer
Upper
Lower
1996, survivors
To 13 Jul
To 23 Sep
Elisa absorbance
Days to death
Relative frequency

Figure 6.

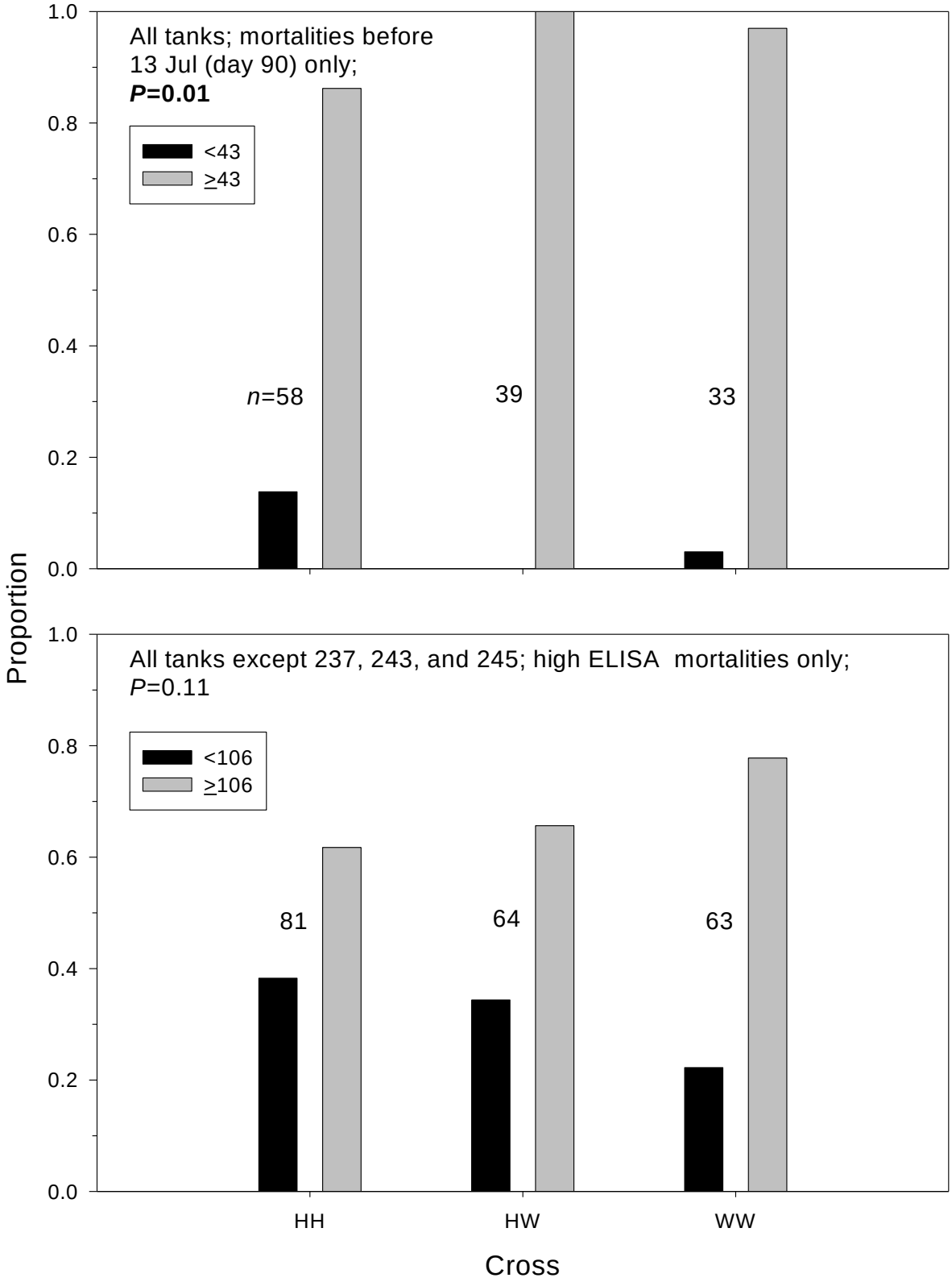
All tanks; mortalities before
13 Jul (day 90) only;
P=0.01
<43
≥43
n=58
39
33
All tanks except 237, 243, and 245; high ELISA mortalities only;
P=0.11
<106
≥106
81
64
63
Proportion
1.0
0.8
0.6
0.4
0.2
0.0
HH
HW
WW
Cross

Figure 7.

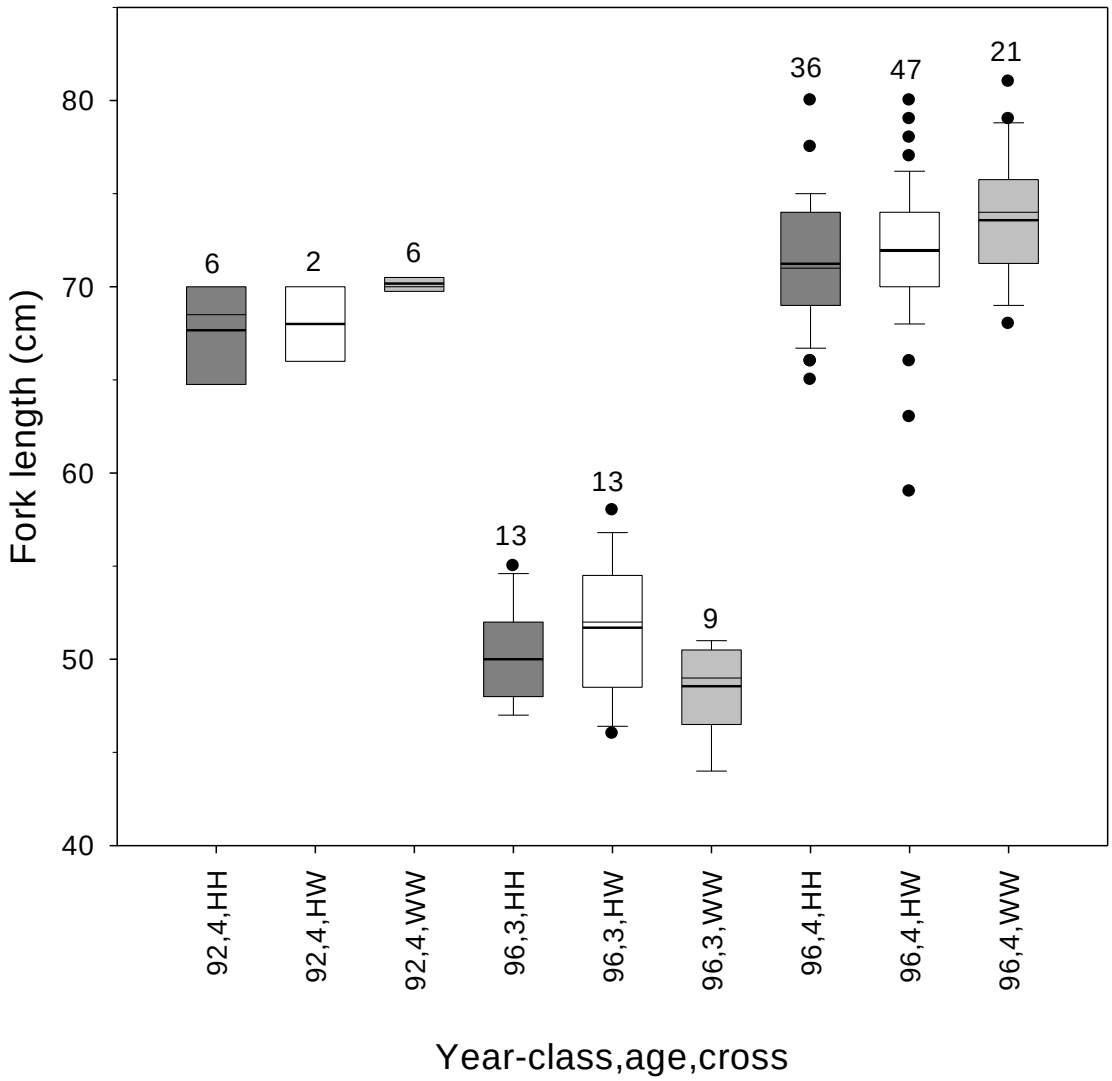
Fork length (cm)
80
70
60
50
40
6
2
6
13
13
9
36
47
21
92,4,HH
92,4,HW
92,4,WW
96,3,HH
96,3,HW
96,3,WW
96,4,HH
96,4,HW
96,4,WW
Year-class,age,cross

Figure 8.

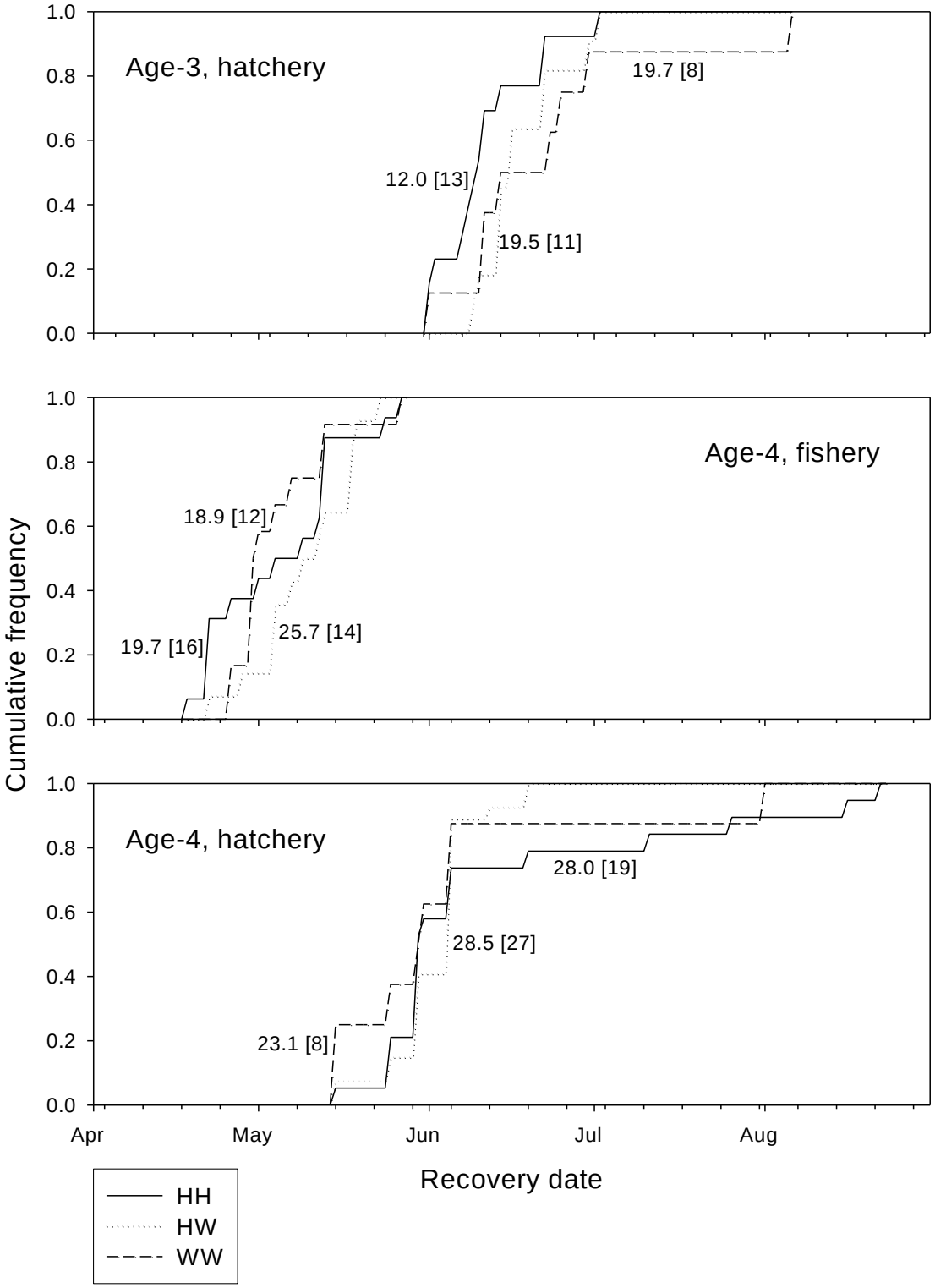
Age-3, hatchery
12.0 [13]
19.5 [11]
19.7 [8]
Age-4, fishery
18.9 [12]
19.7 [16]
25.7 [14]
Age-4, hatchery
23.1 [8]
28.5 [27]
28.0 [19]
Cumulative frequency
1.0
0.8
0.6
0.4
0.2
0.0
Apr
May
Jun
Jul
Aug
Recovery date
HH
HW
WW

Figure 9.

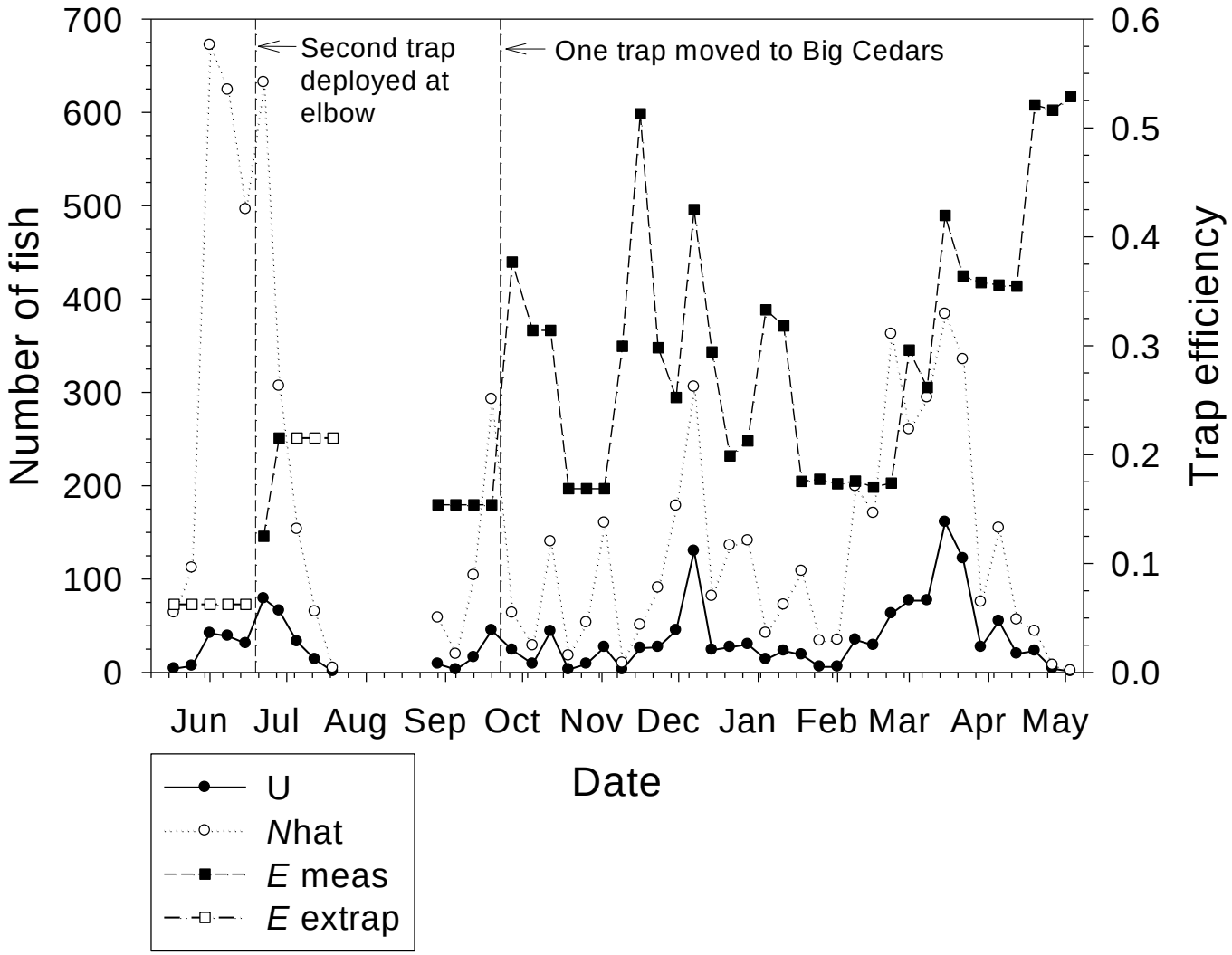
Second trap deployed at elbow
One trap moved to Big Cedars
Number of fish
Trap efficiency
Date
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
U
Nhat
E meas
E extrap

Figure 10.

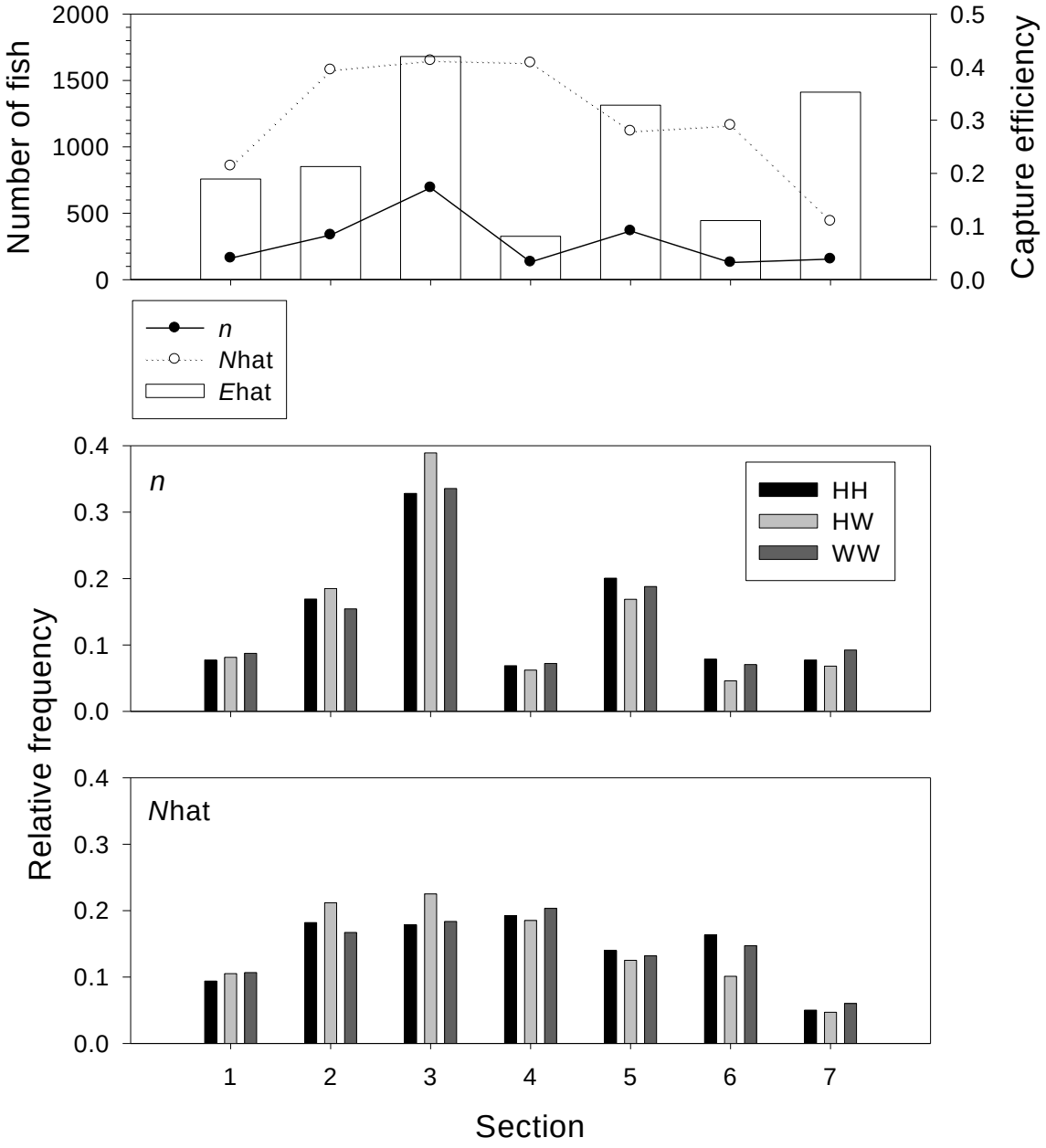
Number of fish
Capture efficiency
2000
1500
1000
500
0
0.5
0.4
0.3
0.2
0.1
0.0
n
Nhat
Ehat
Relative frequency
n
HH
HW
WW
Nhat
0.4
0.3
0.2
0.1
0.0
1
2
3
4
5
6
7
Section

Figure 11.

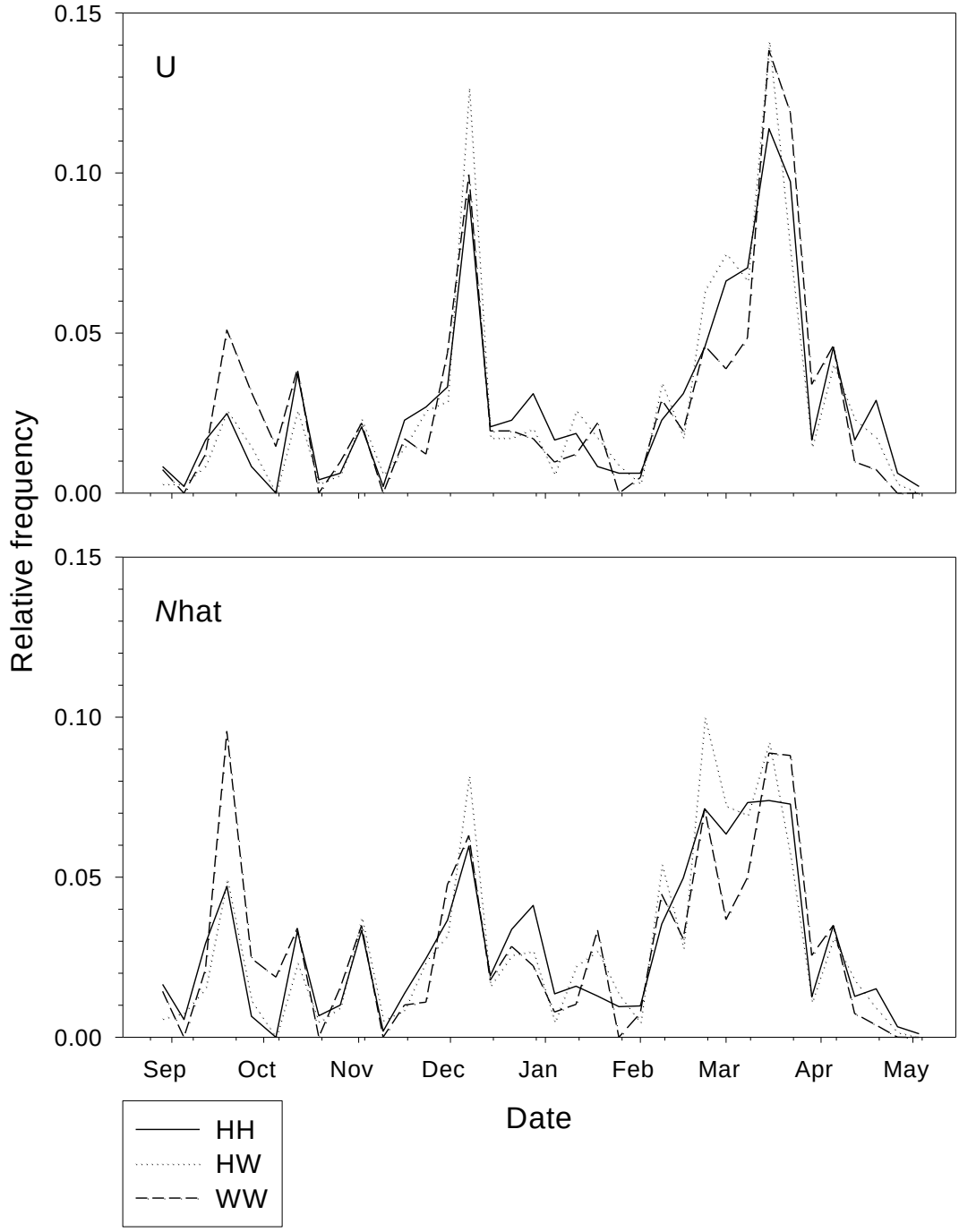
U
Nhat
Relative frequency
0.15
0.10
0.05
0.00
Sep
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Date
HH
HW
WW

Figure 12.

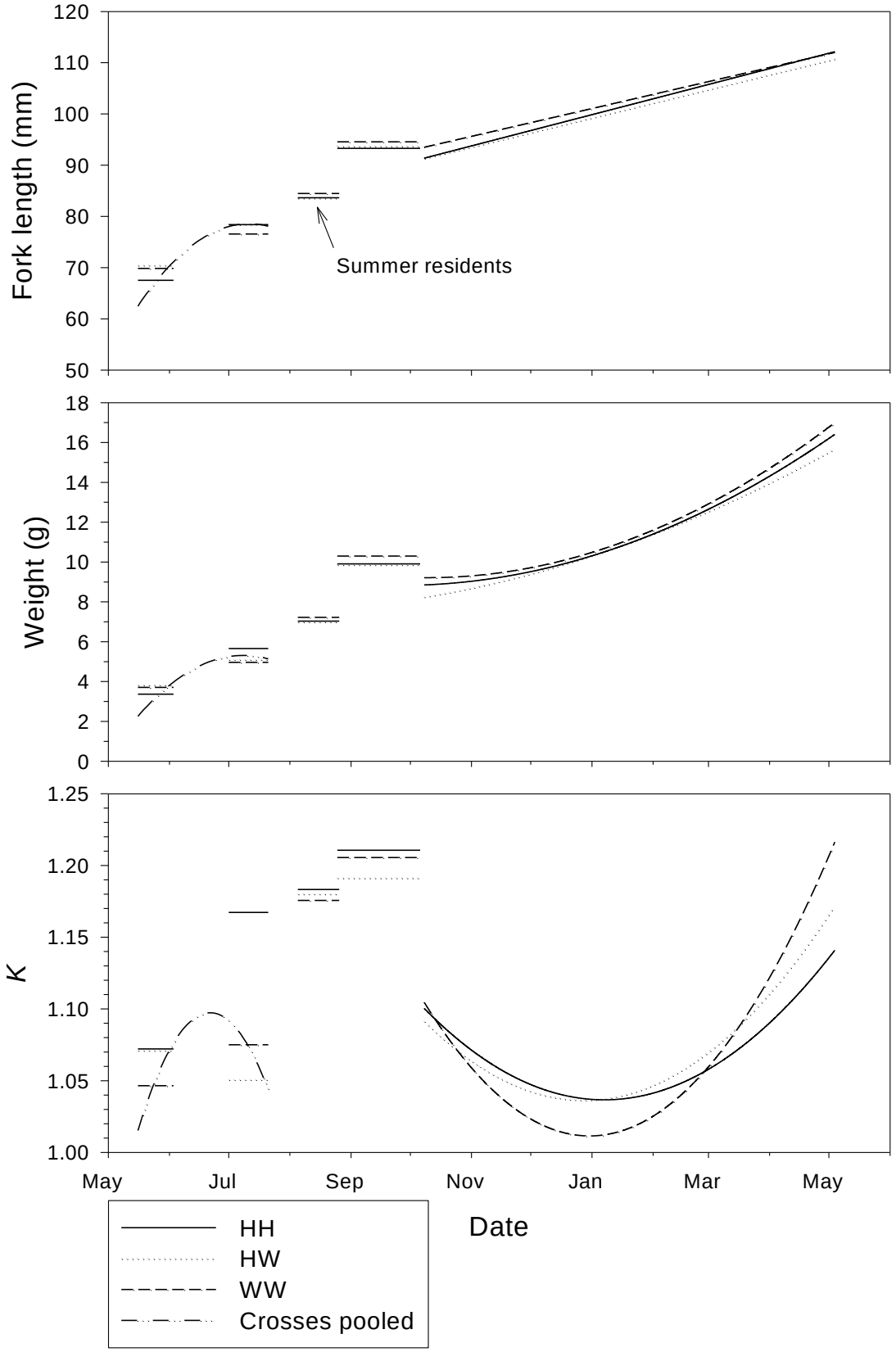
Fork length (mm)
120
110
100
90
80
70
60
50
Summer residents
Weight (g)
18
16
14
12
10
8
6
4
2
0
K
1.25
1.20
1.15
1.10
1.05
1.00
May
Jul
Sep
Nov
Jan
Mar
May
Date
HH
HW
WW
Crosses pooled

Figure 13.

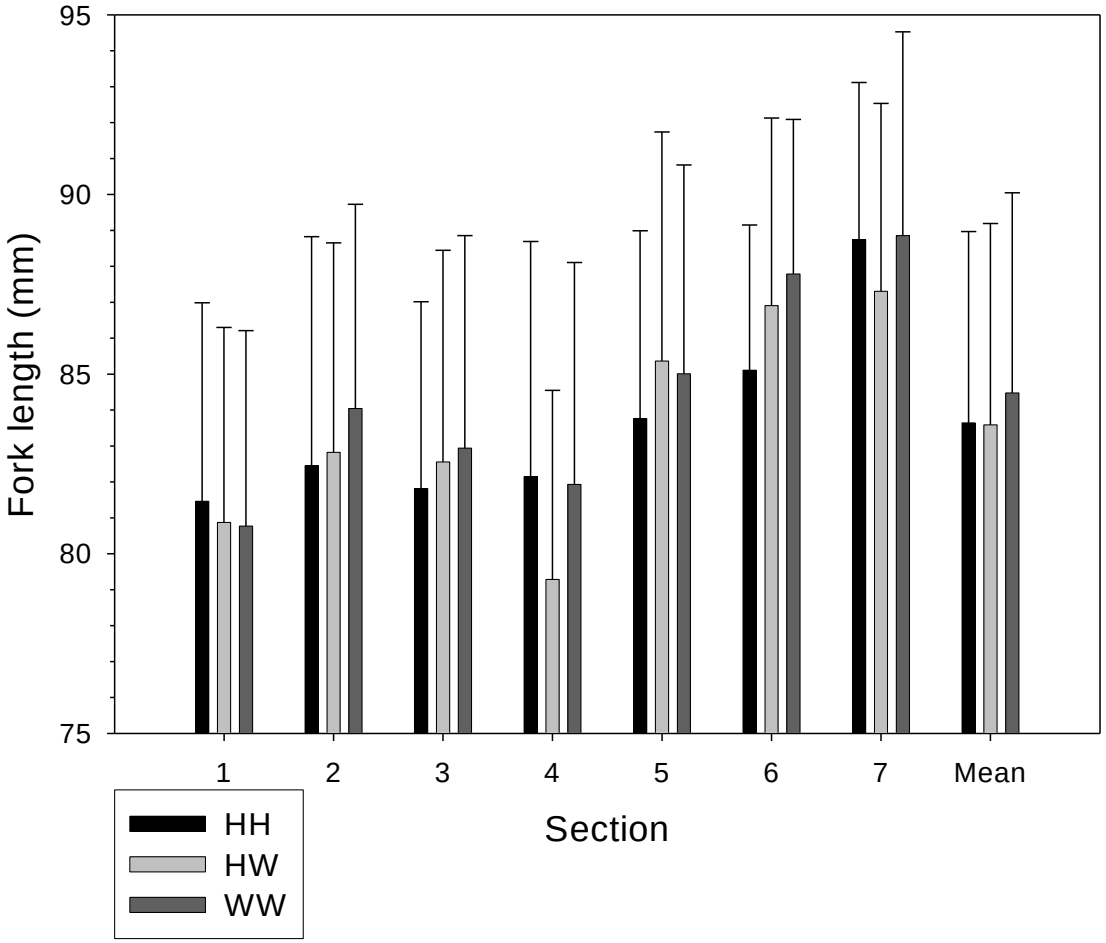

Figure 14.

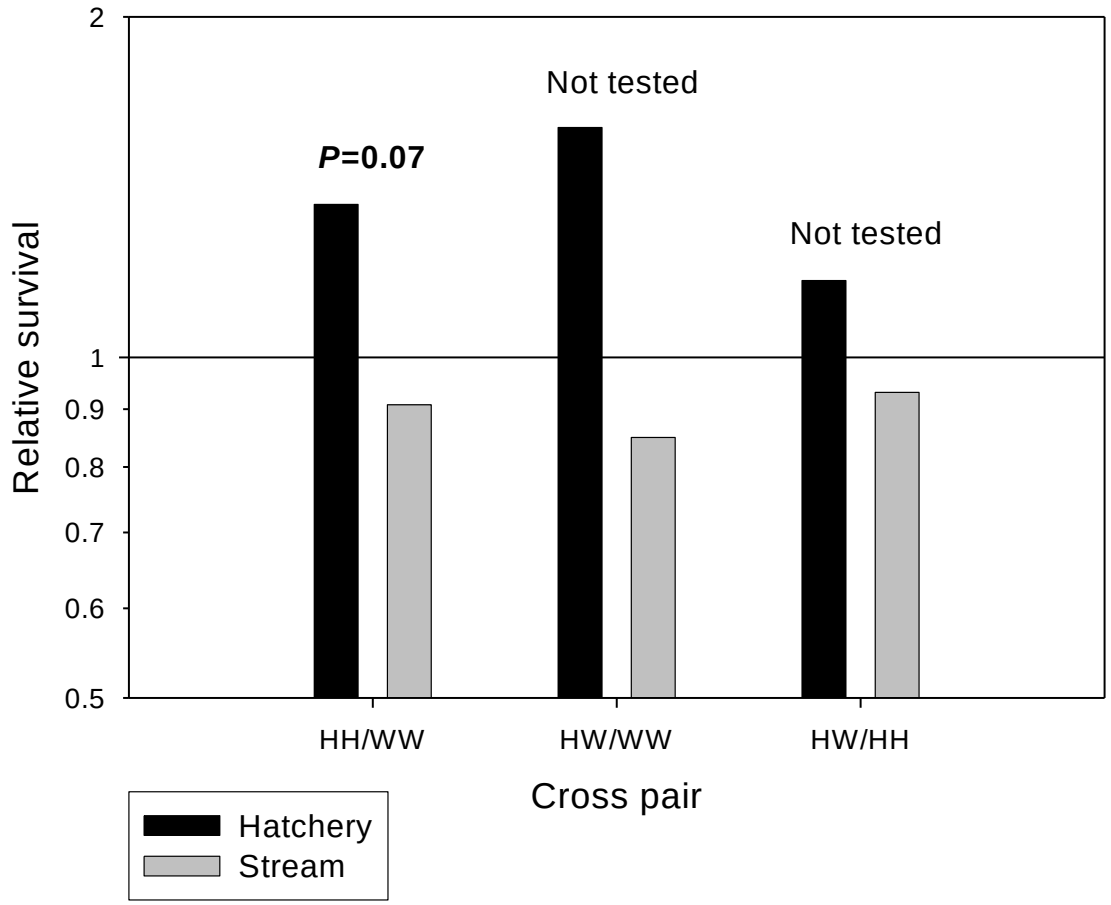
2
1
0.9
0.8
0.7
0.6
0.5
Relative survival
Not tested
P=0.07
Not tested
HH/WW
HW/WW
HW/HH
Cross pair
Hatchery
Stream

Figure 15.

Chapter 9: Effectiveness of an integrated hatchery program in the Warm Springs River, Oregon: Can genetic based performance differences between hatchery and wild Chinook salmon (*Oncorhynchus tshawytscha*) be avoided? *(Study sites: Warm Springs Hatchery and the Metolius River; Stocks: Warm Springs hatchery and Warm Springs River wild; Year class: 2000)*

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Extended abstract

Introduction - We evaluated a 6th generation rearing program for spring Chinook salmon (*Oncorhynchus tshawytscha*) at Warm Springs National Fish Hatchery (NFH), Oregon, USA. The program was designed to protect the local wild stock by minimizing genetic divergence of the hatchery population and preventing hatchery fish from spawning naturally. Techniques included 1) selection of broodstock that represented the natural run including jacks, 2) average of 10% wild fish as hatchery spawners, 3) volitional releases of juveniles, 4) marking and tagging all hatchery fish, and 5) the presence of a barrier dam that allowed marked fish to be retained rather than passed upstream to spawn naturally.

Methods – We evaluated fitness-related traits for the 2000 year class produced from hatchery (H) and wild (W) parents. Three progeny groups were created: HH, WW, and HW (H females mated to W males) to help judge maternal effects. All three groups were reared in hatchery (HRE) and natural rearing (NRE) experiments and evaluated for survival, size, and migration timing at juvenile (HRE and NRE) and adult (HRE only) stages. Study sites were Warm Springs NFH and the Metolius River.

More than 40 families were produced for each cross type (frequency of eyed embryos of each cross type was 0.33 of total). In the HRE all fish were marked and nearly equal numbers of each cross type (≈10,000) were reared in each of three raceways. Volitional releases of juveniles occurred in fall 2001 and spring 2002. Numbers of fall migrants from two raceways (R1 and R-2) were estimated and we simulated the fall release in a third raceway (R-SIM) by siphoning water into an adjacent raceway. Fish remaining in the hatchery were sampled in spring 2002 (pre-smolts). Returning adults were sampled at the hatchery and from fisheries. For the NRE, fry released in winter 2001 were evaluated as downstream migrants from January 2001 – May 2002 and as summer residents (fish residing upstream from migrant traps) in July-August 2001. All HRE and NRE fish were tissue sampled and measured for length. We used DNA-based parentage analysis or otolith marks to identify cross type.

Results – In the HRE 10,900 fish in R-1 and 13,000 fish in R-2 exited the hatchery during the fall volitional release. Only 4,300 fish migrated from R-SIM and cross type frequencies from this raceway were similar to expected frequencies ($P>0.55$). Pre-smolt frequencies for R-1 and R-2 pooled (HH=0.407, HW=0.339, WW=0.255) were significantly different from expected ($P<0.01$) and indicated 2x-3x greater fall emigration by WW than HH fish (HW intermediate). A significant difference in adult frequencies (HH=0.373, HW=0.350, WW=0.277) was also observed ($P<0.04$). The pattern (HH>HW>WW) was similar ($P=0.93$) for all three raceways. Among age-4 fish HH were 43% more abundant than WW fish.

In the NRE there were no significant differences between observed and expected cross type frequencies for summer residents ($P>0.11$). Mean length was also similar among cross types ($P = 0.13$).

Fish captured as migrants were separated into three trap periods: January to early March, early March to mid-July and mid-July 2001 to May 2002. Migrants during January to March were 38% WW, 30% HW, and 32% HH fish (significant difference between WW and HW; $P<0.03$) and composed 74% of total migrants. No other significant differences in frequency or size were observed for migrants.

Discussion – Results of the hatchery rearing experiment indicated frequency differences between HH and WW fish for pre-smolts and for adult returns. Because the fish were reared in a common environment from conception, these differences were not due to environmental differences but rather were from genetic or maternal effects. The largest difference observed between HH and WW fish was for pre-smolts (60% more HH than WW fish). In comparison, HW pre-smolts were intermediate (33% more HW than WW), suggesting that fall emigration differences (which created the pre-smolt differences) were from additive genetic effects. Among adults, HW performance was also intermediate, suggesting that differences in survival to adult were at least in part due to genetic effects. Performance differences were consistent with domestication theory (increased fitness for the hatchery population in the hatchery program), and suggested that selection against fall emigration from the hatchery was a possible mechanism of domestication. In contrast, the NRE indicated few differences between cross types. However, the analysis was confounded by early emigration of large numbers of fry. Although the differences we observed between HH and WW fish indicated genetic differences between the hatchery and wild populations, the differences would likely have been even greater without the use of the various management strategies that were employed at the hatchery. Additional study is needed to evaluate which hatchery techniques are most useful for reducing performance differences and risks to wild populations.

Chapter 10: A test for the relative strength of maternal and stock effects in spring Chinook salmon (*Oncorhynchus tshawytscha*) from two different hatcheries *(Study site: Warm Springs Hatchery; Stocks: Warm Springs Hatchery and Carson Hatchery; Year class: 1993)*

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Abstract

An experiment was undertaken to determine the relative strength of maternal and stock effects in Chinook salmon (*Oncorhynchus tshawytscha*) reared in a common environment, as a companion study to our investigation of hatchery and wild Chinook salmon. Pure-strain and reciprocal crosses were made between two hatchery stocks (Carson and Warm Springs National Fish Hatcheries). The offspring were reared together in one of the hatcheries to the smolt stage, and then were transferred to a seawater rearing facility (USGS-Marrowstone Field Station). Differences in survival, growth and disease prevalence were assessed. Fish with Carson parentage grew to greater size at the hatchery and in seawater than the pure-strain Warm Springs fish, but showed higher mortality at introduction to seawater. The analyses of maternal and stock effects were inconclusive, but the theoretical responses to different combinations of maternal and stock effects may be useful in interpreting stock comparison studies.

Introduction

Maternal effects are the non-genetic effects of the mother on her young (Hallerman 2003). In salmon, maternal effects can include factors that are typically of short duration, such as egg size (Heath et al. 1999; Einum and Fleming 2000) and longer term effects, including egg quality (Berejikian et al. 1999) or vertically transmissible diseases (Pascho et al. 1991). An investigation into possible maternal effects was conducted with spring Chinook salmon (*Oncorhynchus tshawytscha*) from Warm Springs National Fish Hatchery (Warm Springs) in north-central Oregon and from Carson National Fish Hatchery (Carson) in south-central Washington (Figure 1).

We were motivated to investigate maternal effects because of their potential for confounding a companion experiment designed to test genetic differences between hatchery and wild salmon. The companion experiment was designed to use these same hatchery stocks, as well as the wild stock from which the Warm Springs hatchery stock was derived. The current study was undertaken to evaluate the relative importance of maternal and stock effects on survival and growth. We used the two hatchery stocks to make all combinations of pure and hybrid crosses and reared them, so that any subsequent differences in performance were due to maternal or stock rather than to environmental effects. We expected that a stock effect could be expressed where maternal effects were eliminated (due to shared maternity) but paternity differed (e.g. stock A female x stock A male and stock A female x stock B male). Also in this case, the reciprocal hybrid crosses would have similar survival and growth (e.g. AxB and BxA). In the case of a maternal effect, crosses that shared maternity would be the same but the reciprocal hybrid crosses would differ. Expected responses to different combinations of stock and maternal effects are shown in Figure 2.

Methods

Genetic differences between fish stocks reared at Carson and Warm Springs might be expected from their differing origins and hatchery rearing methods. The Carson stock was developed from a heterogeneous population of spring Chinook salmon trapped at Bonneville Dam during the years 1955-1963 (Wahle and Chaney 1981) and is genetically similar to fish from the Upper Columbia and Snake River Basins (NMFS 2003). A spring at the hatchery provides relatively constant rearing temperatures (6.4-8.7^{o}C; Banks 1994) and juveniles are invariably released as yearling smolts. The Warm Springs

stock was initiated with wild adults collected from the Warm Springs River during the years 1978-1981. Since then, the hatchery broodstock has been supplemented with wild fish in most years in the hopes of minimizing genetic differences between wild and hatchery fish (Olson et al. 2004). Rearing water at Warm Springs is obtained from the Warm Springs River and can reach 20°C in the summer and near freezing during the winter (Olson et al. 1995). About 10-30% of the juveniles are released in the fall as subyearlings; the remainder is released as yearlings the following spring (Olson et al. 2004).

There was no overlap in spawning time between the hatcheries, so crosses were made using fresh eggs from the spawning hatchery (Carson-24 August; Warm Springs-1 September 1993) and cryopreserved milt from both hatcheries. Milt was collected and cryopreserved from Warm Springs males in 1992 and Carson males in 1993, following the procedures of Wheeler and Thorgaard (1991). Cryopreserved milt was stored in liquid nitrogen; Warm Springs samples for 11 months and Carson samples for 1 day. A sample of ovarian fluid was taken from each female and tested for the causative agent (Renibacterium salmoninarum) of bacterial kidney disease (BKD) using the Enzyme-Linked Immunosorbant Assay (ELISA; Pascho and Mulcahy 1987). Ovarian samples were analyzed by the U.S. Fish and Wildlife's Lower Columbia River Fish Health Center, Underwood, WA (LCRFHC). Progeny from females with high ELISA absorbance values (>0.3) were excluded from the study.

To reduce founder effects, we collected approximately 200 eggs from up to 20 females for a total of 4,000 eggs per hatchery (Table 1; reflects actual numbers used after ELISA culling). Crosses were made by splitting the eggs from each female in half, and fertilizing each half with milt from a different stock (Table 2) to produce full-sib families for four cross groups: Carson x Carson (CxC), Carson females x Warm Springs males (CxW), Warm Springs females x Carson males (WxC), and Warm Springs x Warm Springs (WxW). Eggs were disinfected with iodophore for 30 minutes and incubated in colanders with individual water supplies until eye-up. Eyed embryos were grouped together according to cross and those from Carson were moved to Warm Springs on 7 October 1993. Each cross was placed in a separate incubator tray and held there through the button-up stage.

Incubation temperatures between fertilization and eye-up were lower at Carson (mean=7.1^{o}C) than at Warm Springs (mean=10.1^{o}C), and upon arrival at Warm Springs, Carson embryos were less developed. To accelerate development, the Carson embryos were held in heated water (3-5°C above ambient) until they caught up to Warm Springs development, based on predictions from an incubation model (Jensen 1988). Each cross was given a unique, thermally-induced otolith mark between hatching and ponding (Brothers 1990; Volk et al. 1990). Mean temperature from fertilization to button-up differed by as much as 0.4^{o}C among crosses (Table 3).

Fry from each cross were hand counted and mixed together in a single nursery tank on 11-12 January 1994. A sample of 20 fry from each cross was preserved in buffered formalin for 10 months; then individuals were measured for fork length, whole body weight, and after dissection, yolk weight. Developmental index ($k_D=10*W^{1/3}*L^{-1}$ where W=weight (mg) and L=fork length (mm); Bams 1970) and percent yolk (by weight) were calculated from these measurements. One hundred fry from each cross were held in separate floating net pens for 1-2 weeks and then sampled to verify the presence and pattern of the otolith marks.

Fish were reared in a nursery tank inside the hatchery until 2 May 1994 when they were transferred to a modified outdoor Burrows pond. Due to the small number of fish, they were confined to half of the pond by a divider, but fish density was still eight times less than normal at the hatchery. On 1 March 1995, fish were crowded and a sub-sample was captured and subsequently tagged with uniquely coded Passive Integrated Transponder (PIT) tags (Prentice et al. 1990) and placed in holding pens (n=445). For each fish we measured fork length (mm) and weight (g). The remaining fish (n=3289) could

not be released into the Deschutes River due to the presence of non-local (Carson) stock and were distributed for consumption to the Confederated Tribes of the Warm Springs.

On 2 March 1995, the PIT tagged fish were transported to the U.S. Geological Survey's Marrowstone Marine Station, Nordland, WA (Marrowstone) for seawater rearing. The fish were placed in four circular tanks (four ft. diam.) with 111-112 fish in each tank. Tanks were initially supplied with fresh water, which was then converted to seawater over a 5-day period (17-21 April 1995). This was accomplished by the standard method used at Marrowstone of mixing flows of freshwater and Puget Sound seawater to achieve one-third seawater on day 1, two-thirds seawater on day 3, and full-strength seawater (approx. 29 ppt) on day 5. During the same week, sixty fish (15/tank) were removed for a different experiment. Mortalities were collected daily, measured for length and weight, scanned for PIT tag code, the head placed in alcohol (for otolith analysis) and the body frozen (for ELISA from kidney samples). The experiment ended on 18 July 1995. The surviving fish (n=124) were euthanized and sampled similarly to mortalities. Otoliths were processed following Volk et al. (1990) and the pattern from each otolith was compared with known samples to determine cross type. We were able to read the otolith mark on 340 of the 378 fish recovered as mortalities or survivors; ten percent of the samples were unreadable due poor otolith quality.

Kidney samples from the mortalities and survivors were analyzed using ELISA in two assays (runs) by the LCRFHC. Absorbance values from both runs were bimodally distributed, but the location of the upper mode differed between runs (Figure 3). We assumed that BKD infection level was similar among heavily infected fish from both runs and that the difference in absorbance resulted from interassay variation (Pascho et al. 1987). In order to compare values among crosses and tanks, the upper mode absorbance values were adjusted to a single curve using the mean of the medians. The mean upper modal absorbance was computed from the median of each run and then averaged (run 1 median=3.38; run 2 median=2.72; mean=3.05). The ratios used to make the adjustments were 0.902 and 1.121, from runs 1 and 2 respectively. All analyses of ELISA data were conducted with adjusted absorbance values similar to Pascho et al. (1987).

We calculated an index of relative survival (RS), to indicate if the abundance in the hatchery of the CxW, WxC or the WxW crosses changed through time relative to the CxC cross:

$$RS = (p_{1,t2} * p_{2,t1})/(p_{1,t1} * p_{2,t2}),$$

where p = the proportion of a cross, and t = time (t_1=ponding; t_2=hatchery sampling prior to transfer to Marrowstone). An RS value of 1.0 indicated no change in frequency between crosses, or equal survival. Relative survival among groups was then tested with a *G*-test for goodness of fit (Sokal and Rohlf 1995). All two-way contingency tables, including survival at Marrowstone, were tested for independence using the *G*-test (Sokal and Rohlf 1995). Exact *P*-values of goodness of fit and independence tests were obtained from StatXact-3 (Cytel Software Corporation 1997). The effects of two factors on a binary response variable (e.g. of cross, initial length, and their interaction on survival at Marrowstone) were tested with logistic models (logistic procedure; SAS Institute Incorporated 2000). Continuous data were analyzed by analysis of variance (AOV) if normality and equal variance assumptions held or by Kruskal-Wallis (K-W; based on ranks) if not. Rate of growth in length at Marrowstone was tested with analysis of covariance (AOCV) to account for effects of initial length and survival status (surviving or not). For all tests, all six paired comparisons between the four crosses were made; *P*-values were adjusted by the Tukey-Kramer method for AOV and AOCV or by the non-sequential Bonferonni method for all other tests (Sokal and Rohlf 1995).

Small sample sizes in some tanks influenced the results of the seawater rearing experiment. Therefore, we pooled data from four rearing tanks for our primary analyses, and then conducted

secondary analyses on key variables to test whether including tank effect changed the results. For secondary tests not meeting assumptions of AOV, we conducted two-way AOV on ranks to account for the cross-tank interaction.

Results

Hatchery Rearing

Egg weight and ELISA absorbance were similar between Carson and Warm Springs female spawners (Table 3). Survival to eye-up was greater for eggs fertilized by Carson than by Warm Springs males. Fry length differed among crosses (P=0.04). The shortest and longest fry were from the CxC and WxW crosses, respectively, but none of the pair-wise comparisons were significant. Means for fry weight, k_D, and percent yolk were similar among crosses.

Survival at the hatchery was 74% (3,734 yearlings/5,053 fry), with no difference in relative survival among crosses. Differences were found for final length and weight (Table 4) using K-W rank comparisons because all of the crosses showed a bimodal length distribution; fish were either <169 or >174 mm long (Figure 4). The percent of fish in the upper mode compared to the lower mode was greater for CxC and WxC than for WxW fish, with CxW intermediate.

Seawater Rearing

There were no significant differences in seawater survival among crosses (Table 5) even when length modes were considered separately (Figure 5). Fish mortalities did not occur until the tank water was converted from fresh to seawater. Thereafter fish mortalities occurred in "early" and "late" time periods (before and after 77 days post-hatchery sampling based on a natural break in the data; Figure 6). Mean number of days to death was similar among crosses, but the percent of mortalities in the early time period was significantly lower for WxW fish than for the other crosses (Table 5). Final length and weight of survivors was significantly different among crosses, but only one paired comparison was different (WxC greater than WxW fish; Table 5). Growth rate (mm/day) was higher for survivors than for mortalities, and within each group growth rate appeared to be inversely correlated with initial length (Figure 6). Mean growth rate was significantly greater (16%) for WxC than for WxW fish when we accounted for the effects of survival status (surviving or not) and initial length (Table 5); no other comparisons were significant.

The distribution of ELISA absorbance values was bimodal in the overall sample (Figure 3) and when mortalities and survivors were considered separately (Figure 6). ELISA absorbance and frequency of occurrence in the upper ELISA mode (absorbance > 2.2) was similar among crosses for all fish (survivors and mortalities combined) (Table 5). The percentage of fish in the upper ELISA mode was significantly greater for fish from the upper initial length mode than the lower length mode, the exception being WxW in the lower length mode (P=0.048; Figure 5, bottom). Most fish that died early had low absorbance values. Thereafter, most of the mortalities had high absorbance values (Figure 7).

There were differences among crosses in the four rearing tanks for survival (range 4-79%; P<0.01), mean growth rate (range 0.122-0.157 mm/d; P=0.03), ELISA absorbance (range 0.5-2.7; P<0.01), and days to death (range 91-108; P=0.054). However, results of comparisons among crosses for these variables were mostly unchanged by including tank effect in analyses. There was never a significant interaction between tank and cross (P>0.10). The crosses differed in growth rate (WxC highest and WxW lowest; P=0.02) but not in survival or days to death (P>0.10), the same results as were obtained by pooling over tanks (Table 5). Only results for ELISA absorbance changed. Including tank effect resulted in a difference among crosses (CxC>WxC; CxW and WxW intermediate; 2-way AOV on ranks; P=0.03) whereas none was found by pooling over tanks (Table 5).

Discussion

Our goal was to evaluate maternal and stock effects on growth and survival of Chinook salmon during hatchery and seawater rearing. The results (summarized in Figure 8) did not conform to any of the expected responses shown in Figure 2 and are inconclusive. However, we were able to isolate trends by comparing the results from pairs of experimental groups to corresponding pairs in the expected responses from Figure 2. We found similarity between reciprocal hybrids (CxW and WxC), suggesting no maternal effect. There were significant differences between the WxC and WxW crosses, indicating a stock effect. In contrast, the similarity of CxC and CxW crosses suggested no stock effect. With CxC and CxW being similar and WxW being different a maternal effect is indicated. Performance measures for the three crosses with at least one Carson parent (CxC, CxW, and WxC) were always statistically similar. One possibility was that stock effect was not additive, as in our theoretical model, but non-additive, with Carson genetics being dominant. Alternatively, the observed performance patterns (CxC=CxW=WxC>WxW) could have been caused by an interaction between maternal and stock effects; if the female parent was from Warm Springs, then the genetic difference between stocks was expressed, but if the female was from Carson, no stock effect was expressed.

It is unlikely that the differences among crosses we observed were due to effects other than maternity or stock. Progeny from a large number of parents were tested, ruling out a founder effect. Survival to eye-up at the hatchery was greater for crosses using Carson males rather than Warm Springs males. This result was most likely due to a difference in the quality of the preserved milt, either due to inadvertent technique differences or long-term storage effects; eggs surviving past eye-up had similar survival. Environmental conditions among crosses were similar during incubation and were identical during hatchery and seawater rearing, ruling out an environmental effect. Differences among crosses at Marrowstone were not confounded by tank effects and evidence for a paternal effect in this study was inconclusive; CxC and WxC were similar, but CxW and WxW were different.

Final size was lower for WxW fish than for the other crosses, even though they were initially the longest fry, indicating faster growth for fish with Carson parents. Growth differences may indicate hatchery-specific adaptations. Fish reared at Carson typically attain a release size of 25g under cool summer-growth conditions, while Warm Springs fish typically attain a size of 38g at a similar release time under warmer conditions (Banks 1994; Olson et al. 1995). When reared together at Warm Springs NFH, the crosses with at least one Carson parent attained mean weights 40-50% greater than that of WxW fish. Perhaps this difference reflects past selection for faster growth at Carson, where cool rearing temperatures may require “fast” growth (i.e. fast for the given temperature) to achieve adequate size at release, than at Warm Springs.

There was no direct evidence that the fast growth and large final size of CxC, CxW, and WxC fish were either beneficial or detrimental; they had higher initial seawater mortality, but similar overall seawater survival compared with WxW fish. Large size at release can be related to increased smolt-to-adult return rates (Bilton et al. 1982; Martin and Wetheimer 1989; Tipping 1997; Ward and Slaney 1988), with spring growth rate perhaps being the most important contributor (Beckman et al. 1999). However, the low early mortality of the WxW cross may provide indirect evidence of negative effects of fast growth, which can lead to early maturation and residualism in salmonids (Larsen et al. 2010). We did not find evidence for this, with only one fish having obvious signs of early maturation and early mortality being unrelated to size within group. Condition factor (a combination of length and weight) is an indicator of smoltification, but we found no differences among groups. Early mortalities could have been caused by a delayed effect from handling and transportation (i.e. from scale loss) or other factors, including the activation of latent diseases (Wedemeyer 1996) or the physiological stress of adjusting to seawater, especially for fish affected by BKD (Banner et al. 1985). Higher prevalence of BKD in the upper

compared to the lower length mode for CxC, CxW, and WxC fish (Figure 5, bottom) may also indicate a disadvantage to fast growth at the hatchery. However, these crosses were similar to WxW in overall BKD prevalence and BKD was similar between lower and upper length modes of WxW fish sampled at the hatchery.

The distribution and time series of ELISA absorbance shown in Figures 5, 6, and 7 suggest four related conclusions. First, fish with high absorbance values likely died from BKD whereas those with low absorbance values did not. We assigned fish to high and low ELISA groups based on the bimodal distribution of ELISA values, with the cutoff value being 2.2. This is a conservative value when compared to Elliott et al. (1995) who classified absorbance values $\geq$ 1.0 as indicating high infection levels. They identified fish as dying from BKD by high FAT (fluorescent antibody technique) counts of bacteria. Pascho et al. 1987 showed a significant positive correlation ($P<0.001$) between ELISA absorbance levels and FAT counts. Second, BKD was generally low for early mortalities (mean OD=0.872, 82% in the lower ELISA mode) and was not the major cause of death. Apparently, an extended period of seawater rearing was required for BKD to progress to the point of causing mortality. Third, survivors with high absorbance values likely would not have survived much longer had the experiment been continued. Assuming that this was true, we recalculated survival from the seawater rearing experiment using only fish from the low ELISA mode and found that survival was similar among crosses (Table 5). And fourth, at least for crosses other than WxW, lethal or potentially lethal cases of BKD were contracted more frequently by large fish that grew fast in the hatchery than by smaller fish that grew more slowly (Fig 6, lower panel). There did not appear to be any maternal or stock effects associated with ELISA levels.

In conclusion, differences in growth and early seawater survival were observed among experimental groups, but we were unable to identify maternal or stock effects as the cause. While we made efforts to reduce environmental differences between groups through common rearing, some differences could have occurred during marking. Future maternal effects experiments could be improved by creating full diallel crosses, using advances in individual marking technologies or genetic techniques to track individual or family level performance, and by increasing sample size. Choosing stocks with overlapping spawning timing would eliminate the need for using cryopreserved milt, reducing mortality. The exercise examining expected responses to stock and maternal effects is useful for interpreting results from the hatchery/wild companion experiment. Special attention should be paid to the interpretation, given that only one of the reciprocal crosses could be made in that experiment.

Acknowledgements

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References

Bams, R.A. 1970. Evaluation of a revised hatchery method tested on pink and chum salmon fry. Journal of the Fisheries Research Board of Canada 27:1429-1452.

Banks, J. L. 1994. Raceway density and water flow as factors affecting spring Chinook salmon (*Oncorhynchus tshawytscha*) during rearing and after release. Aquaculture 119(2-3), 201-217.

Banner, C.R., J.S. Rohovec, and J.L.Fryer. 1983. *Renibacterium salmoninarum* as a cause of mortality among Chinook salmon in salt water. Journal of the World Mariculture Society 14:236-239.

Beckman, B. R., W.W. Dickhoff, W.S. Zaugg, C. Sharpe, S. Hirtzel, and R. Schrock. 1999. Growth, smoltification, and smolt-to-adult return of spring Chinook salmon from hatcheries on the Deschutes River, Oregon. Transactions of the American Fisheries Society 128(6): 1125-1150.

Berejikian, B. A., E.P. Tezak, S.L. Schroder, T.A. Flagg, and C.M. Knudsen. 1999. Competitive differences between newly emerged offspring of captive-reared and wild coho salmon. Transactions of the American Fisheries Society 128(5), 832-839.

Bilton, H.T., D.F. Alderdice, and J.T. Schnute. 1982. Influence of time and size at release of juvenile coho salmon (*Oncorhynchus kisutch*) on returns at maturity. Canadian Journal of Fisheries and Aquatic Sciences 39:426-447.

Brothers, E.D. 1990. Otolith Marking. American Fisheries Society Symposium 7:203-215.

Cytel Software Corporation. 1997. StatXact-3, Version 3.1. Copyright 1989-1997.

Einum, S. and I.A. Fleming. 2000. Highly fecund mothers sacrifice offspring survival to maximize fitness. Nature 405:565-567.

Elliott, D. G., R.J. Pascho, and A.N. Palmisano. 1995. Brood stock segregation for the control of bacterial kidney disease can affect mortality of progeny chinook salmon (*Oncorhynchus tshawytscha*) in seawater. Aquaculture 132(1-2), 133-144.

Hallerman, E. M. 2003. Quantitative genetics. Pages 261-287 *in* E. M. Hallerman, editor. Population genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, Maryland.

Heath, D.D., C.W. Fox, and J.W. Heath. 1999. Maternal effects on offspring size: variation through early development of Chinook salmon. Evolution 53(5): 1605-1611.

Jensen, J.O.T. 1988. A microcomputer program for predicting embryonic development on Pacific salmon and steelhead trout. World Aquaculture 19(4): 80-81.

Larsen, D.A., B.R. Beckman, and K.A. Cooper. 2010. Examining the conflict between smolting and precocious male maturation in spring (stream-type) Chinook salmon. Transactions of the American Fisheries Society 139: 564-578.

Martin, R.M. and A. Wertheimer. 1989. Adult production of Chinook salmon reared at different densities and released as two smolt sizes. The Progressive Fish-Culturist 51:194-200.

National Marine Fisheries Service (NMFS). 2003. Hatchery broodstock summaries and assessments for chum, coho and Chinook salmon and steelhead stocks within Evolutionary Significant Units listed under the Endangered Species Act. NOAA Fisheries Salmon and Steelhead Hatchery Assessment Group, Northwest and Southwest Fisheries Science Centers.

Olson, D. E., B. C. Cates, and D. H. Diggs. 1995. Use of a national fish hatchery to complement wild salmon and steelhead production in an Oregon stream. Pages 317-328 *in* H. L. Schramm, Jr. and R. G. Piper, editors. Uses and effects of cultured fishes in aquatic ecosystems. American Fisheries Society, Symposium 15. Bethesda, Maryland.

Olson, D. E., B. Spateholts, M. Paiya, and D. E. Campton. 2004. Salmon hatcheries for the 21st century: a model at Warm Springs National Fish Hatchery. Pages 585-602 *in* M. J. Nickum, P. M. Mazik, J. G. Nickum, and D. D. MacKinlay, editors. Propagated fish in resource management. American Fisheries Society, Symposium 44. Bethesda, Maryland.

Pascho, R.J., D.G. Elliott, R.W. Mallett, and D. Mulcahy. 1987. Comparison of five techniques for the detection of *Renibacterium salmoninarum* in adult coho salmon. Transactions of the American Fisheries Society 116:882-890.

Pascho, R. J., D.G. Elliott, and J.M. Streufert. 1991. Brood stock segregation of spring Chinook salmon *Oncorhynchus tshawytscha* by use of the enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody technique (FAT) affects the prevalence and levels of *Renibacterium salmoninarum* infection in progeny. Diseases of Aquatic Organisms 12(1), 25-40.

Pascho, R.J. and D. Mulcahy. 1987. Enzyme-linked immunosorbent assay for a soluble antigen of *Renibacterium salmoninarum*, the causative agent of salmonid bacterial kidney disease. Canadian Journal of Fisheries and Aquatic Sciences 44:183-191.

Prentice, E.F., T.A. Flagg, C.S. McCutcheon, and D.F. Brastow. 1990. PIT-tag monitoring systems for hydroelectric dams and fish hatcheries. In: Parker, N.C., A.E. Giorgi, R.C. Heidinger, D.B. Jester, Jr., E.D. Prince, and G.A. Winans (eds), pp. 323-334. Fish-marking techniques. American Fisheries Society Symposium 7, Bethesda, MD.

SAS Institute Inc. 2000. SAS Online DOC®, Version 8. SAS Institute, Cary, North Carolina.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry, 3rd edition. W. H. Freeman and Company, New York.

Tipping, J.M. 1997. Effect of smolt length at release on adult returns of hatchery-reared winter steelhead. The Progressive Fish-Culturist 59:310-311.

Volk, E.C., S.L. Schroder, and K.L. Fresh. 1990. Inducement of unique otolith banding patterns as a practical means to mass-mark juvenile Pacific salmon. American Fisheries Society Symposium 7:203-215.

Wahle, R. J. and E. Chaney. 1981. Establishment of nonindigenous runs of spring Chinook salmon, *Oncorhynchus tshawytscha* , in the Wind River drainage of the Columbia River, 1955-63. Fisheries Bulletin Seattle 79(3), 507-516.

Ward, B.R. and P.A. Slaney. 1988. Life history and smolt-to-adult survival of Keogh River steelhead trout (*Salmo gairdneri*) and the relationship to smolt size. Canadian Journal of Fisheries and Aquatic Sciences 45:1110-1122.

Wedemeyer, G. A. 1996. Physiology of Fish in Intensive Culture Systems. Chapman & Hall, New York.

Wheeler, P.A. and G.H. Thorgaard. 1991. Cryopreservation of rainbow trout semen in large straws. Aquaculture 93:95-100.

Tables

Table 1. Number of adults spawned.

Stock	Females	Males
Carson	16	20
Warm Springs	19	18

Table 2. Number of males used to produce various combinations of full-sib families by stock and cross in the maternal effects experiment.

		Number of Times Each Male Used[a]			
Male stock	Males	CxC	CxW	WxC	WxW
Carson (n=20)	1	1	--	0	--
	4	0	--	1	--
	15	1	--	1	--
Warm Springs (n=18)	1	--	1	--	0
	1	--	1	--	2
	1	--	2	--	2
	3	--	0	--	1
	12	--	1	--	1

[a]First letter in each cross designation indicates female cross used, e.g. WxC = female Warm Springs x male Carson

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Table 3. Mean (SD) female, egg, and fry characteristics. Means without a letter in common differ (P<0.05).

Variable	CxC	CxW	WxC	WxW	P[a]
Mean ELISA absorbance[b] (SD)[c]	0.063 (0.006)		0.074 (0.023)		--
Individual egg weight (mg)[c]	229 (51) a		231 (40) a		>0.10
Average incubation temperature (^{o}C)	7.4	7.4	7.8	7.8	--
Accumulated temperature units (^{o}C)	1036	1037	1039	1038	--
Survival to eye-up (%)[d]	76.7 (12.0) a	55.0 (17.1) c	73.2 (19.5) ab	58.6 (21.2) bc	<0.01
Survival from eye-up to button-up (%)	90.8	86.9	97.9	90.1	--
Fry length (mm)[e]	33.9 (1.1) a	34.0 (1.1) a	34.5 (1.2) a	34.7 (0.8) a	<0.04
Fry weight (mg)[e]	315 (59) a	330 (63) a	342 (56) a	346 (35) a	>0.10
Developmental index (k_D)[ef]	2.00 (0.07) a	2.02 (0.08) a	2.02 (0.05) a	2.02 (0.05) a	>0.10
Percent yolk[eg]	9.9 (3.4) a	10.5 (4.4) a	9.8 (2.8) a	10.0 (3.5) a	>0.10

[a]P-value of test for overall difference among crosses.
[b]ELISAs were run separately for Carson and Warm Springs females so absorbance values may not be directly comparable; no statistical test conducted.
[c]Standard deviation among female parents.
[d]Standard deviation among families.
[e]Standard deviation among individuals.
[f]$k_D = 10*W^{1/3}*L^{-1}$; W = weight (mg); L = fork length (mm) (Bams 1970).
[g]Yolk weight as percent of whole body weight.

Table 4. Survival and final size characteristics for fish reared at Warm Springs. For tests with $P<0.05$, means without a letter in common differ.

Variable	Cross				
	CxC	CxW	WxC	WxW	P[a]
Proportion of fry ponded (*N*=5053)	0.266	0.191	0.320	0.223	--
Proportion of smolts sampled (n=340) (*N*=340)	0.232	0.185	0.362	0.221	--
Relative survival	1.00	1.11	1.30	1.13	>0.10
Mean fork length (mm) (mean rank, SD)	157 (171, 40) ab	154 (178, 33) ab	156 (183, 33) a	141 (140, 24) b	0.02
Mean weight (g) (mean rank, SD)	51.1 (169, 38.2) ab	45.7 (178, 31.8) a	47.1 (182, 31.8) a	32.8 (137, 19.8) b	0.02
Percent in upper length mode (>170 mm)	36.7 a	25.4 ab	31.7 a	10.8 b	<0.01

[a]*P*-value of test for overall difference among crosses.

Table 5. Survival, growth, and ELISA absorbance of fish reared in seawater. For tests with $P<0.05$, means without a letter in common differ.

Variable	Cross				
	CxC	CxW	WxC	WxW	P[a]
Survival (%)	39.2 a	30.2 a	36.6 a	38.7 a	>0.10
Mean days to death (mean rank, SD) [b]	99 (96, 23)	102 (101, 22)	104 (114, 23)	111 (121, 12)	P>0.10
Percent of mortalities in "early" period (Fig. 6)	18.7 a	15.9 a	17.9 a	0.0 b	<0.01
Mean length of survivors (mm) (mean rank, SD) [c]	183 (63, 35) ab	185 (68, 34) ab	184 (70, 27) a	166 (46, 21) b	0.04
Mean weight of survivors (g) (mean rank, SD)[c]	84 (64, 50) ab	83 (68, 49) ab	81 (70, 37) a	58 (46, 24) b	0.04
Mean growth in length (mm/d)[d]	0.141 ab	0.152 ab	0.160 a	0.138 b	0.02
Mean ELISA absorbance (mean rank, SD)	1.95 (179, 1.32)	1.72 (155, 1.39)	1.83 (172, 1.38)	2.13 (172, 1.29)	--
Percent in upper ELISA mode (Figure 6)	55.7	52.4	54.5	68.0	>0.10
Adjusted survival(%)[e]	27.8	25.4	31.7	29.3	>0.10

[a]*P*-value of test for overall difference among crosses.
[b]K-W rank comparison on fish that died before 18 July. Days to death calculated from hatchery sampling (1 March).
[c]K-W rank comparison on fish that survived to 18 July;.
[d]Least square means from a two-way AOCV; class variables were cross and survival status (surviving to 18 July or not) and covariate was initial length; interactions among cross, survival status, and length were all non-significant ($P>0.10$).
[e]Estimated survival % after removal of survivors in the upper ELISA mode (they were counted as mortalities on the assumption that they would have died soon after the end of the experiment).

Figures

Figure legends

Figure 1. Map of the study area denoting hatchery locations (NFH=National Fish Hatchery).

Figure 2. Expected responses to different combinations of stock and maternal effects. For this exercise, we assumed that effects are additive and that beneficial maternity is equivalent to one unit of beneficial stock; hybrids have one unit and stock A two units of beneficial stock. AxA=stock A female x stock A male; AxB=stock A female x stock B male; BxA=stock B female by stock A male; BxB=stock B female x stock B male. Columns without a lower case letter in common differ.

Figure 3. ELISA absorbance distributions from two different assays (runs). The distribution from each run was bimodal. Vertical dashed lines separate the lower and upper modes of each run.

Figure 4. Relative length frequency distributions of yearling Chinook salmon sampled at Warm Springs. The vertical dashed line separates the upper and lower length modes.

Figure 5. Percent survival (upper panel) and percent occurrence in the upper ELISA mode (lower panel) for fish in each of the initial length modes (shown in Figure 4) of Chinook salmon in the seawater rearing experiment.

Figure 6. Scatter plots of days to death, growth rate, and ELISA absorbance of Chinook salmon mortalities and survivors in the seawater rearing experiment. The x-axis for each graph represents the initial lengths of fish sampled at the hatchery prior to transfer to Marrowstone. Vertical dashed lines separate the lower and upper length modes. The horizontal lines in the days to death plot separate early from late mortalities and show the day that seawater conversion was initiated. The horizontal line in the ELISA absorbance plot separates the lower and upper ELISA modes.

Figure 7. ELISA absorbance versus days to death for Chinook salmon from the seawater rearing experiment. Vertical and horizontal dashed lines separate the early and late mortality periods and the upper and lower ELISA modes, respectively.

Figure 8. Graphs summarizing the significant differences in growth and survival among experimental groups.

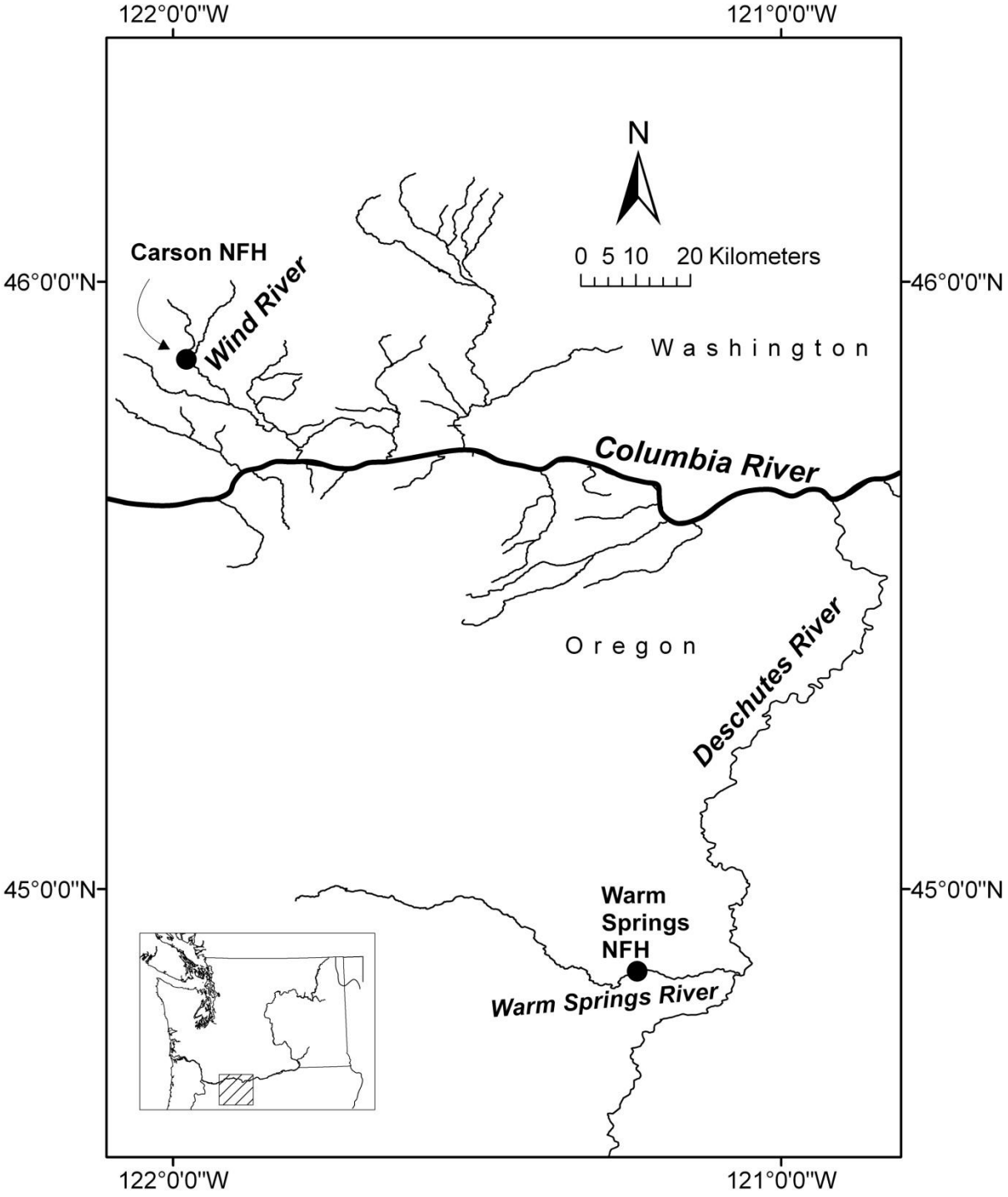
122°0'0"W
121°0'0"W
N
0 5 10 20 Kilometers
Carson NFH
Wind River
46°0'0"N
46°0'0"N
Washington
Columbia River
Oregon
Deschutes River
45°0'0"N
45°0'0"N
Warm
Springs
NFH
Warm Springs River
122°0'0"W
121°0'0"W

Figure 1.

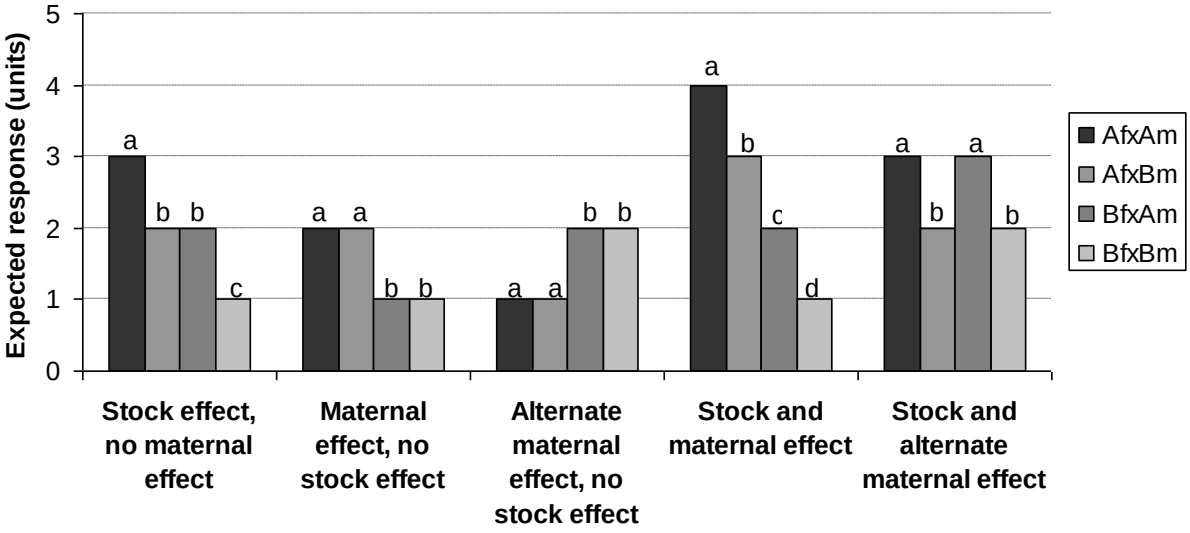
Expected response (units)
5
4
3
2
1
0
a
b b
c
a a
b b
a a
b b
a
b
c
d
a
b
a a
b
Stock effect, no maternal effect
Maternal effect, no stock effect
Alternate maternal effect, no stock effect
Stock and maternal effect
Stock and alternate maternal effect
AfxAm
AfxBm
BfxAm
BfxBm

Figure 2.

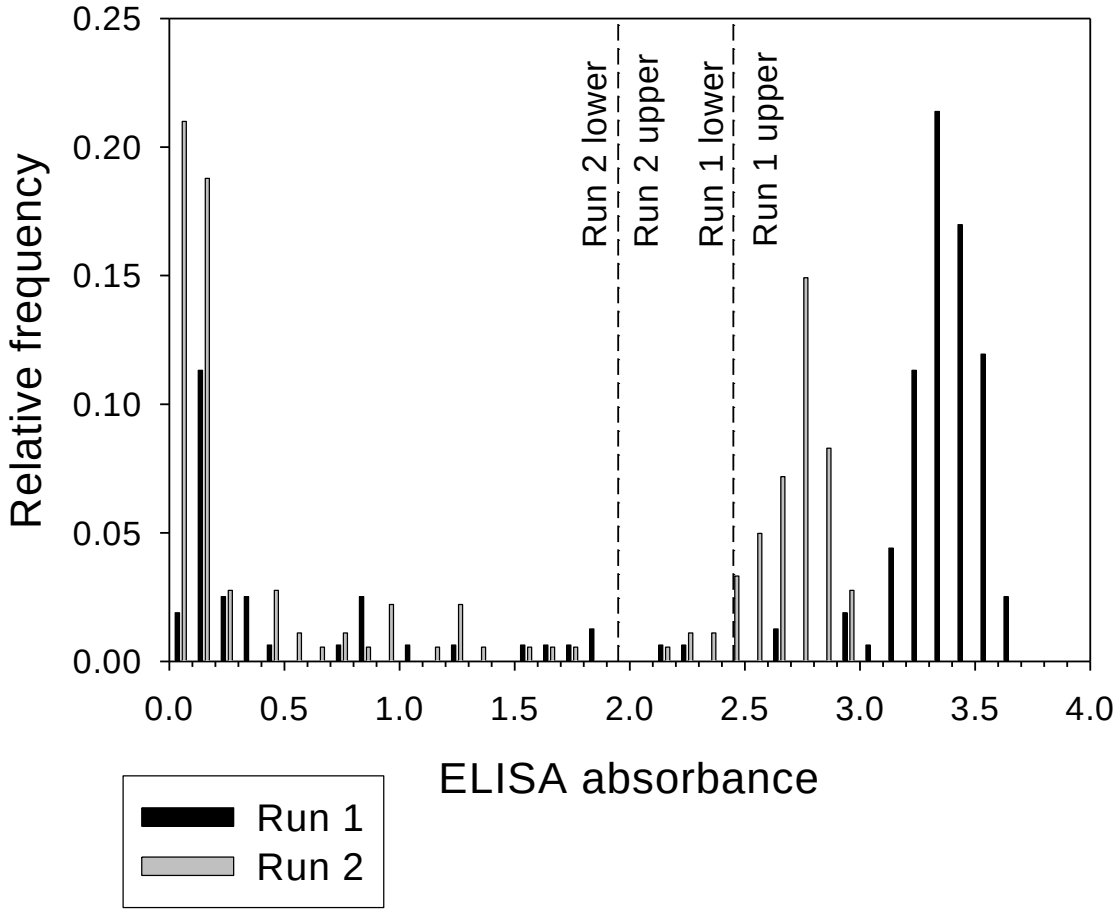
0.25
0.20
0.15
0.10
0.05
0.00
Relative frequency
Run 2 lower
Run 2 upper
Run 1 lower
Run 1 upper
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
ELISA absorbance
Run 1
Run 2

Figure 3.

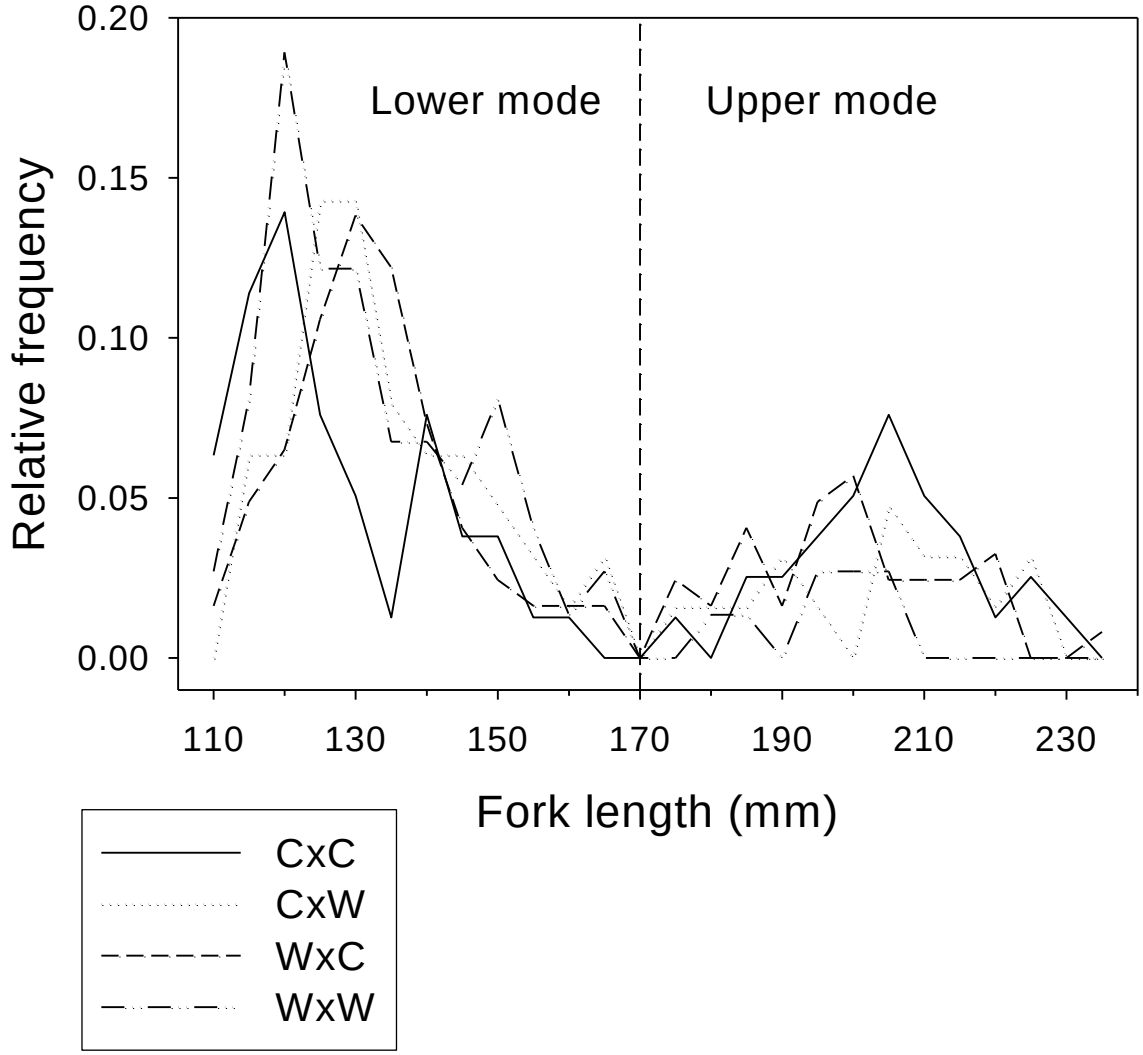
0.20
0.15
0.10
0.05
0.00
Relative frequency
Lower mode
Upper mode
110
130
150
170
190
210
230
Fork length (mm)
CxC
CxW
WxC
WxW

Figure 4.

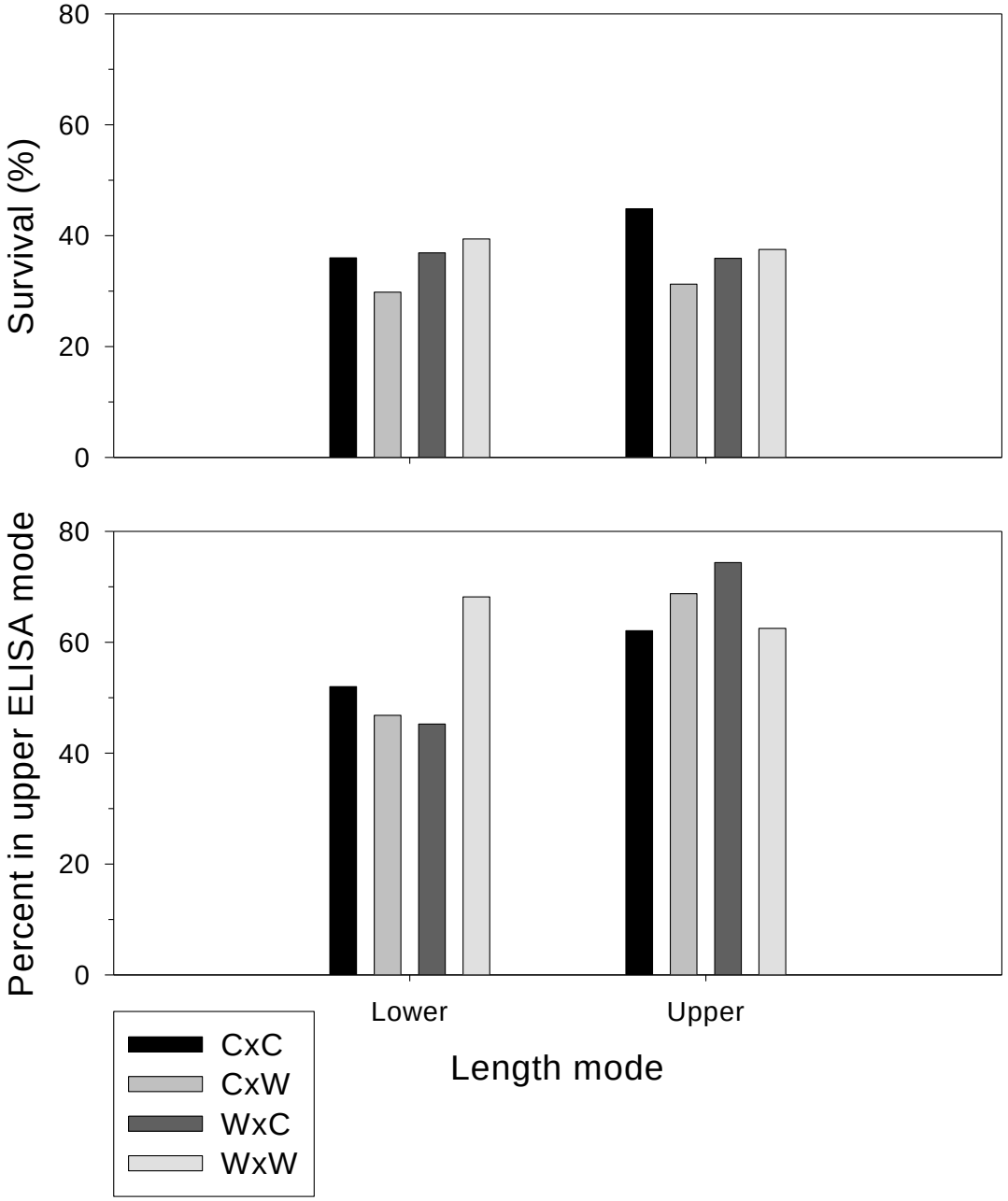
80
60
40
20
0
Survival (%)
80
60
40
20
0
Percent in upper ELISA mode
Lower
Upper
Length mode
CxC
CxW
WxC
WxW

Figure 5.

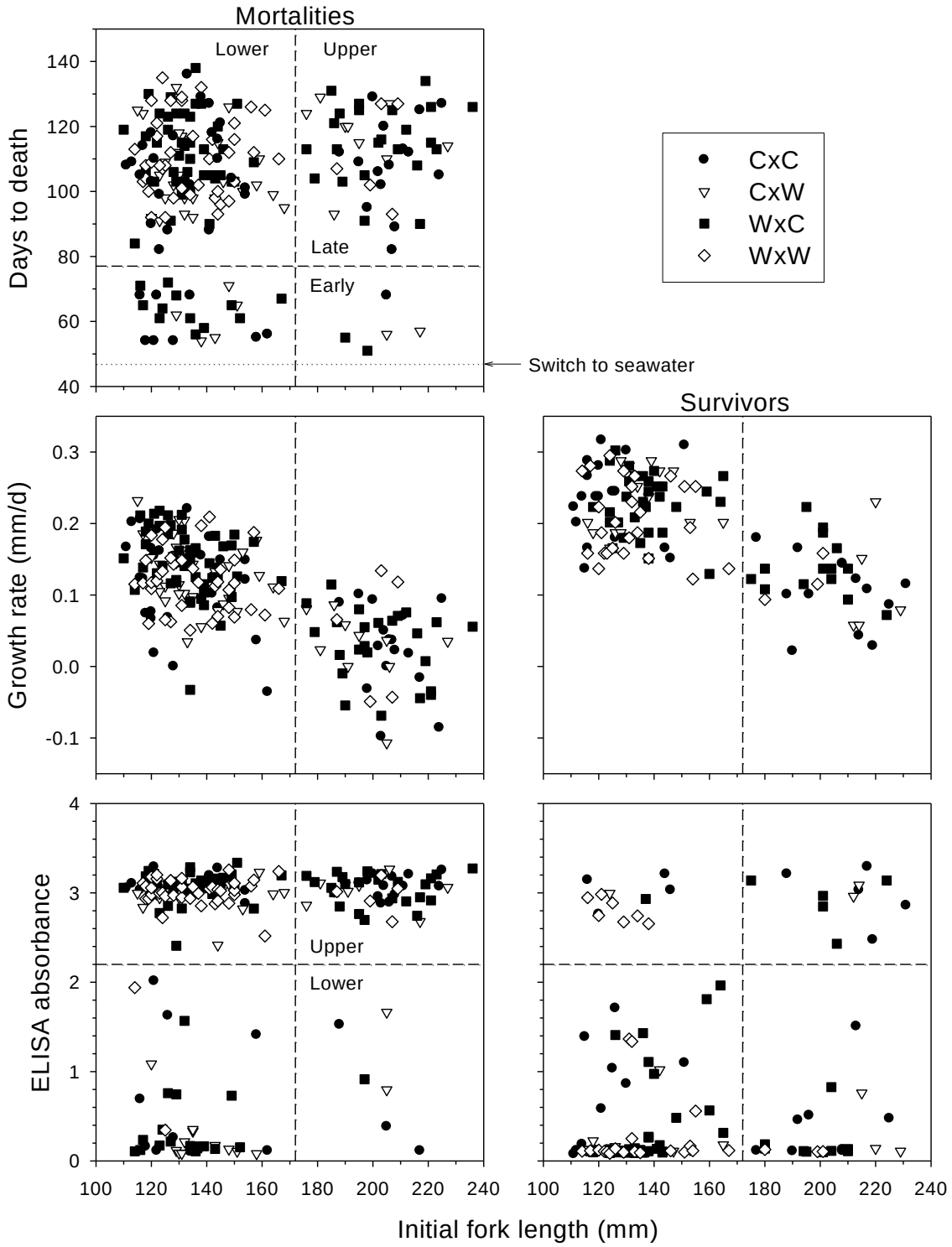
Mortalities
Survivors
Lower
Upper
Late
Early
Switch to seawater
CxC
CxW
WxC
WxW
Days to death
Growth rate (mm/d)
ELISA absorbance
Upper
Lower
Initial fork length (mm)

Figure 6.

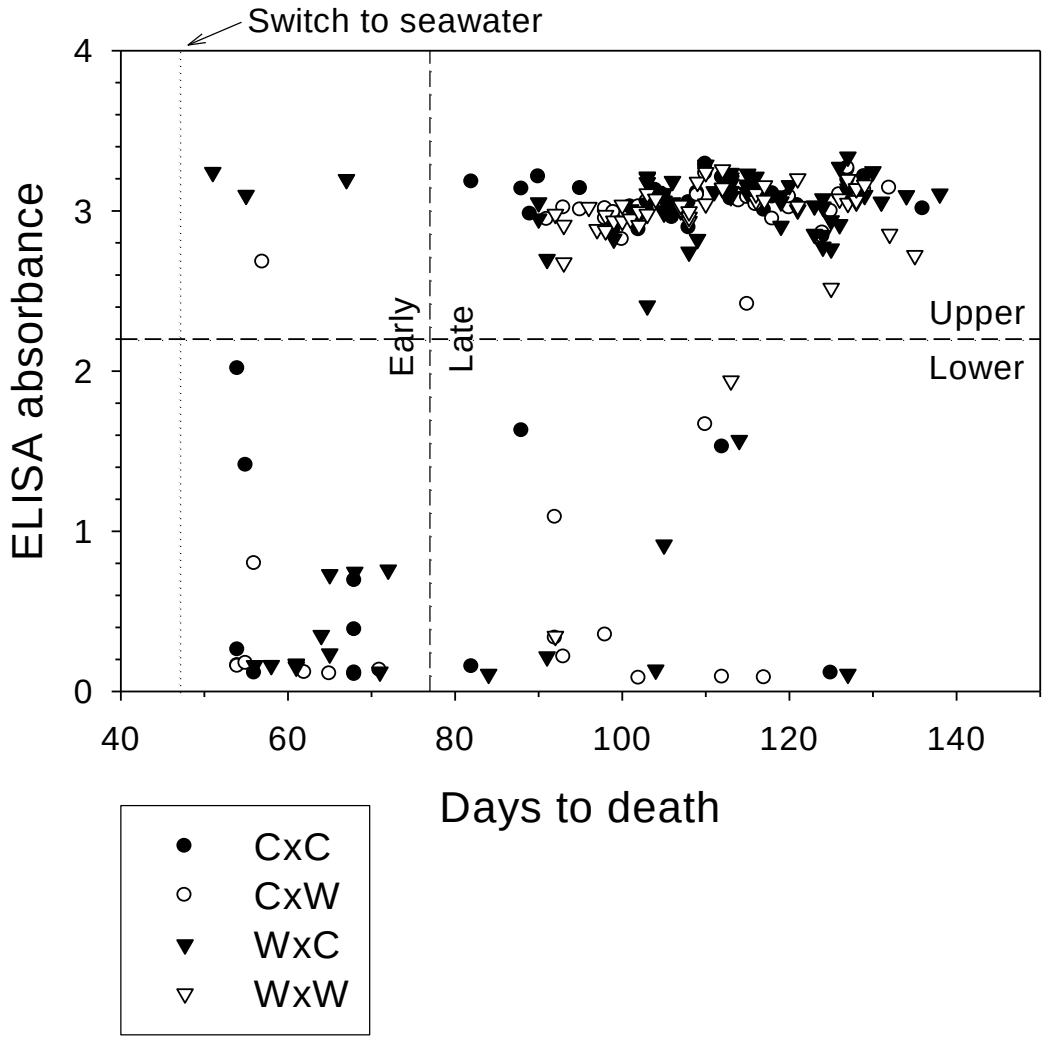
Switch to seawater
ELISA absorbance
Days to death
Early
Late
Upper
Lower
CxC
CxW
WxC
WxW

Figure 7.

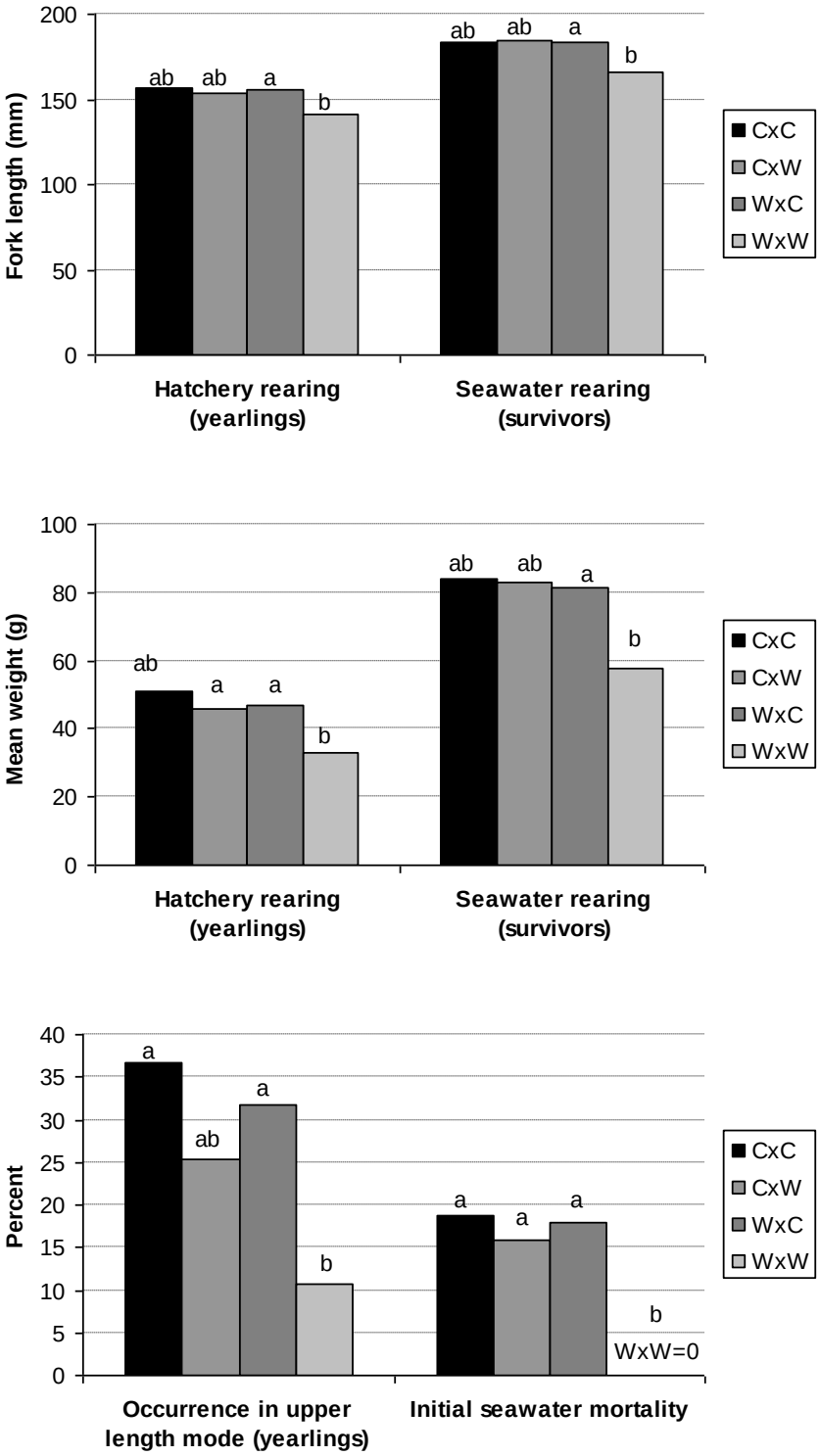
Fork length (mm)
200
150
100
50
0
ab
ab
a
b
ab
ab
a
b
Hatchery rearing (yearlings)
Seawater rearing (survivors)
CxC
CxW
WxC
WxW
Mean weight (g)
100
80
60
40
20
0
ab
a
a
b
ab
ab
a
b
Hatchery rearing (yearlings)
Seawater rearing (survivors)
CxC
CxW
WxC
WxW
Percent
40
35
30
25
20
15
10
5
0
a
ab
a
b
a
a
a
b
WxW=0
Occurrence in upper length mode (yearlings)
Initial seawater mortality
CxC
CxW
WxC
WxW

Figure 8.

Citations and abstracts for previously published papers

Reisenbichler, R. R., M. C. Hayes, S. P. Rubin, L.A. Wetzel, and B. M. Baker. 2006. *PEPA** genotype affects return rate for hatchery steelhead. Transactions of the American Fisheries Society 135:28-39.

Abstract: Allozymes continue to be useful as genetic markers in a variety of studies; however, their utility often hinges on the selective neutrality of the allelic variation. Our study tested for neutrality between the two most common alleles ('100 and '110) at the cytosol nonspecific dipeptidase locus (PEPA- 1') in steelhead Oncorhynchus mykiss from Dworshak National Fish Hatchery in Idaho. We tested for differential growth and survival among fish with the '100/100, '100/ 110, and '110/110 genotypes rearing in a hatchery or a natural stream. We repeated the study for two year-classes, using heterozygous ('100/110) adults to make the experimental crosses. This design avoided differences in family contribution among genotypes because each cross produced all three genotypes. We divided the progeny from each family into two groups. One group was reared in a hatchery for 1 year and then released for migration to the sea and subsequent return to the hatchery as adults. The other group was released into a natural stream and monitored for 3 years. We found no significant differences in size or survival among PEPA-1' genotypes for either the naturally reared fish or the hatchery-reared fish immediately prior to release as smolts. For females, survival to returning adult also was similar among genotypes; however, hatchery-reared males with the '110/110 genotype returned at a higher rate than did males with the '100/ 100 genotype; heterozygous males were intermediate. These results indicate that selection occurs at the PEPA-1' locus or at one or more loci tightly linked to it. The finding of nearly equal frequencies for these two alleles in the source population suggests that selection differentials among genotypes reverse or vary from year to year; otherwise, steady directional selection would drive the '100 allele to low frequencies or extinction. Locus PEPA-1' seems inappropriate for genetic marks in studies of steelhead that span the full life cycle and probably should be avoided for any portion of the life cycle. Inferences about gene flow and population structure from studies that are substantially influenced by PEPA-1' allele frequencies might be misleading.

Hayes, M. C., R. R. Reisenbichler, S. P. Rubin, and L.A. Wetzel. 2011. Differential survival among *sSOD-1** genotypes in Chinook salmon. Transactions of the American Fisheries Society 140:1305-1316.

Abstract: Differential survival and growth were tested in Chinook salmon *Oncorhynchus tshawytscha* expressing two common alleles, *∗–100* and *∗–260*, at the superoxide dismutase locus (*sSOD-1∗*). These tests were necessary to support separate studies in which the two alleles were used as genetic marks under the assumption of mark neutrality. Heterozygous adults were used to produce progeny with *–100/–100*, *–100/–260*, and *–260/–260* genotypes that were reared in two natural streams and two hatcheries in the states of Washington and Oregon. The latter also were evaluated as returning adults. In general, the genotype ratios of juveniles reared at hatcheries were consistent with high survival and little or no differential survival in the hatchery. Adult returns at one hatchery were significantly different from the expected proportions, and the survival of the *–260/–260* genotype was 0.56–0.89 times that of the *–100/–100* genotype over four year-classes. Adult returns at a second hatchery (one year-class) were similar but not statistically significant: survival of the *–260/–260* genotype relative to the *–100/–100* genotype was 0.76. The performance of the heterozygote group was intermediate at both hatcheries. Significant differences in growth were rarely observed among hatchery fish (one year-class of juveniles and one age-class of adult males) but were consistent with greater performance for the *–100/–100* genotype. Results from two groups of juveniles reared in streams (one year-class from each stream) suggested few differences in growth, but the observed genotype ratios were significantly different from the expected ratios in one stream. Those differences were consistent with the adult data; survival for the *–260/–260* genotype was 76% of that of the *–100/–100* genotype. These results, which indicate nonneutrality among *sSOD-1∗* genotypes, caused us to modify our related studies and suggest caution in the interpretation of results and analyses in which allozyme marks are assumed to be neutral.

Hayes, M. C., S. P. Rubin, J. E. Hensleigh, R. R. Reisenbichler, and L. A. Wetzel. 2005. Performance of juvenile steelhead trout (*Oncorhynchus mykiss*) produced from untreated and cryopreserved milt. Aquaculture 249:291-302.

Abstract: Despite the expanding use of milt cryopreservation in aquaculture, the performance of fish produced from this technique has not been fully explored beyond initial rearing stages. We compared the performance of juvenile steelhead Oncorhynchus mykiss produced from untreated (UM) and cryopreserved milt (CM) and reared for 4-9 months. For the 1996 brood, CM alevins were heavier (similar to 1.7%, P<0.01) than UM alevins and length was influenced by a significant milt-by-family interaction (P<0.03) suggesting a greater treatment effect for some families. No significant differences were found in length or weight (P>0.05) for 1997 brood alevins and percent yolk was similar for both broods (P>0.34). In growth and survival experiment I (GSE-I, 1996), UM and CM juveniles reared in separate tanks and fed to satiation (130 days) showed no significant differences in survival, length or weight (P>0.05) between milt groups. In contrast, for UM and CM siblings reared in the same tank for 210 days on a low food ration (GSE-II), survival was similar (P>0.05), but length (UM 4%>CM, P<0.05) and possibly weight (UM 15%>CM, P=0.08), were influenced by cryopreservation. Fish from the 1997 brood (GSE-III) were reared for 313 days in a repeat of GSE-II and no differences were found in survival (P=0.47), length (P=0.75) or weight (P=0.76) suggesting considerable heterogeneity between broods. Performance of the 1996 brood was also tested for response to stress and a disease challenge. Cortisol responses of juveniles exposed to acute stress were not significantly different (P=0.19), but mean cortisol was consistently and significantly greater (P<0.01) for CM than UM fish exposed to a 48-h stress (increased density). After exposure to three dosages of the bacteria, Listonella anguillarum, we found similar mortality proportions (P=0.72) for UM and CM fish. Variable juvenile performance for the parameters tested indicated significant differences among broods and families and suggests a cautionary approach to the widespread use of cryopreservation for steelhead.

Reisenbichler, R. R., S. P. Rubin, L.A. Wetzel, and S. R. Phelps. 2004. Natural selection after release from a hatchery leads to domestication in steelhead, *Oncorhynchus mykiss*. Pages 371-383 In K.M. Leber, H.L. Blankenship, S. Kitada, and T. Svåsand [editors] Stock Enhancement and Sea Ranching: developments, pitfalls, and opportunities. 2nd edition. Blackwell Science Ltd, Oxford.

Abstract: Genetic theory and data suggest that sea ranching of anadromous salmonids (*Oncorhynchus* spp. and *Salmo* spp.) results in domestication (increased fitness in the hatchery program) accompanied by a loss of fitness for natural production. We tested for genetic differences in growth, survival, and downstream migration of hatchery and wild steelhead (*O. mykiss*) reared together in a hatchery. We found little or no difference in survival during hatchery rearing but substantial differences in growth and subsequent downstream migration. Intense natural selection after release from the hatchery favored fish that had performed well (e.g. grew fast) in the hatchery. This selection in the natural environment genetically changes (domesticates) the population because at least some of the performance traits are heritable. Domestication should improve economic efficiency for producing adult hatchery fish but compromise conservation of wild populations when hatchery fish interbreed with wild fish.